Ref. No. [UMCES] CBL 2017-050
ACT VS17-05

PERFORMANCE VERIFICATION STATEMENT
For Real Tech Real Nitrate Analyzer GL Series

TECHNOLOGY TYPE: Nutrient Sensors

APPLICATION: In situ estimates of NO_3 for coastal moored deployments

PARAMETERS EVALUATED: Accuracy, precision, range response and reliability

TYPE OF EVALUATION: Laboratory and Field Performance Verification

DATE OF EVALUATION: Testing conducted from January 2015 to November 2016

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EXECUTIVE SUMMARY

The Alliance for Coastal Technology (ACT) conducted a sensor verification study of in situ nutrient analyzers during 2016 to characterize performance measures of accuracy, precision and reliability. The verification including a week of laboratory testing along with three moored field deployments in freshwater, estuarine, and oceanic coastal environments. Laboratory tests of accuracy, precision, and range were conducted at the University of Maryland's Chesapeake Biological Laboratory (CBL) in Solomons, MD. A series of five tests were conducted to evaluate performance under controlled challenge conditions including: concentration range, temperature, salinity, turbidity, and dissolved organic carbon. All laboratory tests were conducted in 250 L polypropylene tanks using RO water as the initial matrix, within a temperature controlled room. Instruments sampled from a common, well-mixed, test tank maintained at a documented level of known challenge condition. Instruments were set-up by the manufacturer daily prior to the start of each individual laboratory test, exposed to each test condition for a period of three hours, and programmed to sample at a minimum frequency of 30 minutes. Reference samples were collected every 30 minutes for five timepoints during corresponding instrument sampling times for each test.

For the laboratory concentration range challenge the absolute difference between the Real Tech Real-NO3 and reference measurement across all timepoints for trials C0 – C5 ranged from -0.217 to 0.490 mgN/L, with a mean of 0.185 ±0.168 mgN/L. A linear regression of the measurement difference versus concentration was significant ($p=0.0192$; $r^2=0.193$), but with a low regression coefficient due to a reversal in direction for the C4 trial. In general, the Real-NO3 increasingly over-predicted concentrations as they increased in the test. An assessment of precision was performed by computing the standard deviations and coefficients of variation of the five replicate measurements for C1 – C5 concentration trials. The standard deviation of the mean ranged from 0.010 to 0.022 mgN/L across the five trials, and the coefficient of variation ranged from 0.20 to 6.47 percent. For the laboratory temperature challenge at 5 ^{o}C, the absolute difference between instrument and reference measurement across all timepoints for trials C2 – C4 ranged from -0.0880 to 0.4381 mgN/L, with a mean of 0.056 ±0.115 mgN/L. Measurement differences at both C2 and C3 were significantly lower at 5 ^{o}C (0.017 and 0.058) versus 20 ^{o}C (0.020 and 0.237) ($p<0.01$). Differences were not statistically significant across temperatures at the C4 level. Similar to test results at 20 ^{o}C, the measurement offset increased in a positive direction as concentration increased. For the laboratory salinity challenge performed at the C3 concentration level, the absolute difference between instrument and reference measurement across all timepoints for the three added salinity levels ranged from 0.146 to 0.483 mgN/L, with a mean of 0.272 ±0.095 mgN/L. A linear regression of the measurement differences versus salinity was significant ($p=0.004$; $r^2=0.38$) with a slope of 0.005 and intercept of 0.184. The average offset at salinity 30 was 0.16 mgN/L higher than the average for the 10 and 20 salinity trials. For the laboratory turbidity challenge, performed at the C3 concentration level, the absolute difference between instrument and reference measurement across all timepoints for the two added turbidity levels ranged from 0.028 to 0.135 mgN/L, with a mean of 0.096 ±0.036 mgN/L. The effect of turbidity on measurement accuracy was mixed, when compared against RO water results, however, the magnitude of over-prediction approximately doubled between the 10 and 100 NTU trials. For the laboratory DOC challenge, performed at the C3 concentration level, the absolute difference between instrument and reference measurement across all timepoints for the two added DOC levels ranged from 0.099 to 0.482 mgN/L, with a mean of 0.292 ±0.193 mgN/L. The measurement difference increased positively by a factor of four between the 1 and 10 DOC trials. A linear

regression of the measurement differences versus DOC concentration was significant (p=0.008; r^2=0.43), with a slope of 0.013 and intercept of 0.118.

A 32 day field deployment occurred from May 26 through June 27 in the Maumee River, at the facilities of the Bowling Green, Ohio Water Treatment Plant. The Real-NO3 operated successfully during 31 of the total 32 day deployment, sampling at 5 minute intervals. The instrument shut down on 5/31 and was rebooted on 6/1 per manufacturer's instructions resulting in the loss of one day of data. The Real-NO3 generated 8827 accepted observations out of a possible 9156 for a data completion result of 96.4%. In total, 11 were omitted as outliers due to extreme range (<-0.01 or >25 mgN/L) and 318 values were missing from the inoperable period. The average and standard deviation of the measurement difference between instrument and reference NO_3 measurements for each matched pair (n=47 of a possible 51 observations) over the total deployment was 0.312 ± 1.029 mgN/L with a total range of -3.35 to 1.15 mgN/L. There was no significant trend in measurement difference over time as estimated by linear regression (p= 0.28; r^2=0.026). A linear regression of instrument versus reference measurement was highly significant (p<0.0001; r^2 = 0.75) with a slope of 0.96 and intercept of 0.38.

An 84 day moored field test was conducted in Chesapeake Bay from July 18 to October 10, 2016. The Real-NO3 operated continuously for 69 days until 9/24 when air purge system malfunctioned. The system was bypassed per manufacturer's instructions and the instrument restarted on 9/30. The instrument returned 22,345 observations out of a possible 24,144 based on approximate 5 minute sampling intervals for a data completion rate of 93%. The average and standard deviation of the measurement difference between instrument and reference NO_3 measurements for each matched pair (n=100 of a possible 103 observations) over the total deployment was 0.083 ±0.022 mgN/L, with the total range of differences between 0.018 to 0.166 mgN/L. There no significant trend in measurement difference over time during the deployment (p=0.681; r^2=0.002). A linear regression of the data was significant (p=0.0002; r^2 = 0.132), with a slope of 0.680 and intercept of 0.085. For the calibration set-up at this field test, the Real-NO3 significantly over-predicted concentrations.

A one month long moored field test was conducted in Kaneohe Bay from October 3, 2016 to November 2, 2016. The Real-NO3 was not deployed at HIMB at the manufacturer's decision.

BACKGROUND AND OBJECTIVES

The Alliance for Coastal Technologies (ACT) is a NOAA- and EPA-funded partnership of research institutions, state and regional resource managers, and private sector companies that are interested in developing, improving, and applying sensor technologies for studying and monitoring coastal environments. ACT was established on the premise that instrument validation of existing and emerging technologies is essential to support both coastal science and resource management. The overall goals of ACT's verification program are to provide industry with an opportunity to have a third-party test their instruments in both controlled laboratory settings and in diverse field applications within a range of coastal environments, and to provide users of this technology with an independent and credible assessment of instrument performance.

ACT partnered with the multi-agency Challenging Nutrients Coalition on the Nutrient Sensor Challenge to help address the environmental and ecological problems associated with nutrient pollution. A critical step in this process is facilitating the development and adoption of the next-generation of *in-situ* nutrient sensors and analyzers. To that end, the ACT Technology Verification model was applied to the Nutrient Sensor Challenge to test instrument performance in laboratory and field tests against reference water samples analyzed using EPA-approved standard methods.

The report within contains the test results for Real Tech's Real Nitrate Sensor during the ACT Performance Verification. A synthesis of the testing protocols and reference sample analysis are provided below. A complete copy of the verification protocols is available on the ACT website at the following link: http://www.act-us.info/nutrients-challenge/Download/Nutrient_Challenge_Test%20Protocols_PV16_01.pdf

INSTRUMENT TECHNOLOGY TESTED

Spectrophotometry is a well-known analytical technique that uses light in the ultraviolet (UV) and visible (VIS) wavelength range to measure substances that absorb or reflect light across a range of wavelengths. Nitrate ions have a natural absorbance peak in the 200-210 nm wavelength range in the UV spectrum and strongly absorb light between 200 and 240 nm. As the concentration of nitrates in water increases, the absorbance of light in this distinct wavelength range will also increase.

Real Tech's Real Nitrate Sensor (denoted as Real-NO3 throughout the report) operates by shining UV-VIS light from a xenon flash lamp through a quartz measurement cell and the absorbance between 200-240 nm is measured for nitrate concentration. In addition, reference wavelengths in the UV-VIS spectrum are also measured and used to compensate for common interferences with nitrate measurement, such as organic compounds, iron, and turbidity or suspended solids. The absorbance data are then converted to a milligram per liter (mg/L) concentration value using chemometrics, and custom software algorithms programmed in the sensor controller. The sensor comes factory calibrated with a standard nitrate algorithm. The calibration can be further improved upon by incorporating site-specific data into the existing data set. This way, the sensor can learn the characteristics of the on-site water and improve its accuracy over time. No reagents are used for nitrate detection purposes.

To compensate for drift and instability associated with volatile light sources, the sensor continuously rotates from a test position to a reference position. This proprietary measurement technique allows for a high degree of accuracy and repeatability.

The instrument is a bypass design that pulls a sample from a pressurized source or open channel to the sensor inside a cabinet for measurement. The cabinet provides easy access to the sensor for visual inspection and routine maintenance. The bypass design also allows for the sample to be diluted with Real Tech's proprietary dilution system to achieve a greater measurement range. Another advantageous feature specific to the cabinet design is the ability to clean the measurement cell with chemicals. All optical sensors are prone to fouling over time which will impact the accuracy of measurement. Mechanical methods, such as the use of wipers or brushes, tend to wear down and are often ineffective for many fouling agents. Chemical cleaning provides the flexibility to use a chemical that is ideal for removing the site-specific fouling agents. Depending on the site-specific requirements, used cleaning chemicals can be diverted and stored in a container for future disposal.

Multiple product configurations are available for nitrate monitoring applications. The monitoring systems are designed for and most commonly used at groundwater blending stations, municipal drinking water plants, and municipal and industrial wastewater treatment facilities.

PERFORMANCE EVALUTION TEST PLAN

These Test Protocols are based on consensus recommendations of the ACT Technical Advisory Committee, ACT staff, and participating Manufacturers. In summary, the test:

- utilized standard, approved laboratory analytical methods to provide best possible measure of the 'true' nutrient concentration from reference samples, which served as performance standards against which instrument estimations were compared internally by the individual developer;
- conducted all reference sample analysis at the state certified Nutrient Analytical Services Laboratory (NASL) of the Chesapeake Biological Laboratory (CBL), Solomons, MD to determine true nutrient concentrations using USEPA approved methodologies (see details below);
- included a laboratory evaluation of instrument performance;
- included three moored/dock-based field trials under a wide range of environmental conditions including freshwater, estuarine and marine ecosystems with varying nutrient concentrations and water quality characteristics (e.g. turbidity).

All ACT personnel involved in the Nutrient Sensor Verification were trained on standardized water sample collection, storage and shipping methods. ACT staff was available to assist in the physical deployment and recovery of all submitted test instruments and were responsible for the data management of test instrument results. Challenge participants were responsible for initial set-up and calibration of their instrument. If requested, ACT provided the chemicals and nutrient standards needed for instrument set-up and calibration. All laboratory nutrient analyses of the independent reference samples were conducted at the CBL NASL using standardized automated wet chemistry. All numerical data were recorded to three significant decimals where appropriate and nutrient concentrations reported in elemental mass units as mgN/L or mgP/L for nitrate+nitrite (NO_{23}), nitrate (NO_3^-) or phosphate (PO_4^{3-}), respectively.

Laboratory Tests

Laboratory tests of accuracy, precision, and range were conducted at the University of Maryland's Chesapeake Biological Laboratory (CBL) in Solomons, MD. A series of five tests

were conducted to evaluate performance under controlled challenge conditions including: concentration range, temperature, salinity, turbidity, and dissolved organic carbon (details below). All Laboratory tests were conducted in polypropylene tank using RO water as the initial matrix, within a temperature controlled room. All instruments sampled from a common, well-mixed, test tank of approximately 250L volume, maintained at a documented level of known challenge condition. Instruments were set-up by the manufacturer daily prior to start of each individual laboratory tests. Instruments were exposed to each test condition for a period of three hours and programmed to sample at a minimum frequency of 30 minutes. Reference samples were collected every 30 minutes for five timepoints during instrument sampling times for each test. Laboratory tests included the following 'controlled' challenge conditions:

Test 1: Accuracy and Precision over a broad concentration range

- Tested response across a broad range of concentrations representative of natural waters.
  - Concentration levels for NO_3 (mgN/L): 0.005, 0.1, 1.0, 5, 10, and 50
  - Concentration levels for PO_4 (mgP/L): 0.002, 0.01, 0.05, 0.1, 0.5, and 2.0
- The range test was split into two separate tests with concentrations for levels 1-4 conducted on day 1 and the last two concentrations tested on day 6 due to time constraints. Note that the starting level on day 6 was mistakenly set to 5 mgN/L and the 10 mgN/L level was not actually tested.
- Three hour sampling windows were provided at each of the six concentrations during which instruments measured concentrations at a minimum frequency of every 30 minutes.
- Discrete reference samples were collected every 30 minutes, corresponding to instrument sampling times, to generate five comparative measurements to assess accuracy and precision against reference values.
- RO water was used as the test matrix to which known amounts of nutrient salts (KNO_3 and K_2HPO_4) were added. Analysis of ambient blanks indicated a small amount of inorganic nutrients in the RO water.
- Tests were conducted at 20 ^oC in a temperature controlled room with samples drawn from a common well-mixed 250L test tank.

Test 2: Temperature Response

- Instrument response was tested for three concentrations, corresponding to levels C2, C3, and C4 from the range test, at temperatures of 5 ^oC versus the temperature of 20 ^oC on the first day.
- Temperature was regulated and maintained within a temperature controlled room and independently verified in the test tank with an YSI EXO2 reading at 15 min intervals.
- Instruments were equilibrated to the new 5 ^oC test temperature overnight.
- Instruments were exposed for three hours at each of the 3 concentrations with reference samples collected every 30 minutes following an initial 30 minute equilibration period to each condition.

Test 3: Salinity Response

- Accuracy and precision was tested over three additional salinities (10-20-30) at the C3 concentration level of the range test at 20^oC.

- Salinity levels were developed using Instant Ocean additions to the RO water matrix, which could have contributed trace amounts of nutrients, but would have measured in the final reference samples.
- Instruments were exposed for three hours at each of the 3 concentrations with reference samples collected every 30 minutes following an initial 30 minute equilibration period to each condition.

Test 4: Turbidity Response

- Accuracy and precision were tested over two elevated turbidity levels (approximately 10 and 100 NTU) at the C3 concentration level of the range test at 20 ^{o}C.
- Test tanks were continuously mixed with submersed pumps but there was some settling of the material as noted by continuous monitoring with the EXO2 sonde and analysis of discrete turbidity samples on the Hach 2100.
- Turbidity concentrations were established using Elliot Silt Loam reference material (cat # 1B102M) available from the International Humic Substances Society (http://www.humic-substances.org) added into RO water matrix.
- Instruments were exposed for three hours at each of the 3 concentrations with reference samples collected every 30 minutes following an initial 30 minute equilibration period to each condition.

Test 5: DOC Response

- Accuracy and precision were tested against two DOC levels (1 and 10 mg/L) at the C3 concentration level of the range test at 20 ^{o}C.
- DOC concentrations were established using the Upper Mississippi River Natural Organic Matter standard (cat# 1R110N) available from the International Humic Substances Society (http://www.humic-substances.org) added to RO water matrix.
- Instruments were exposed for three hours at each of the 3 concentrations with reference samples collected every 30 minutes following an initial 30 minute equilibration period to each condition.

Field Tests

In situ field performance evaluations of the test instruments were conducted under extended mooring deployments at three ACT Partner Institution sites covering freshwater, estuarine, and marine conditions. Site specific details for each test site were as follows:

Freshwater Deployment: The freshwater deployment occurred on the Maumee River in Waterville, OH for one month duration and provided a high nutrient, high turbidity test environment. The ACT Partner at the University of Michigan established a flow-through system on the Maumee River near Waterville Ohio (83.74 ^{o}N; 41.48 ^{o}W), located within the pump house of the City of Bowling Green Municipal Water Treatment Plant. Instruments were deployed in a 180 gallon flow-through tank with a water depth of approximately 0.8m and exchange time of approximately 10 minutes. The Maumee River main stem flows 137 km before flowing into the Maumee Bay of Lake Erie at the city of Toledo, Ohio. The Maumee watershed is the largest watershed of any Great Lakes river with 8,316 square miles. The majority of the watershed is cultivated crop land, mostly corn and soybeans, though concentrated areas of pasture are located in the northwestern and southeastern areas of the watershed.

Estuarine deployment : The estuarine deployment occurred at the research pier of the Chesapeake Biological Laboratory in Solomons, MD for three month duration and provided for variable salinity and nutrient levels within a highly productive and biofouling environment. The ACT Partner at Chesapeake Biological Laboratory (CBL), University of Maryland Center for Environmental Science, has established a Technology Verification Field Test Site on a fixed pier (38.32 ^{o}N;76.45 ^{o}W), with an average depth of 2.1 m at the mouth of the Patuxent River, a tributary of the Chesapeake Bay. The deployment frame was arranged so that all of the sample inlets for the instruments remain at a fixed depth of 1 m below the water surface using a floating dock. The Chesapeake is a nutrient rich estuary with a watershed that encompasses portions of six states and the District of Columbia. Water temperatures at the test site ranged from 20 to 31°C and salinity ranged from 12.7 to 16.9 psu during the Verification.

Marine deployment: The marine deployment occurred in Kaneohe Bay at the Hawaii Institute of Marine Biology field lab for one month duration and provided a full salinity, low nutrient test condition. The ACT Partner at the Hawaii Institute of Marine Biology (HIMB) is part of the University of Hawaii with a field site established on the Kaneohe Bay Barrier Reef flat (21.43 ^{o}N;157.79 ^{o}W) in waters ~16 m deep. The deployment frame was arranged so that all of the sample inlets for the instruments remain at a fixed depth of 1 m below the water surface using a floating dock. Kaneohe Bay sits on the northeast, or windward, side of Oahu. Water temperatures at this site varied between 24.5 and 27.9°C and salinities were between 27.3 and 34.8 psu during the Verification.

Instrument Setup - Prior to deployment, all instruments were set up and calibrated as required at the field sites by a manufacturer representative, with assistance provided by ACT staff as necessary. The manufacturer supplied or specified to ACT all specific materials and hardware (chemicals, power cords, cables, weights, etc.) needed to deploy the test instrument according to requirements defined for each field site. ACT staff worked with the manufacturer to design an appropriate sensor deployment configuration at each site and arranged instruments in a manner so that a single representative field sample could be collected without the potential of interference between instruments. No servicing of the instruments was to occur during the test deployment period unless observed physical damage had occurred from natural events and a repair or replacement was deemed necessary. Instruments were set up as self-recording, either internally or to an external data logger, and programmed to record data based on a time interval that allowed instruments to function for the specified number of days for the respective deployment. Specific sampling intervals varied among test instruments, but with a stated goal of 15 minute sampling intervals if possible and two-hour intervals at maximum. A sampling schedule was established so that all instruments being tested at the same time had a common sampling time point at a minimum frequency of 2 hours. Internal clocks were set to local time and synchronized against the time standard provided by www.time.gov.

Reference Water Sampling Schedule – The reference sampling schedule generated between 50 - 100 comparative reference samples and was structured to examine changes in nutrient concentrations over daily to monthly time scales. Specifically, once each week ACT staff conducted an intensive sampling event that consisted of four consecutive samples spaced at two-hour intervals. For the remaining four days of the week, ACT staff sampled once or twice per day, spaced out to cover early morning and late-afternoon timepoints or anticipated flow or tidal events. The initial intensive sampling event occurred within the first two days of the deployment after all instruments had been deployed, and the final intensive sampling event occurred during the last two days of the deployment.

Reference Water Sample Collection - A standard 2L Van Dorn bottle was used at the CBL and HI field sites to collect reference water samples for laboratory nutrient concentration analysis. For the riverine test site a 1L acid-cleaned, polypropylene bottle was filled directly from the flow-through tank. For the tank sampling, the sampling bottle was rinsed three times before filling. For the mooring sites, the Van Dorn bottle was lowered to the same depth and as close as physically possible to the sampling inlets of all instruments and less than 1 m from any individual sampling inlet and soaked at sampling depth for 1 minute prior to sampling. The water sample was then transferred to an acid washed 1L polypropylene bottle after three initial rinses of the field sample. All environmental reference samples were processed within 10 minutes of collection while wearing clean laboratory gloves to minimize potential sources of contamination. The sample was filtered through a 47mm Whatman GFF filter into an acid cleaned vacuum flask. The first 50 ml of filtrate were discarded as a rinse. The remaining filtrate was distributed into 8 individual acid-cleaned, 30 ml polypropylene bottles to provide three analytical replicates each for NO_3 and PO_4 plus two replicates to hold as back-ups. All final sample bottles were rinsed once before filling and filled no more than ¾ full to allow adequate headspace for freezing. The final reference samples were immediately frozen and remained so until shipment to CBL-NASL for analysis.

Sample Handling and Chain of Custody - All collected reference samples at each test site were dated and coded according to site and sample sequence. Each sample container was labeled with a number for identification. The reference sample number was used in all laboratory records and Chain-of-Custody (COC) forms to identify the sample. Samples were shipped on dry ice to CBL-NASL for nutrient analysis within approximately two weeks of collection. Shipping containers were sent for next morning delivery, or the soonest possible delivery time possible from a given shipping location. All samples, including the condition shipped and received, were recorded onto Chain of Custody (COC) forms and a copy sent with the samples. The COC specified time, date, sample location, unique sample number, requested analyses, sampler name, and required turnaround time, time and date of transaction between field and laboratory staff, and name of receiving party at the laboratory. NASL confirmed receipt and condition of samples within 24 hours of their arrival by signing and faxing a copy of the form to the test site.

Reference Sample Analysis

Phosphate concentrations for all reference and quality control samples were determined by the NASL at CBL following their Standard Operating Procedures Manual (CEES, UMD, Publication Series No. SS-80-04-CBL). The methodology is based on U.S. EPA Method 365.1, 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). In brief, ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of phosphate to form an antimony-phospho-molybdate complex. The complex is reduced to an intensely blue-colored complex by ascorbic acid. The color produced is proportional to the phosphate concentration present in the sample.

Nitrate and nitrite concentrations for all reference and quality control samples were determined by the NASL at CBL following their Standard Operating Procedures Manual (CEES, UMD, Publication Series No. SS-80-04-CBL). The methodology 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). In brief, nitrate is reduced to nitrite using the cadmium reduction method. The nitrite is then determined by diazotizing with sulfanilamide and coupling with N-1-

naphthylethylenediamine di hydrochloride 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 cadmium reduction procedure.

All laboratory nutrient analyses were conducted on an Aquakem 250 auto-analyzer. For phosphates, a statistically-determined method of detection limit for this instrument of 0.0007 mgP/L was established by prior laboratory studies for a wide range of salinities. An expected working concentration range for this Verification and SOP was between 0.002 and 1.48 mgP/L. The detection limits for nitrate and nitrite were similarly established at 0.0007 mgN/L and 0.0006 mgN/L respectively. 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. The system contains an auto-dilutor to bring any higher concentrations down to the established linear calibration range. A sample reagent blank is analyzed in conjunction with every sample as part of the routine operation of the Aqaukem 250. Approximately 40 samples per hour can be analyzed. All internal standards were verified and calibrated using certified external nutrient standards (such as Spex Certi-Prep or NIST). In addition, Field Trip Blanks and Field Sample Spike Additions (defined below) were conducted once per week by ACT as part of established quality assurance/quality control (QA/QC) protocols.

RESULTS OF LABORATORY TEST

Accuracy

Real-NO3 measurements and corresponding reference measurements for the lab concentration range challenge are shown in figure 1. Results for the highest concentration are excluded from any numerical or statistical comparisons because of its extreme range, but were included in the test to help identify maximum detection potential. The absolute difference between instrument and reference measurement across all timepoints for trials C0 – C5 ranged from -0.217 to 0.490 mgN/L, with a mean of 0.185 ±0.168 mgN/L. The means for each trial are given in Table 1. A plot of the absolute difference between Real-NO3 and reference measurement is shown in the bottom panel of figure 1. There was significant trend in instrument offset versus concentration as estimated by linear regression ($p=0.0192$; $r^2=0.193$). There is no known explanation for the reversal in measurement offset for the C4 trial. Without that exception Real-NO3 measurement increasingly over-predicted concentration as test concentrations increased.

Table 1. Accuracy results for laboratory testing of the Real-NO3 analyzer assessed by absolute difference (mgN/L) and percent error between instrument and reference measurements for the concentration range test.

Trial	Reference	**Real-NO3**	Absolute Diff	% Error
C0	0.0224	0.0401	0.0177	78.8
C1	0.0282	0.1887	0.1604	568.2
C2	0.1330	0.3345	0.2015	151.6
C3	1.1005	1.3378	0.2373	21.6
C4	5.6629	5.6358	-0.0270	0.5
C5	4.4573	4.9103	0.4530	10.2

Precision

An assessment of precision was performed by computing the standard deviations and coefficients of variation of the five replicate measurements for each of the concentration challenges. The standard deviation of the mean ranged from 0.010 to 0.022 mgN/L across the five trials, and the coefficient of variation ranged from 0.20 to 6.47 % (Table 2).

Table 2. Precision assessment of the Real-NO3 analyzer during the laboratory concentration range test. Variance is reported as the standard deviation and coefficient of variation of five replicate measurements collected at 30 minute intervals in a well-mixed tank maintained at known uniform conditions.

	Mean NO_3 (mgN/L)		**Standard Deviation**		**Coefficient of Variation**	
Trial	*Reference*	*Real-NO3*	*Reference*	*Real-NO3*	*Reference*	*Real-NO3*
C1	0.0282	0.1887	0.0032	**0.0122**	11.45	**6.47**
C2	0.1330	0.3345	0.0020	**0.0174**	1.50	**5.21**
C3	1.1005	1.3378	0.0087	**0.0102**	0.79	**0.76**
C4	5.6629	5.6358	0.1243	**0.0113**	2.19	**0.20**
C5	4.4573	4.9103	0.0195	**0.0220**	0.44	**0.45**

Lab Concentration Range Challenge

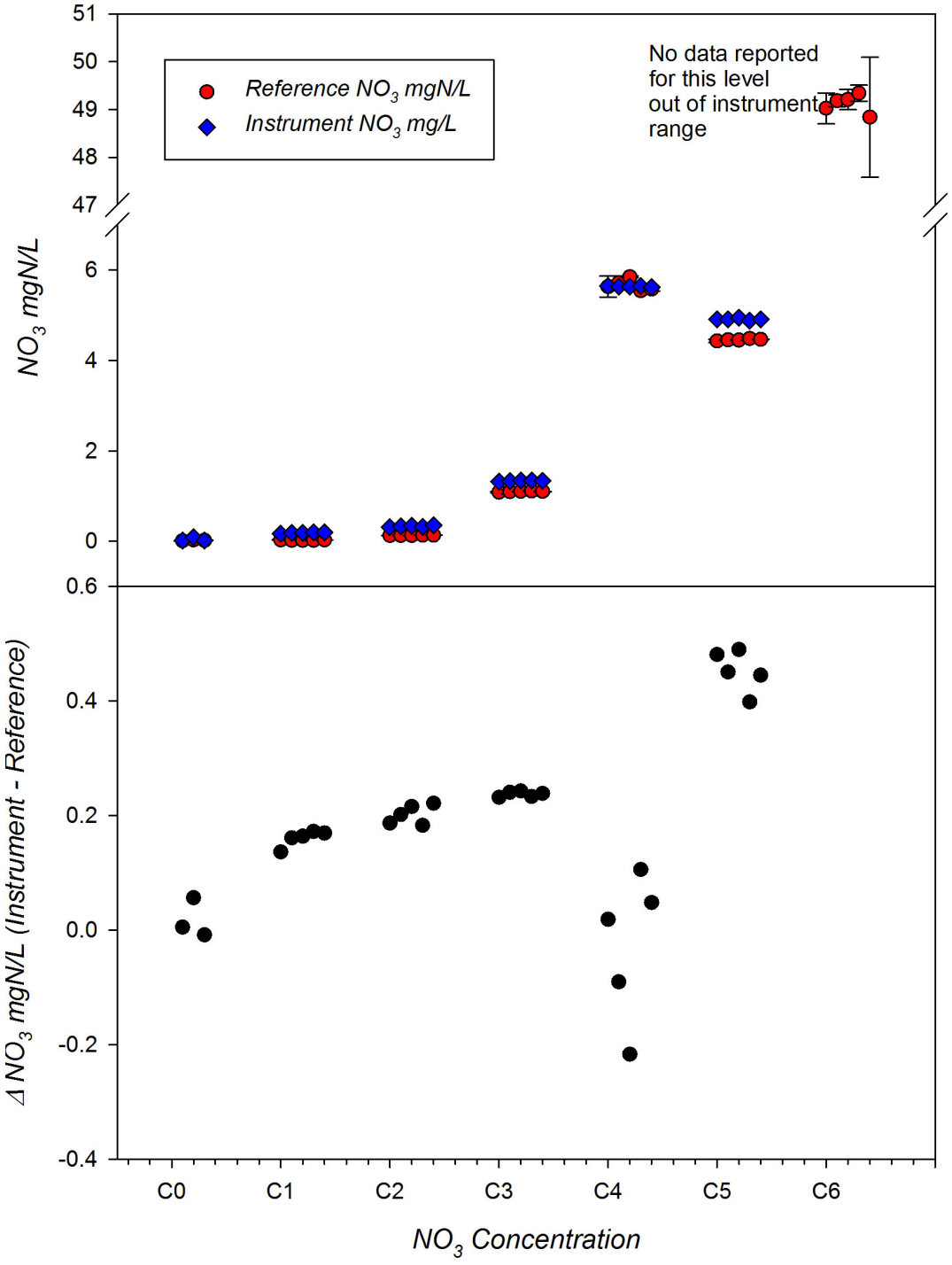


Figure 1. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO_3 in the laboratory concentration range challenge covering ambient plus 6 concentration ranges. Five replicate measurements were made at each concentration level along with three measurements at ambient level. *Bottom Panel:* Plot of the absolute difference in mgN/L between Real-NO3 and reference measurement.

Time series results of ambient water quality conditions for the salinity, turbidity, and DOC matrix challenges are presented in figure 2. Final test concentrations of turbidity and DOC were slightly below the stated target levels, and there was noticeable settling of turbidity at the highest addition level, but confirm the overall challenge conditions being tested.

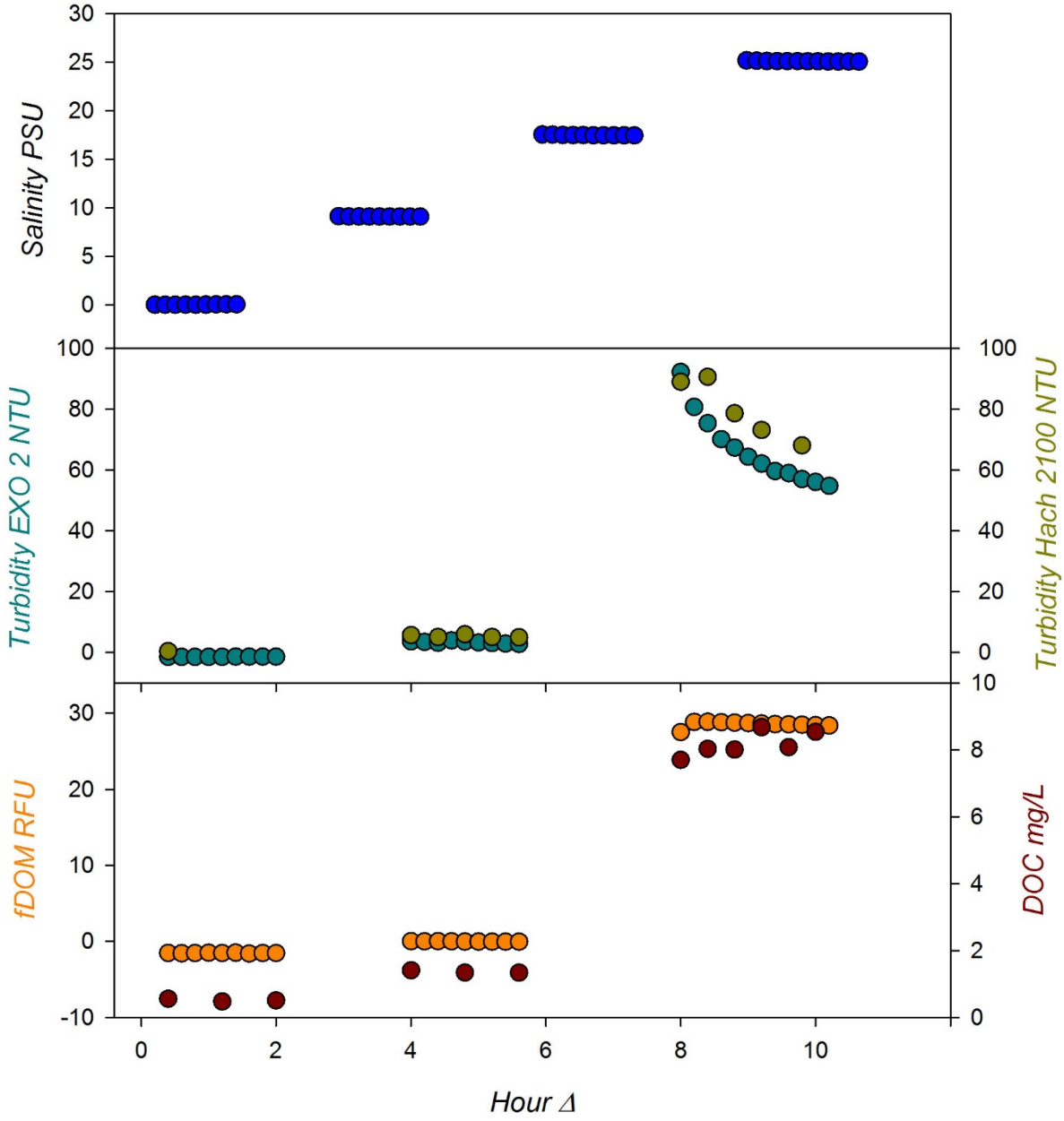


Figure 2. *Top Panel: In situ* salinity measured by EXO2 sonde in the laboratory salinity challenge covering ambient plus 3 salinity ranges. *Middle Panel: In situ* turbidity measured by EXO2 sonde (teal) and on grab samples by a Hach 2100 Turbidimeter (olive) during the laboratory turbidity challenge covering ambient plus 2 additions. *Bottom Panel: In situ* fDOM measured by EXO2 sonde (orange) and DOC of discrete samples (dark red) during the DOC challenge covering ambient plus 2 additions.

Results of the laboratory temperature challenge at 5 ^{o}C are shown in figure 3. The absolute difference between instrument and reference measurement across all timepoints for trials C2 – C4 ranged from -0.0880 to 0.4381 mgN/L, with a mean of 0.056 ±0.115 mgN/L. The means for each trial are given in Table 3. Measurement differences at both C2 and C3 were significantly lower at 5 ^{o}C (0.017 and 0.058) versus 20 ^{o}C (0.020 and 0.237) ($p<0.01$). Differences were not statistically significant across temperatures at the C4 level. Similar to test results at 20 ^{o}C, the measurement offset increased in a positive direction as concentration increased during the 5 ^{o}C test.

Table 3. Summary of accuracy results for temperature trials assessed by absolute difference (mgN/L) and percent error between instrument and reference measurements.

Trial	Reference	**Real-NO3**	Absolute Diff	% Error
C2	0.1162	0.1328	0.0166	14.3
C3	1.0627	1.1203	0.0576	5.4
C4	5.4630	5.5565	0.0935	1.7

Results of the laboratory salinity challenge at the C3 concentration level are shown in figure 4. The absolute difference between instrument and reference measurement across all timepoints for the three added salinity levels ranged from 0.146 to 0.483 mgN/L, with a mean of 0.272 ±0.095 mgN/L. The means for each salinity trial are given in Table 4. The zero salinity results are taken from the initial concentration challenge on day 1. There was a significant increase in measurement offset (more positive) at a salinity of 30, whereas differences were quite similar at the 0, 10, and 20 salinity levels. A linear regression of the measurement differences versus salinity was significant ($p=0.004$; $r^2=0.38$) with a slope of 0.005 and intercept of 0.184, clearly reflecting the strong difference for the salinity 30 test. The average offset at salinity 30 was around 0.16 mgN/L higher than the average for the other trials, which corresponded to a doubling of the relative error to nearly 42%.

Table 4. Summary of accuracy results for salinity trial assessed by absolute difference (mgN/L) and percent error between instrument and reference measurements.

Trial	Reference	**Real-NO3**	Absolute Diff	% Error
0	1.1005	1.3378	0.2373	21.6
10	0.9358	1.1565	0.2207	23.6
20	1.0227	1.2329	0.2102	20.6
30	0.9222	1.3064	0.3842	41.7

Results of the laboratory turbidity challenge at the C3 concentration level are shown in figure 5. The absolute difference between instrument and reference measurement across all timepoints for the two added turbidity levels ranged from 0.028 to 0.135 mgN/L, with a mean of 0.096 ±0.036 mgN/L. The means for each turbidity trial are given in Table 5. Results for the zero turbidity level are taken from the initial concentration challenge on day 1. The measurement difference increased positively by a factor of two between the 10 and 100 NTU trials. However offsets for both trials were substantially lower than the results seen for C3 on day 1 using RO water. Due to the higher offset in the zero trial, a linear regression of the measurement differences versus turbidity was significant ($p=0.02$; $r^2=0.34$), with a slope of -0.005 and intercept of 0.209, however the negative slope contradicts the increased positive offset seen between the two turbidity addition trials.

Table 5. Summary of accuracy results for turbidity trials assessed by absolute difference (mgN/L) and percent error between instrument and reference measurements.

Trial	Reference	**Real-NO3**	Absolute Diff	% Error
0	1.1005	1.3378	0.2373	21.6
10	1.0002	1.0662	0.0660	6.6
100	0.9798	1.1066	0.1268	12.9

Results of the laboratory DOC challenge at the C3 concentration level are shown in figure 6. The absolute difference between instrument and reference measurement across all timepoints for the two added DOC levels ranged from 0.099 to 0.482 mgN/L, with a mean of 0.292 ±0.193 mgN/L. The means for each of the DOC trials are given in Table 6. Results for the zero DOC level are taken from the initial concentration challenge on day 1. The measurement difference increased positively by a factor of four between the 1 and 10 DOC trials. A linear regression of the measurement differences versus DOC concentration was significant ($p=0.008$; $r^2=0.43$), with a slope of 0.013 and intercept of 0.118. The measurement offset was 0.37 mgN/L more positive at 10 versus 1 mg/L DOC, and corresponded to a relative error of approximately 48% versus 11%, respectively.

Table 6. Summary of accuracy results for Laboratory testing assessed by absolute difference (mgN/L) and percent error between instrument and reference measurements for each individual trial condition within each matrix challenge.

Trial	Reference	**Real-NO3**	Absolute Diff	% Error
0	1.1005	1.3378	0.2373	21.6
1	1.0013	1.1102	0.1089	10.9
10	0.9870	1.4621	0.4751	48.1

Lab Temperature Challenge

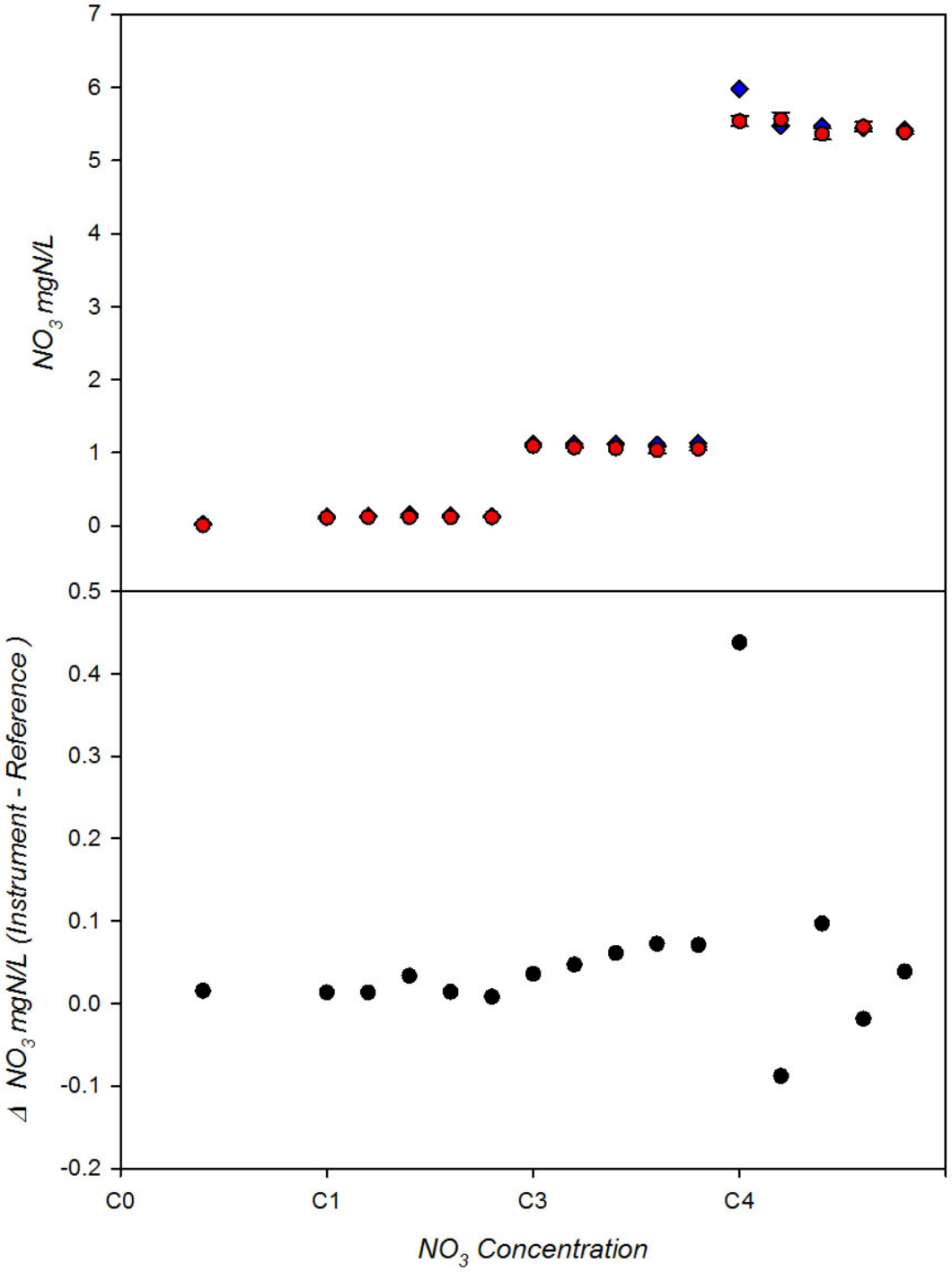


Figure 3. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO_3 (mgN/L) in the temperature response challenge covering concentration ranges C2 – C4 measured at 5 ^oC test conditions. Five replicate measurements were made at each concentration level along with one measurement at ambient level. *Bottom Panel:* Plot of the absolute difference between Real-NO3 and reference measurement.

Lab Salinity Challenge

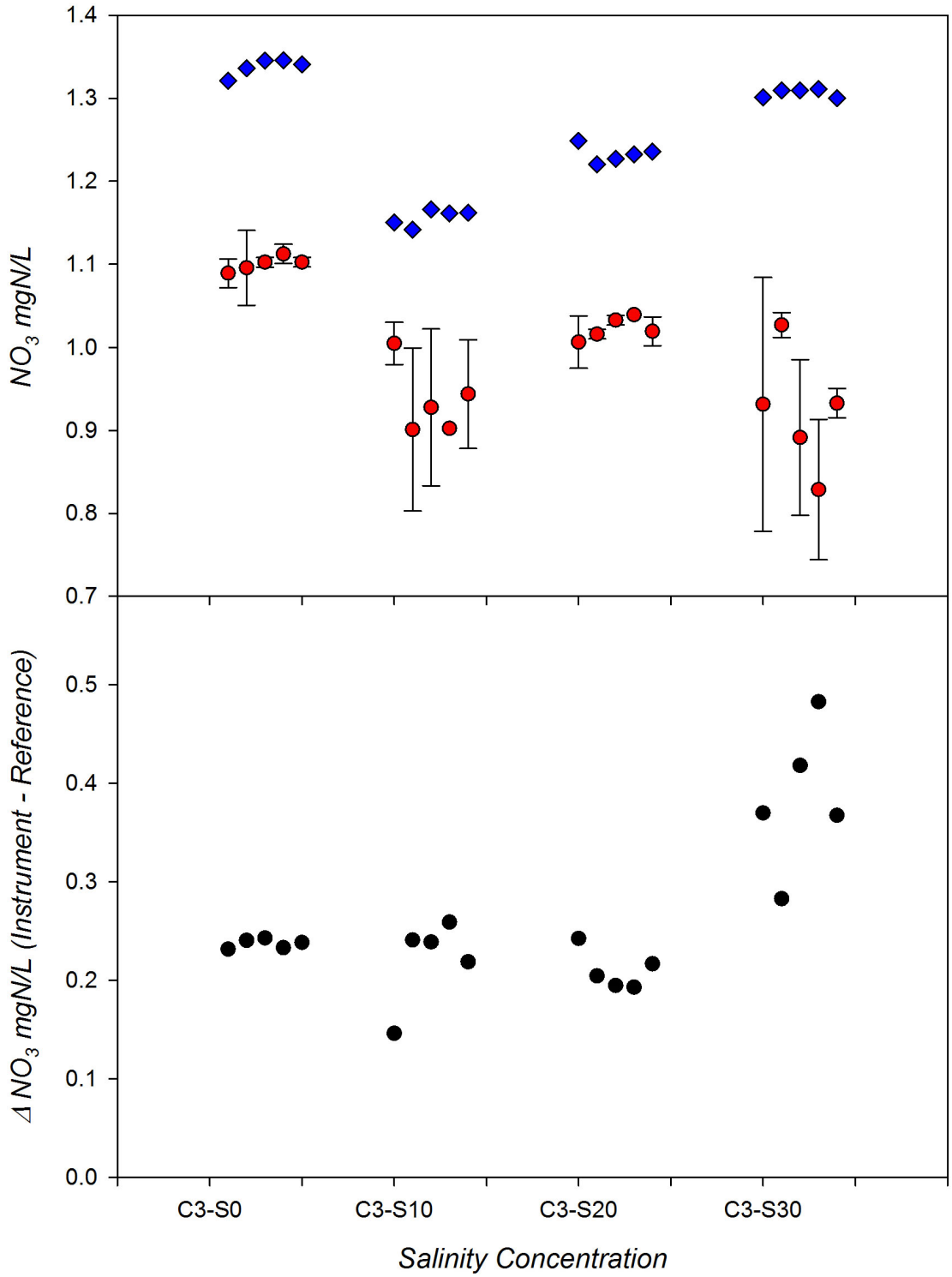


Figure 4. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO_3 (mgN/L) at four salinity levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between Real-NO3 and reference measurement.

Lab Turbidity Challenge

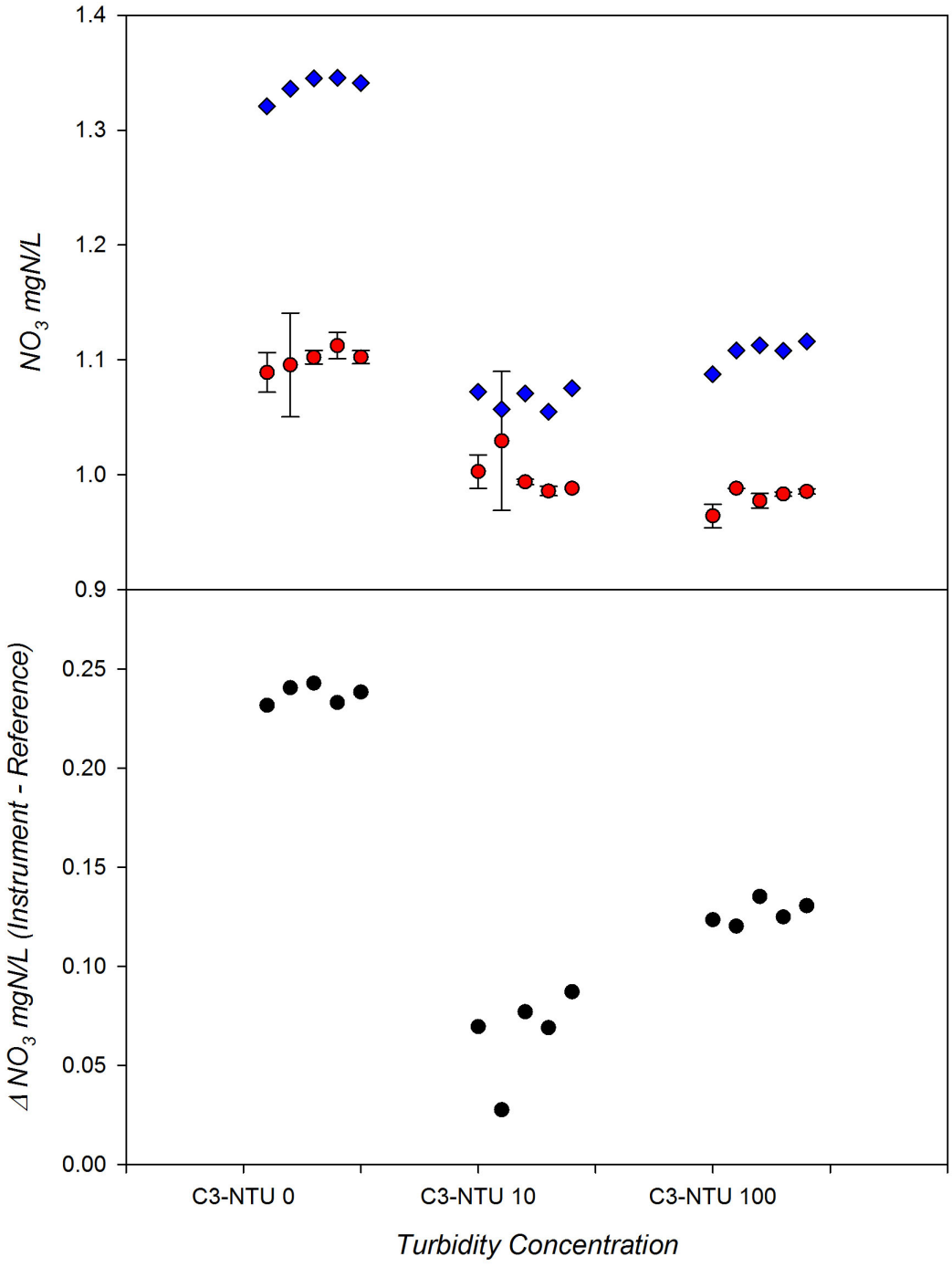


Figure 5. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO_3 (mgN/L) at three turbidity levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between Real-NO3 and reference measurement.

Lab DOC Challenge

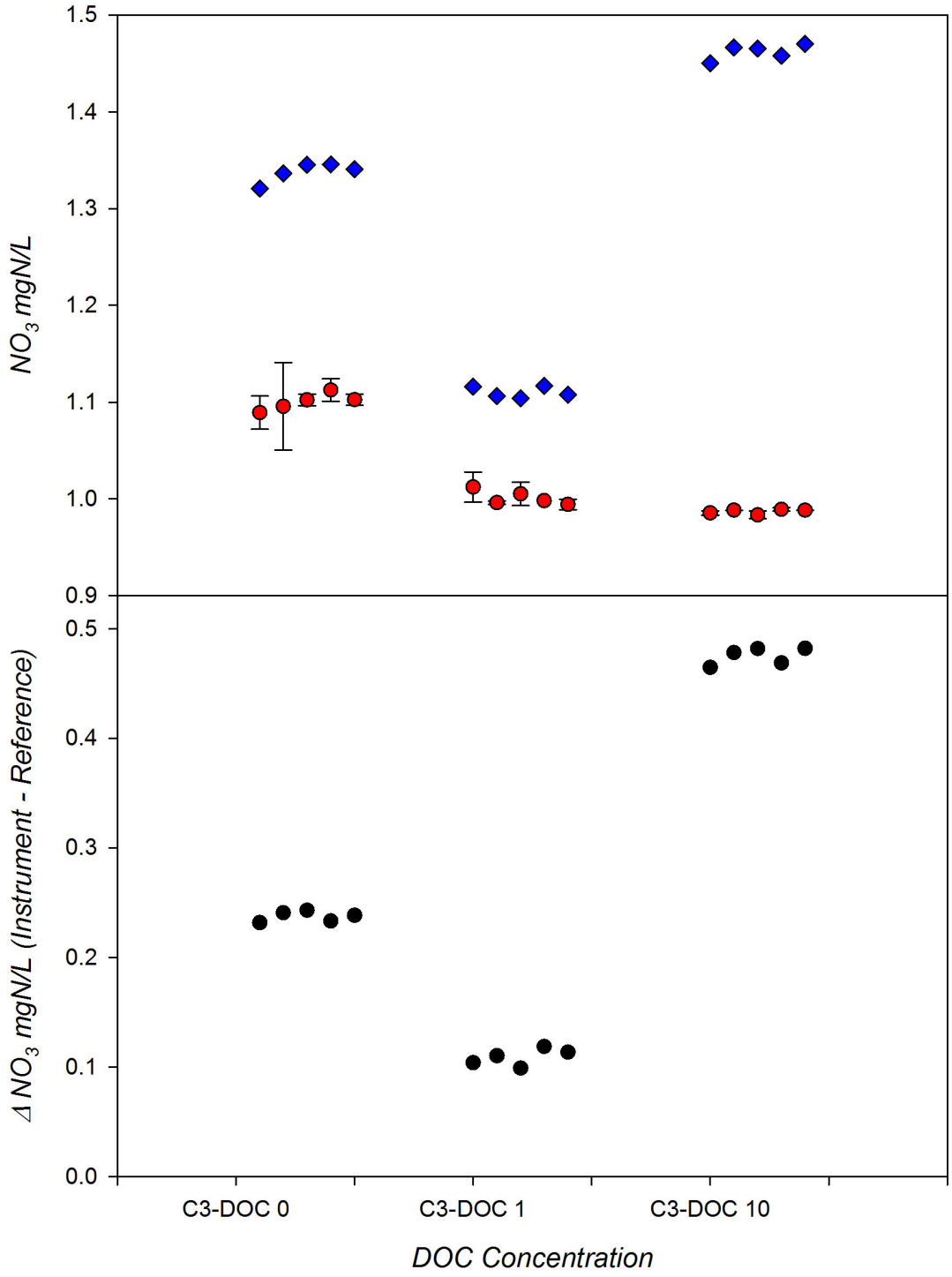


Figure 6. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO_3 (mgN/L) at three DOC levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between Real-NO3 and reference measurement.

A summary of measurement differences between the Real-NO3 and reference sample for each trial of each laboratory challenge is presented together in figure 7. With one exception for the C4 trial in the concentration range challenge the Real-NO3 over-predicted nitrate concentrations throughout the Laboratory testing. The magnitude of the offset ranged from 0.02 – 0.48 mgN/L. Measurement difference generally increased in a positive direction with increasing concentration at both 5 and 20 ^{o}C. Measurement differences also increased at the highest addition levels for salinity, turbidity, and DOC. Larger offsets occurred for the C3 concentration trial on day 1, which was used as the 'zero' addition level for the matrix challenges, and made it harder to establish predictable response effects to challenge additions. Results of measurement differences averaged across all trials within each of the challenge matrices are presented in Table 7.

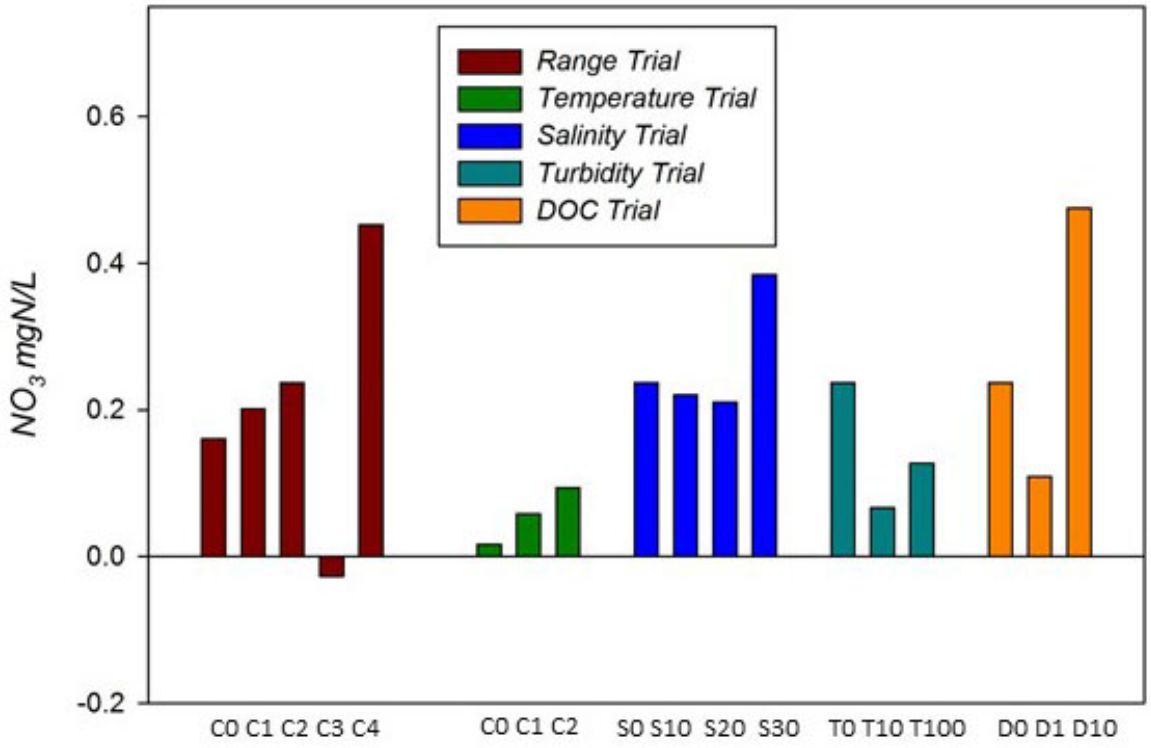


Figure 7. Global summary of difference between instrument and reference measurements for all laboratory tests at each trial conditions for the Real-NO3 analyzer.

Table 7. Measurement differences in mgN/L (min, max, mean, stdev) between instrument and reference concentrations averaged across all trials within a laboratory challenge.

Real-NO3	Range	Temp	Salinity	Turbidity	DOC
min	-0.0270	0.0166	0.2102	0.0660	0.1089
max	0.4530	0.0935	0.3842	0.1268	0.4751
mean	0.2050	0.0559	0.2717	0.0964	0.2920
stdev	0.1720	0.0385	0.0976	0.0430	0.2589

RESULTS of FIELD TESTS

Moored field tests were conducted to examine the performance of the Real-NO3 to consistently track natural changes in NO_3 over extended field deployments with durations of 31-84 days. In addition, field tests examined the reliability of the instrument, i.e., the ability to maintain integrity or stability of data collection over time. Reliability was determined by quantifying the percent of expected data that was recovered and useable. The performance of the Real-NO3 was examined in three separate field tests at various ACT Partner sites to include a range of biogeochemical conditions. The range and mean for temperature and salinity for each test site is presented in Table 8. The reference temperature and conductivity data was measured by RBR thermistors and a SeaBird SBE 26 or Xylem EXO2 sonde that were mounted at the same sampling depth as the test instrument. Immediately before and after each deployment, samples of the on-board standards were taken from the instrument for comparison against a reference measurement and to assess their stability over the course of the deployment (Table 9). The Real-NO3 was calibrated and programmed for deployment by the manufacturer representative.

Table 8. Range and average for temperature, and salinity at each of the test sites during the sensor field deployments. Temperature and salinity were measured by RBR temperature loggers and a SeaBird SBE 26 or a Xylem EXO2 mounted on the instrument rack or in the tank for the duration of the deployment.

SITE (deployment period/duration)		**Temperature** (°C)	**Salinity** (PSU)
Maumee River	Min.	20.1	0.0
26May – 27Jun	Max.	27.7	0.3
(n = 32 days)	Mean	24.3	0.2
Chesapeake Bay	Min.	20.0	12.7
18Jul – 10Oct	Max.	31.1	16.9
(n = 84 days)	Mean	27.2	14.7
Kaneohe Bay	Min.	24.5	27.3
3Oct – 2Nov	Max.	27.9	34.8
(n = 31 days)	Mean	26.3	34.2

Deployment at Maumee River Bowling Green, Ohio

A 32 day deployment occurred from May 26 through June 27 in the Maumee River, at the facilities of the Bowling Green, Ohio Water Treatment Plant (Figure 8). The deployment site was located at 41.48° N, 83.74° W, in a flow-through tank located in the water treatment plant pump house. The pump house is located above the Maumee, approximately 200 m up river from the water treatment intake and approximately 35 km from the Maumee outflow into Lake Erie. River water was continuously pumped into a 180 gallon test tank where it was mixed using two submerged pumps. The residence time in the tank was approximately 10 minutes. The instrumentation was suspended within the tank with the sampling inlet 0.2 m off the bottom.

Figure 8. Aerial view of the Maumee River (left) and the flow through deployment tank (right).

Time series results of ambient conditions for river discharge, temperature, specific conductivity, turbidity and chlorophyll are given in figure 9. Temperature ranged from 20.5 – 27.7°C, specific conductivity from 423 - 689 µS/cm, turbidity from 8 – 681 NTU, and chlorophyll from 4.5 – 131 µg/L over the duration of the field test.

The Real-NO3 operated successfully during 31 days of the total 32 day deployment sampling at approximately 5 minute intervals. The instrument shut down on 5/31 and was rebooted on 6/1 per manufacturer's instructions resulting in the loss of a day of data. The Real-NO3 generated 8827 accepted observations out of a possible 9156 for a data completion result of 96.4%. In total, 11 were omitted as outliers due to extreme range (<-0.01 or >25 mgN/L) and 318 values were missing from the inoperable period. Time series results of the Real-NO3 measurements and corresponding reference NO_3 results are given in figure 9 (top panel). NO_3 measured by the Real-NO3 ranged from 0.00 to 19.08 mgN/L compared to a range of 1.16 to 12.72 mgN/L within the reference samples.

The time series of the difference between instrument and reference NO_3 measurements for each matched pair (n=47 of a possible 51 observations) is given in the bottom panel of figure 10. Four of the 51 possible comparisons were lost because of missing instrument data. The average and standard deviation of the measurement difference over the total deployment was 0.312 ± 1.029 mgN/L with a total range of -3.35 to 1.15 mgN/L. There was no significant trend in measurement difference over time as estimated by linear regression ($p= 0.28$; $r^2=0.026$).

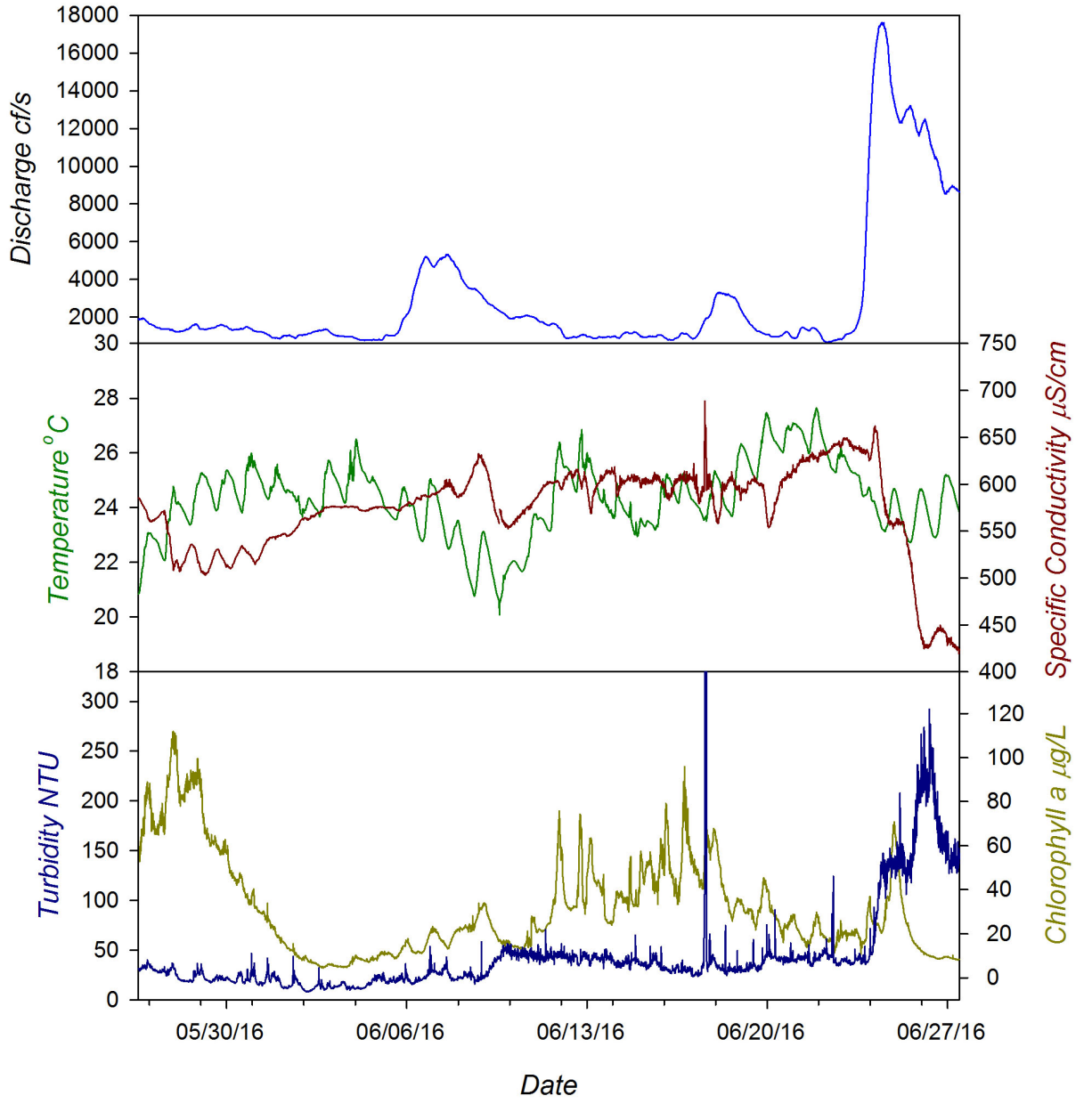


Figure 9. Environmental conditions encountered during the 32 day freshwater deployment in the Maumee River at Waterville, OH. *Top Panel:* Variation in river discharge over the term of the deployment. *Middle Panel:* Variation in temperature (green) and Conductivity (red) at the depth of the sensors, measured by an EXO 2 Sonde. *Bottom Panel:* Time series of turbidity (blue) and chlorophyll (dark yellow) as measured by the EXO 2 Sonde. The large spike in turbidity (681 NTU) was produced during a nutrient addition test when sediment accumulated on the bottom was stirred up from additional mixing of the tank.

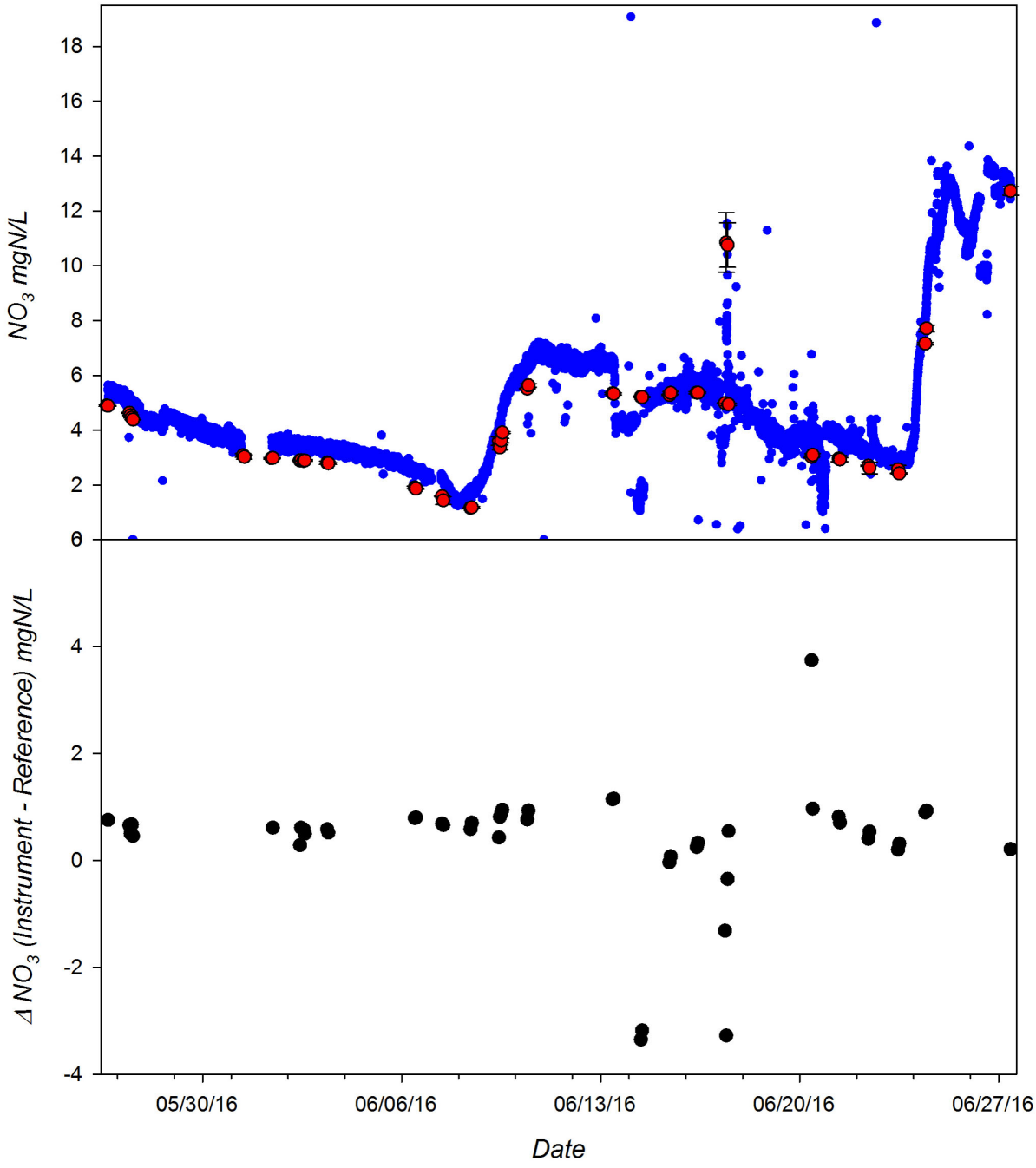


Figure 10. *Top Panel:* Time series plot of the Real-NO3 measurement (blue dots) and reference measurements (red dots) of nitrate in mgN/L. The green crosses at the top of figure represent flagged data (not values) and are plotted on the date of occurrence. *Bottom Panel:* Time series plot of the difference between the Real-NO3 and reference measurements of nitrate in mgN/L (instrument – reference) during the freshwater deployment in the Maumee River at Waterville, OH.

A cross-plot of all matched observations for the deployment is given in figure 11. A linear regression of instrument versus reference measurement was highly significant ($p<0.0001$; $r^2 = 0.75$) with a slope of 0.96 and intercept of 0.38.

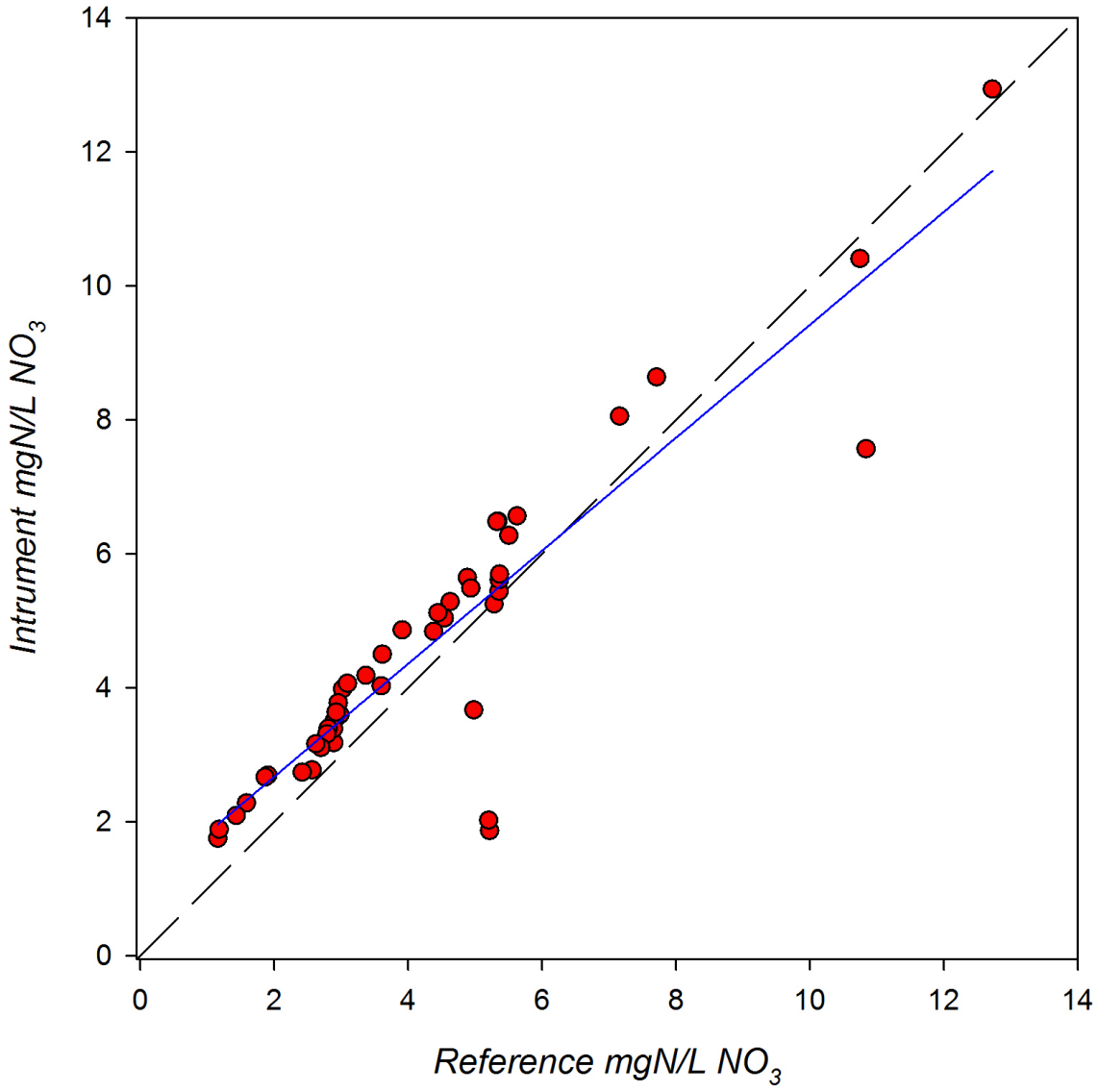


Figure 11. Maumee River field response plot for the 32 day deployment of the Real-NO3 compared to reference NO_3 samples. The plotted line represents a 1:1 correspondence, the blue line represents the linear regression.

Photographs of test instrument before and after the field deployment to indicate potential impact of biofouling (Figure 12).

Figure 12. Photographs of the Real-NO3 nitrate analyzer and the filter intake from the flow-through test tank following a 32 day field test in the Maumee River.

Deployment at Chesapeake Biological Laboratory (CBL)

An 84 day moored field test was conducted in Chesapeake Bay from July 18 to October 10, 2016. The deployment was located at 38.32°N, 76.45°W attached to the side of a floating pier at the mouth of the Patuxent River (Figure 13.) The site was brackish with an average water depth of 2.2 m at the test site.

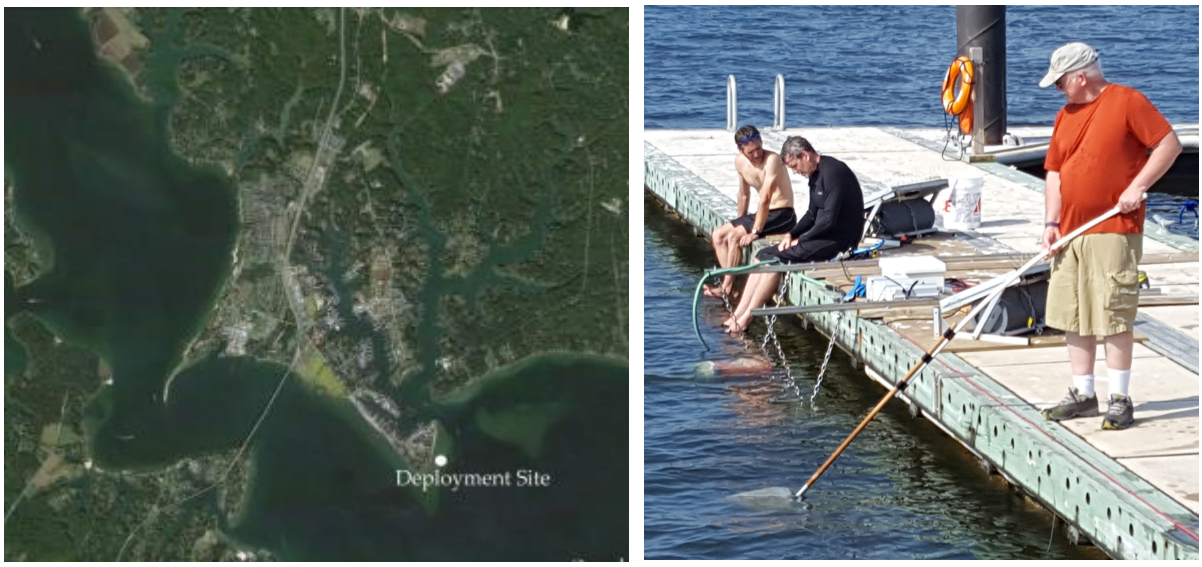


Figure 13. Aerial view of CBL deployment site (left) and instrument deployment rack off the dock during deployment (right).

Time series results of ambient conditions for tidal height, temperature, salinity, turbidity and chlorophyll are given in figure 14. Temperature ranged from 20.0 to 31.3°C, salinity from 12.7 to 16.9 PSU, turbidity from 0.5 to 936.3 NTU and chlorophyll from 0.2 to 97.1 µg/L over the duration of the field test.

The Real-NO3 operated continuously for 69 days until 9/24 when air purge system malfunctioned. The system was bypassed per manufacturer's instructions and the instrument restarted on 9/30. The instrument returned 22,345 observations out of a possible 24,144 based on approximate 5 minute sampling intervals for a data completion rate of 93%. For the entire deployment, 1796 data points were missing, and 3 were flagged as bad. Time series results of the Real-NO3 and corresponding reference NO_3 results are given in figure 15 (top panel). For the interval deployed, the range of accepted values reported by the Real-NO3 was 0.000 to 0.254 mgN/L, compared to 0.001 to 0.038 mgN/L within reference samples.

The bottom panel of figure 14 presents the time series of the difference between the Real-NO3 and reference NO_3 for each matched pair (n=100 comparisons out of a total of 103 with 3 missing instrument results during the inoperable). The average and standard deviation of the measurement difference for the deployment was 0.083 ±0.022 mgN/L, with the total range of differences between 0.018 to 0.166 mgN/L. There no significant trend in measurement difference over time during the deployment ($p=0.681$; $r^2=0.002$).

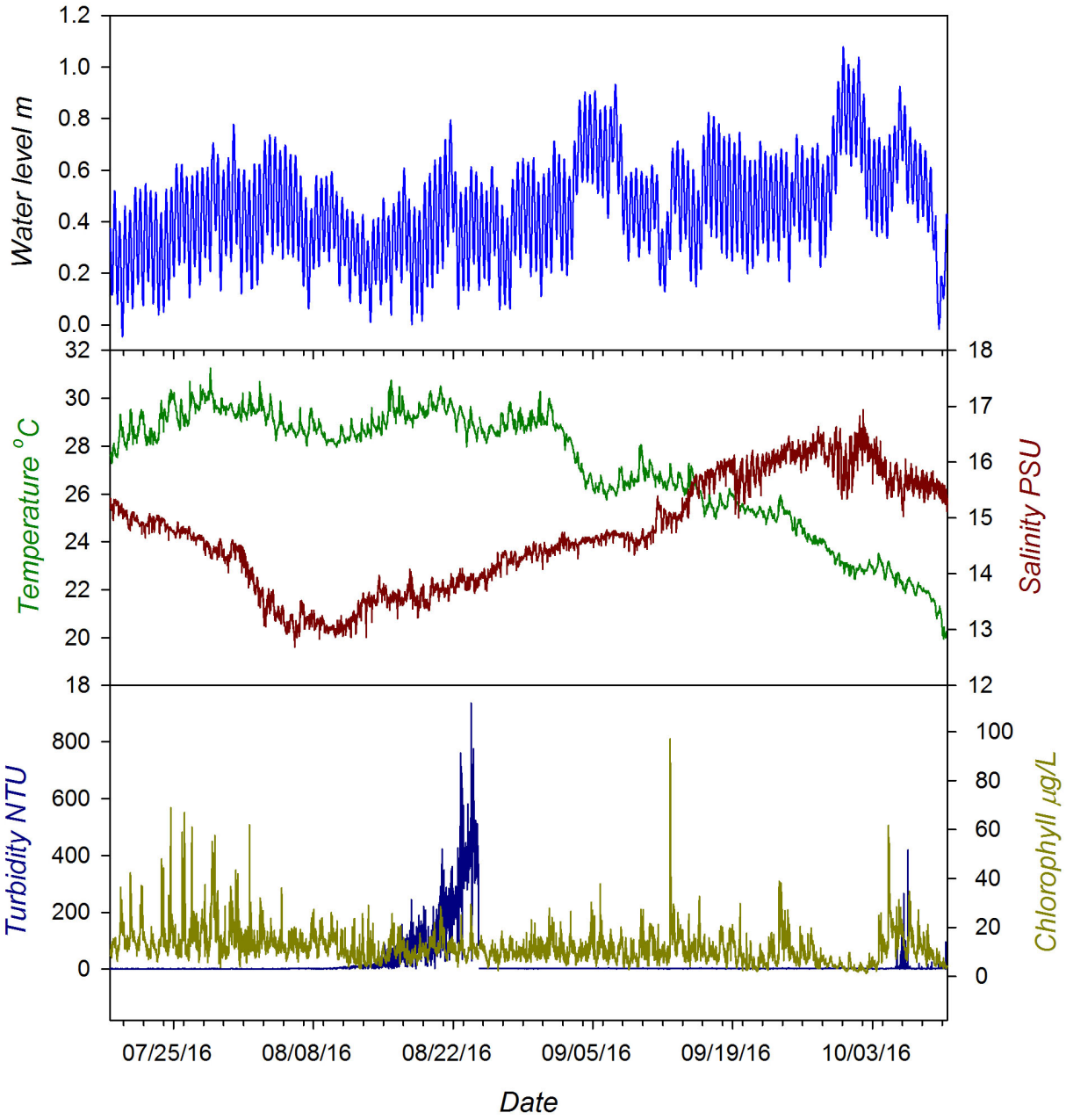


Figure 14. Environmental conditions encountered during the 84 day CBL floating dock deployment. Test sensor array deployed at 1 m fixed depth, variation in local tidal heights indicate active water flow around instrument (*Top Panel*). Variation in temperature (green) and salinity (red) at depth of instrument sensor detected by an EXO2 sonde and two RBR Solo thermistors (*Middle Panel*). Variation in turbidity (blue) and chlorophyll (dark yellow) at depth of instrument sensor detected by an EXO2 sonde (*Bottom Panel*).

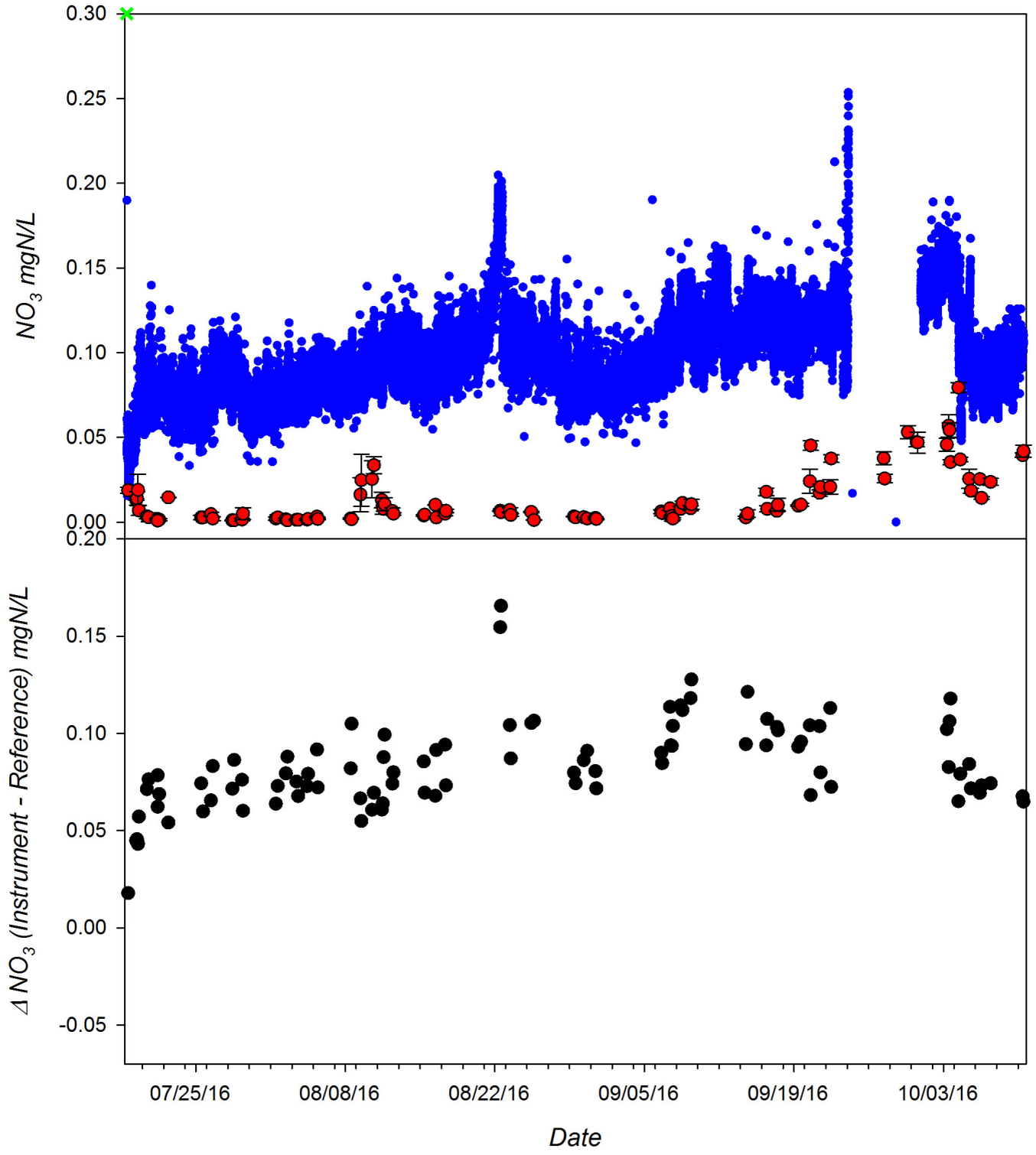


Figure 15. Time series of NO_3 measured by the Real-NO3 during the 84 day CBL field trial. *Top Panel*: Continuous NO_3 recordings from instrument (blue circles) and NO_3 of adjacent grab samples (red circles). *Bottom Panel*: The difference in measured NO_3 relative to reference samples (Instrument mgN/L – Reference mgN/L) observed during deployment.

A cross-plot of the matched observations for the deployment is given in figure 16. A linear regression of the data was significant (p=0.0002; $r^2 = 0.132$), with a slope of 0.680 and intercept of 0.085. For the calibration set-up at this field test, the Real-NO3 significantly over-predicted concentrations.

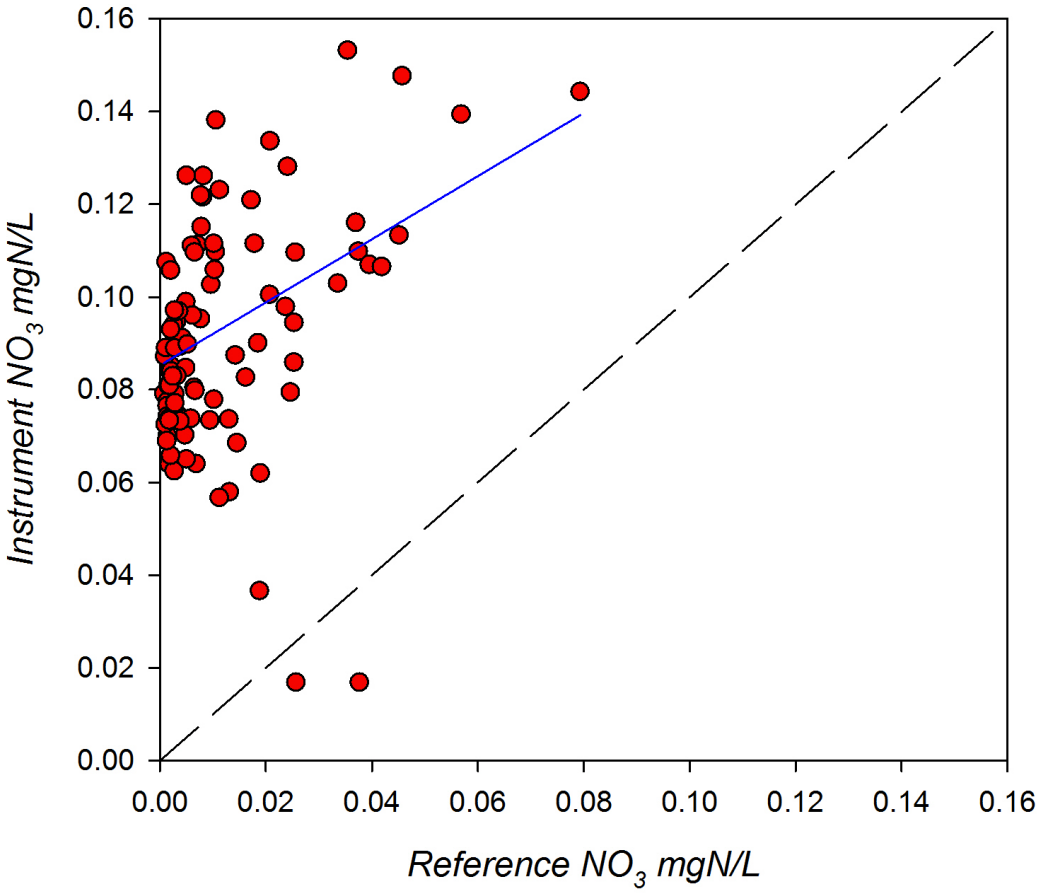


Figure 16. CBL field response plot for Real-NO3 compared to reference NO_3 samples. The plotted line represents a 1:1 correspondence, the blue line represents the linear regression.

Photographs of the Real-NO3 system and the filter intake after the 84 day field deployment to indicate potential impact of biofouling (Figure 17).

Figure 17. Photographs of the Real-NO3 instrument prior to and following the CBL field trial.

Deployment off Coconut Island in Kaneohe Bay, Hawaii

A one month long moored field test was conducted in Kaneohe Bay from October 3, 2016 to November 2, 2016. The deployment site was located at 21.43° N x 157.79° W, on a floating dock anchored off Coconut Island (HIMB) in a depth of approximately 16 meters (Figure 18). Kaneohe Bay, located on the eastern side of Oahu, Hawaii, is a complex estuarine system with a large barrier coral reef, numerous patch reefs, fringing reefs, and several riverine inputs. Tides in Kaneohe Bay are semi-diurnal with mean tidal amplitude of approximately 68 cm day.

Figure 18. Aerial view of HIMB deployment site (left) and instrument rack in-situ (right).

Time series results of ambient conditions for tidal height, temperature, and salinity are given in figure 19. Temperature at the sensor level ranged from 24.5 to 27.9 °C and salinity from 27.3 to 34.8 PSU over the duration of the field test

The Real-NO3 was not deployed at HIMB at the manufacturer's decision.

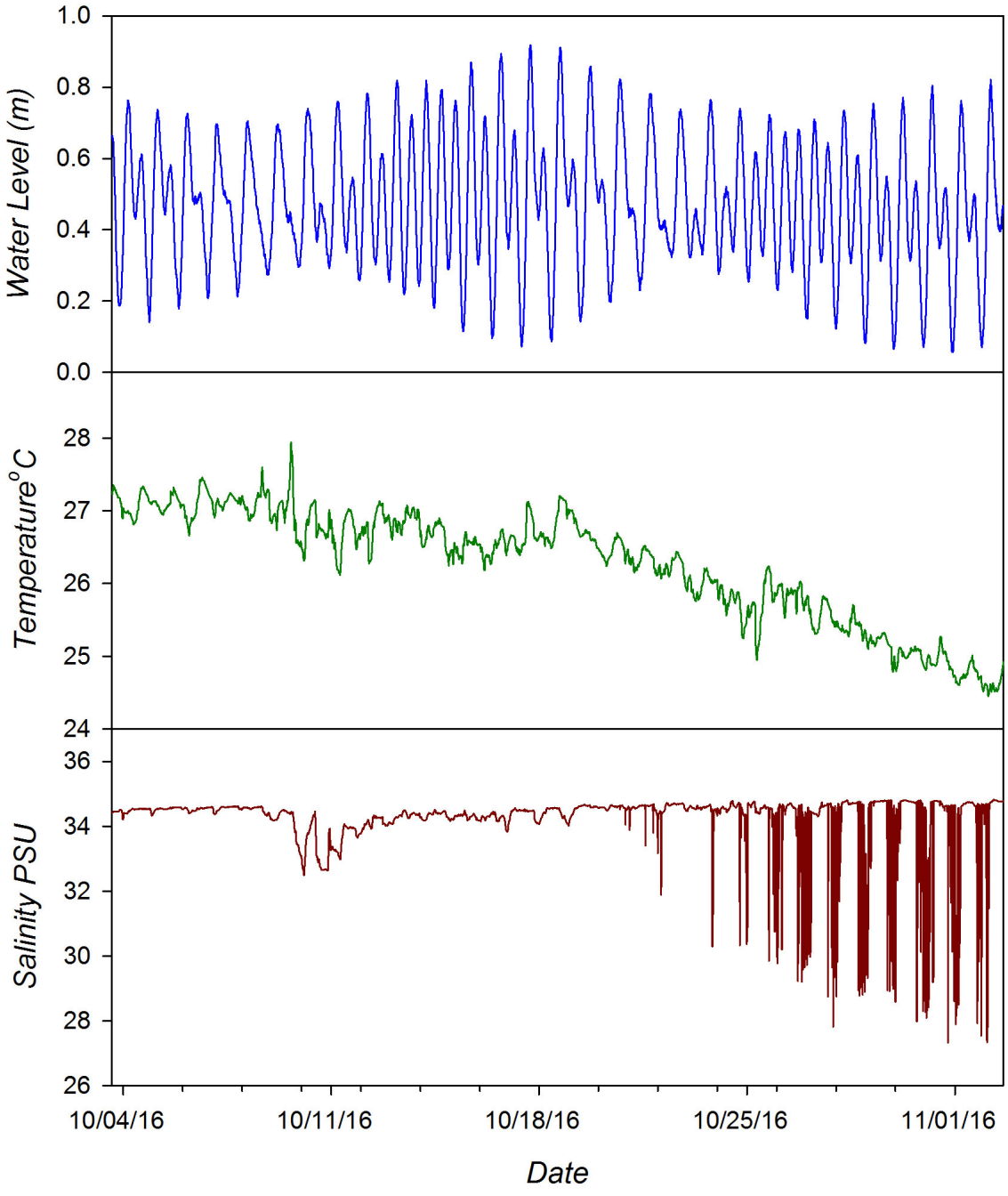


Figure 19. Environmental conditions encountered during the one month HIMB deployment on a floating dock off Coconut Island Test sensor array deployed at 1 m fixed depth, variation in local tidal heights indicate active water flow around instrument (*Top Panel*). Variation in temperature (green) and Salinity (red) at depth of instrument sensor detected by an SBE 26 and two RBR Solo thermistors (*Middle Panel and Bottom Panel*).

A global summary of instrument versus reference readings for the two field deployment sites are plotted in figure 20. The Real-NO3 response showed good linearity for the Maumee River deployment, with higher variability and a noted offset for the brackish test in Chesapeake Bay (see insert). Due to the spread generated within the Maumee River test, a linear regression of instrument and reference measurements for the two field tests combined was highly significant ($p<0.0001$; $r^2 = 0.94$) with a slope of 0.99 and intercept of 0.167. The data comparison across the two field tests covered a concentration range of 0.007 to 12.7 mgN/L.

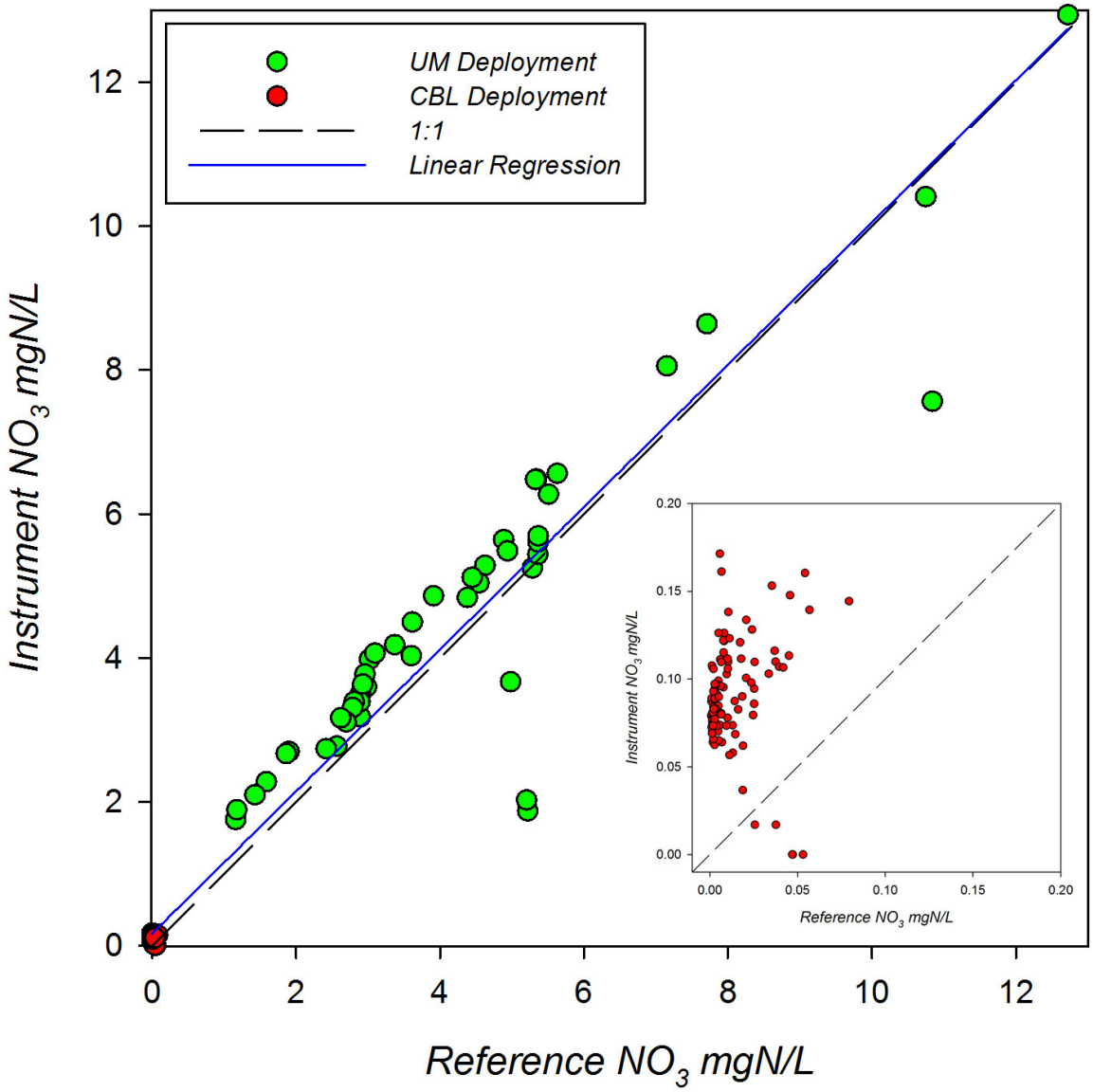


Figure 20. Global response plot for the Real-NO3 observed during the three ACT field trials. Insert shows the CBL and HIMB deployments enlarged. Black dotted line represents a 1:1 correspondence, the blue line represents the linear regression.

QUALITY ASSURANCE AND QUALITY CONTROL

All technology evaluations conducted by ACT comply with its Quality Management System (QMS), which includes the policies, objectives, procedures, authority, and accountability needed to ensure quality in work processes, products, and services. A QMS provides the framework for quality assurance (QA) functions, which cover planning, implementation, and review of data collection activities and the use of data in decision making, and quality control. The QMS also ensures that all data collection and processing activities are carried out in a consistent manner, to produce data of known and documented quality that can be used with a high degree of certainty by the intended user to support specific decisions or actions regarding technology performance. ACT's QMS meets U.S. Environmental Protection Agency quality standards for environmental data collection, production, and use, and the requirements of ISO/IEC 17025:2005(E), *General requirements for the competence of testing and calibration laboratories*.

An effective assessment program is an integral part of a quality system. The ACT Quality Assurance (QA) Manager independently conducted Technical Systems Audits (TSA) of field tests at Maumee River field trial during May 25-28, 2016, a TSA of the Laboratory test at the Chesapeake Biological Laboratory during July 10-18, 2016 and a data quality review of the reference data sets from all tests conducted during the Nutrient Challenge.

Technical System Audits

A TSA is a thorough, systematic, on-site qualitative audit of sampling and measurement processes and procedures associated with a specific technology evaluation. The objectives of the TSAs conducted during this evaluation were to assess and document the conformance of on-site testing procedures with the requirements of the Test Protocols, the ACT Quality Assurance Project Plan (QAPP), and associated Standard Operating Procedures (SOPs).

The TSA was conducted in accordance with the procedures described in n EPA's *Guidance on Technical Audits and Related Assessments for Environmental Data Operations (EPA QA/G-7)* and ISO 19011, *Guidelines for Quality and/or Environmental Management Systems Auditing*. A TSA checklist based on the Test Protocols was prepared prior to the audits and reviewed by the ACT Director and Senior Scientist. The TSA assessed ACT personnel, the test and analytical facilities, equipment maintenance and calibration procedures, sample collection, analytical activities, record keeping, and QC procedures. Reference sample handling and chain-of-custody by NASL were observed during the laboratory test at CBL.

During the audits, the QA Manager met with ACT technical staff involved in the evaluation and asked them to describe the procedures followed. All procedures were observed; and logbooks, data forms, and other records were reviewed.

Key components of the audit included:

- Assessment of Quality Assurance/Quality Control:
  - Adequacy of procedures, and
  - Adherence to procedures.
- Assessment of Sample System:
  - Sample collection,

  - Analytical procedures, and
  - Documentation.
- Assessment of Data and Document Control:
  - Chain of custody, and
  - Documentation.

The TSAs' findings were positive. The field and laboratory tests were implemented consistent with the Test Protocols, QAPP, and SOPs. Minor deviations were documented in laboratory records. There were no deviations which may have had an effect on data quality for the test. All phases of the implementation of the tests reviewed during the audits were acceptable and performed in a manner consistent with ACT data quality goals. The overall quality assurance objectives of the test were met.

ACT personnel are well-qualified to implement the evaluation and demonstrated expertise in pertinent procedures. Communication and coordination among all personnel was frequent and effective. Internal record keeping and document control was well organized. The ACT staff understands the need for QC, as shown in the conscientious development and implementation of a variety of QC procedures.

All samples were collected as described in the Test Protocols and SOPs. Examination of maintenance and calibration logs provided evidence of recent and suitable calibration of sampling and analytical equipment.

Data Quality

Data Verification, Validation, and Assessment.

Data review is conducted to ensure that only sound data that are of known and documented quality and meet technology evaluation quality objectives are used in making decisions about technology performance. Data review processes are based in part on two EPA guidance documents: *Guidance on Environmental Data Verification and Data Validation* (QA/G-8) [EPA, 2002] and *Guidance on Technical Audits and Related Assessments for Environmental Data Operations* (QA/G-7) [EPA, 2000].

The data were verified and validated to evaluate whether the data have been generated according to the Test Protocols and satisfied acceptance criteria. Data verification evaluates the completeness, correctness, and consistency of the data sets against the requirements specified in the Test Protocols, measurement quality objectives (MQOs), and any other analytical process requirements contained in SOPs.

The ACT QA Manager reviewed the reference data sets from all field and laboratory tests. The number of reference samples collected at each site and the laboratory tests are in Table 10. A total of 346 reference samples were collected for the field and laboratory tests. The overall reference data set included 3,666 distinct analyses.

Table 9. The number of reference samples collected during the laboratory test and at each field site.

Site	No. of Samples	No. of Replicates per Sample	No. of Analytes[1/] Measured in Each Replicate	No. of Measurements
Maumee River	61	3	3	549
CBL – Field	120	3	3	1080
CBL – Lab	92	5	3	1380
Hawaii	73	3	3	657
Total	346			3,666
[1/] NO_2; NO23; PO_4				

The data review verified that the sampling and analysis protocols specified in the Test Protocols were followed, and that the ACT measurement and analytical systems performed in accordance with approved methods, based on:

- The raw data records were complete, understandable, well-labeled, and traceable;
- All data identified in the Test Protocols were collected;
- QC criteria were achieved; and
- Data calculations were accurate.

Data validation uses the outputs from data verification and included inspection of the verified field and laboratory data to determine the analytical quality of the data set. A representative set of approximately 10% of the reference data was traced in detail from 1) raw data from field and laboratory logs, 2) data transcription, 3) data reduction and calculations, to 4) final reported data. Validation of the data sets established:

- Required sampling methods were used;
- Sampling procedures and field measurements met performance criteria; and
- Required analytical methods were used.

The data validation also confirmed that the data were accumulated, transferred, summarized, and reported correctly. There is sufficient documentation of all procedures used in the data collection and analysis to validate that the data were collected in accordance with the evaluation's quality objectives.

A Data Quality Assessment (DQA) is the third and final process of the overall data assessment. It is a scientific and statistical evaluation of validated data to determine if the data are of the right type, quality, and quantity to support conclusions on the performance of the technologies. The DQA determined that the test's data quality objectives, described in Section 7.1 of the Test Protocols and Section 3.4 and Appendix B of the ACT QAPP (ACT, 2016), were achieved. This evidence supports conclusions that:

- The sampling design performed very well and is very robust with respect to changing conditions.
- Sufficient samples were taken to enable the reviewer to see an effect if it were present.

Audit of Data Quality.

The ACT QA Manager conducted an Audit of Data Quality (ADQ) on verified data to document the capability of ACT's data management system to collect, analyze, interpret, and report data as specified in the Test Protocols, QAPP, and SOPs. The ADQ determined that the data were accumulated, transferred, reduced, calculated, summarized, and reported correctly. There is sufficient documentation of all procedures used in the data collection and analysis to verify that the data have been collected in accordance with ACT quality objectives.

Table 10**.** Results of Field Duplicates (FD) for the Maumee River mooring test.

Date/Time	**Rep**	**NO_3**	**Mean**	**Std Dev**	**ABS Diff**	**CV%**
6-16-16 9:00	FD1	5.363	5.369	0.0077	0.011	0.14
	FD2	5.374				
6-17-16 12:00	FD1	4.938	4.876	0.0876	0.124	1.80
	FD2	4.814				
6-20-16 10:00	FD1	3.023	3.036	0.0174	0.025	0.57
	FD2	3.048				
6-23-16 11:00	FD1	2.568	2.486	0.1167	0.165	4.70
	FD2	2.403				

Table 11. Results of Field Duplicates (FD) for the Chesapeake Bay, MD mooring test.

Date/Time	**Rep**	**NO_3**	**Mean**	**Std Dev**	**ABS Diff**	**CV%**
7-20-16 10:00	FD1	0.0033	0.0033	0.0000	0.0001	1.43
	FD2	0.0033				
7-26-16 14:00	FD1	0.0020	0.0014	0.0009	0.0013	65.53
	FD2	0.0007				
8-2-16 10:00	FD1	0.0015	0.0015	0.0000	0.0000	0.00
	FD2	0.0015				
8-10-16 16:00	FD1	0.0336	0.0198	0.0194	0.0274	97.71

	FD2	0.0061				
8-23-16 12:00	FD1	0.0041	0.0038	0.0005	0.0007	12.41
	FD2	0.0035				
9-8-16 10:00	FD1	0.0077	0.0100	0.0032	0.0046	32.45
	FD2	0.0122				
9-16-16 12:00	FD1	0.0078	0.0076	0.0003	0.0005	4.20
	FD2	0.0074				
10-4-16 14:00	FD1	0.0369	0.0404	0.0049	0.0069	12.14
	FD2	0.0439				
10-10-16 10:00	FD1	0.0395	0.0398	0.0005	0.0007	1.18
	FD2	0.0402				

Table 12. Results of Field Duplicates (FD) for the Kaneohe Bay, HI mooring test

Date/Time	Rep	NO_3	Mean	Std Dev	ABS Diff	CV %
10-6-16 14:00	FD1	0.0149	0.0126	0.0033	0.0046	26.11
	FD2	0.0102				
10-12-16 11:00	FD1	0.0117	0.0109	0.0012	0.0017	11.05
	FD2	0.0100				
10-17-16 9:00	FD1	0.0148	0.0130	0.0026	0.0037	20.00
	FD2	0.0111				
10-26-16 9:00	FD1	0.0130	0.0123	0.0010	0.0014	7.87
	FD2	0.0116				
11-1-16 9:00	FD1	0.0223	0.0195	0.0040	0.0057	20.72
	FD2	0.0166				

Table 13. Results of Field Trip Blanks all deployments.

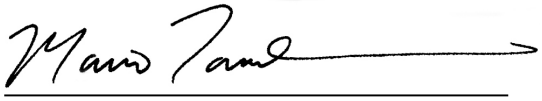
Maumee River		**Chesapeake Bay**		**Kaneohe Bay**	
Field Blank ID	NO_3 (Std Dev)	Field Blank ID	NO_3 (Std Dev)	Field Blank ID	NO_3 (Std Dev)
GLFB1	0.013 (0.004)	CBLFB1	0.0012 (0.0005)	HIFB1	0.0045 (0.0028)
GLFB2	0.007 (0.003)	CBLFB2	0.0003 (0.0002)	HIFB2	0.0013 (0.0010)
GLFB3	0.003 (0.001)	CBLFB3	0.0001 (0.0002)	HIFB3	0.0032 (0.0038)
GLFB4	0.003 (0.001)	CBLFB4	0.0002 (0.0003)	HIFB4	0.0118 (0.0032)
--	--	--	--	HIFB5	0.0083 (0.0024)
Mean (Std Dev)	0.006 (0.005)	Mean (Std Dev)	0.000 (0.001)	Mean (Std Dev)	0.006 (0.004)
Grand Mean (Std Dev)					0.004 (0.004)

ACKNOWLEDGEMENTS:

We wish to acknowledge the support of all those who helped plan and conduct the verification test, analyze the data, and prepare this report. In particular we would like to thank our Technical Advisory Committee, Dr. Suzanne Bricker, National Oceanic and Atmospheric Administration, Dr. Brian Pellerin, U.S. Geological Survey, Dr. Dwane Young, U.S. Environmental Protection Agency, Dr. Matt Cohen, University of Florida, Dr. R. David Holbrook, National Institute for Standards and Technology, Mr. Chris Gross, U.S. Department of Agriculture NRCS, Dr. Joe Rudek, Environmental Defense Fund for their advice and direct participation in various aspects of this evaluation. Earle Buckley also provided critical input on all aspects of this work and served as the independent Quality Assurance Manager. This work has been coordinated with, and funded by, the National Oceanic and Atmospheric Administration, Integrated Ocean Observing System program.

June 1, 2017

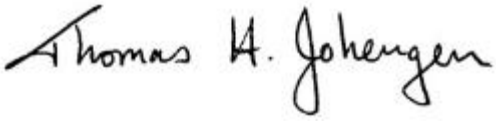
Date



Approved By: Dr. Mario Tamburri
ACT Executive Director

June 1, 2017

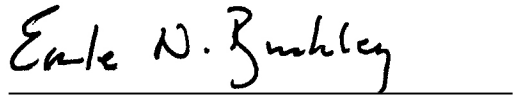
Date



Approved By: Dr. Tom Johengen
ACT Chief Scientist

June 1, 2017

Date



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June 5, 2017
Dr. Thomas H. Johengen, ACT Chief Scientist
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4840 South State Rd
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Re: Nutrient Challenge Company Response Letter

Dear Dr. Johengen,

Real Tech Inc. would like to thank you and the Alliance for Coastal Technologies (ACT) team for the opportunity to participate in the Nutrient Challenge competition and comment on the performance of the Real Nitrate Analyzer GL series.

We are very pleased that ACT has organized this challenge and shined a spotlight on the benefits of real-time sensors for environmental nutrient monitoring. Real Tech's analyzers utilize spectrophotometry to detect nitrate nitrogen in water without the use of reagents, are highly modifiable based on clients' needs, and can generate data at a frequency of every minute. Below we expand on some of the most important aspects of Real Tech's experiences in this challenge.

1) Design Application

Real Tech's nitrate monitoring systems are designed for and most commonly used at well water blending stations, and municipal and industrial water/wastewater treatment facilities. Due to the current regulations and the typical nitrate concentrations encountered in these environments, two of the field tests (Chesapeake Bay, MD and Kaneohe Bay, HI) were not within the design specifications of our analyzer. Both of these field test sites had high salinity and very low nitrate concentrations (less than 0.04 mgN/L compared to 10 mgN/L maximum allowable concentration in drinking water).

2) Custom Calibration

Real Tech's preferred method of deployment for its analyzers involves an initial in-house calibration followed by an on-site audit of the calibration after installation. This allows for an adjustment of the calibration for background water interferences. However, as deployment prior to the actual testing days was not a possibility and collecting audit data during the testing period would interfere with the testing, we were not able to implement a correction based on on-site data. We strongly believe that spectrophotometric measurement techniques work best when a custom calibration is built on-site. For instance, in the Maumee River field test, Real-NO3 measurements appear to have tracked the actual nitrate concentrations well, however, it is evident that a slight offset was present. This offset could have been corrected for with an on-site correction factor on the first day of installation, had on-site data auditing been allowed. Furthermore, continued collaboration between the client and the manufacturer will provide superior data as our analyzers are capable of building a site-specific library which improves performance over time.

3) Range

Real Tech's analyzers come with adjustable flow cell components. This allows for a customized approach to concentration range. A longer path length flow cell increases sensitivity and accuracy at low concentrations while a shorter path length flow cell provides the widest range, but has reduced sensitivity at low concentrations. During the laboratory testing part of the nutrient challenge, we were asked to provide an analyzer that would measure a range of 0.01-50 mgN/L NO_3. We provide analyzers that can measure up to hundreds of mgN/L. However, as explained above, a wider range comes with a trade-off of losing the required sensitivity at low concentrations. For this reason, Real Tech participated in the laboratory testing with an 8-mm flow cell that aimed to maintain a reasonable level of accuracy at low concentrations (0.01-0.1

mgN/L) while providing a relatively wide range (up to 10 mgN/L). In practice, this is not representative for the performance of the instrument since two separate flow cells would be used.

4) Laboratory Testing

The ACT report states that “Instruments were set-up by the manufacturer daily prior to start of each individual laboratory test.”. At Real Tech’s own decision, the Real Tech analyzer was not set up for each laboratory test, but instead for the whole laboratory challenge once at the beginning. A set up performed at the beginning of each laboratory test would have yielded better results. However, this would have been unrealistic as real-world applications are likely to present more than one interfering factor at all times.
It is important to also note that one of the highest absolute differences for a nitrate concentration of 1 mgN/L was observed during the concentration range test. During the range test, no interfering substance was intended to be present in the testing tank water. Therefore, the value obtained from the range test was used as a reference for other laboratory tests. However, all other laboratory test results for the same concentration of 1mgN/L, with the exception of 30 PSU salinity and 10 mg/L DOC, yielded absolute differences less than this reference value despite possible interference from the test matrix. For this reason, we believe that the concentration range test may have been confounded by the presence of an interfering substance in the testing tank water, despite the best efforts of the ACT team. It is possible that the interfering substance may have affected readings of all concentrations tested during the range test.

5) Data Collection Frequency

Although data collection frequencies higher than every 15 min were not deemed an important feature for the Challenge, we believe that in many real-world applications data collection frequencies may play a critical role. For this reason, we would like to clarify that the accurate frequency of data readings for Real Tech’s analyzers ranged between 2 min 34 sec and 3 min 41 sec for the Nutrient Challenge field and laboratory tests. Moreover, data collection frequency of Real Tech’s analyzers is modifiable based on the clients’ needs and can be as high as every minute.

6) Maintenance

Real Tech’s analyzers have the capacity to alarm due to a series of potential problems that may arise and impact the operation of the instrument. As per the Challenge’s protocol, the analyzers were not to be serviced by the manufacturer during the Challenge as durability testing was part of the competition. However, some of the alarms that occurred during testing could have easily been resolved by Real Tech’s staff to ensure high accuracy data collection. Although an instrument should ideally be able to operate without maintenance for long periods, we believe it is equally important that the instrument is intelligent enough to inform the user of any issues that arise during deployment. This two-way communication capacity of Real Tech’s analyzers provides an extra level of confidence for the proper operation of the instrument.
We would like to thank the ACT team again for their hard and thorough work throughout the Nutrient Challenge. It has been an invaluable experience for Real Tech.

Sincerely,

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Applications Research Scientist
Real Tech Inc.
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