Ref. No. [UMCES] CBL 2017-048
ACT VS17-03

PERFORMANCE VERIFICATION STATEMENT
For NOC Nitrate Analyzer

TECHNOLOGY TYPE:	Nutrient Sensors
APPLICATION:	In situ estimates of NO23 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
EVALUATION PERSONNEL:	T. Johengen, H. Purcell, M. Tamburri, D. Loewensteiner, G.J. Smith, D. Schar, Margaret McManus, GordonWalker, and Beth Stauffer

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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 NOC-NO23 and reference measurement across all timepoints for trials C0 – C5 ranged from -1.3061 to 0.0234 mgN/L, with an overall mean of -0.314 ±0.445 mgN/L. There was significant trend in instrument offset versus concentration as estimated by linear regression ($p=0.0006$; $r^2=0.39$). The trend was driven by the substantially higher offsets at the C4 and C5 test concentrations (approximately 5 mgN/L) where the measurement error approached 20%. 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 0.002 to 0.040 mgN/L across the five trials, and the coefficient of variation ranged from 0.36 to 12.9 %. For the laboratory temperature challenge with testing at 5 ^{o}C, the absolute difference between instrument and reference measurement across all timepoints for trials C2 – C4 ranged from -0.629 to 0.056 mgN/L, with a mean of -0.048 ±0.194 mgN/L. The measurement difference at C2 was not significantly different between temperatures; however, the offset at C3 was significant greater at 5 ^{o}C then at 20 ^{o}C (0.032 vs. 0.003 mgN/L). Only one timepoint comparison was generated for the C4 trial so no statistical comparison was possible, however the greater negative offset was similar to test results at 20 ^{o}C. 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 -0.281 to 0.021 mgN/L, with a mean of-0.155 ±0.086 mgN/L. A linear regression between salinity and measurement error was not significant ($p=0.17$; $r^2=0.11$), however, there was a noticeable increase in measurement variability and concentrations were consistently under-predicted at each added salinity level compared to zero. 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 0.010 to 0.050 mgN/L, with a mean of 0.030 ±0.016 mgN/L. A linear regression of the measurement differences versus turbidity was not significant ($p=0.15$; $r^2=0.15$). 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 -0.086 to 0.009 mgN/L, with a mean of -0.039 ±0.042 mgN/L. A linear regression of measurement differences versus DOC concentration was highly significant ($p<0.0001$; $r^2=0.79$), with a slope of -0.004 and intercept of 0.029. The measurement offset was approximately 0.08 more negative at 10 mg/L DOC compared to lab RO

water which corresponded to a relative error of approximately 8%.

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. The NOC-NO23 operated during the entire 32 day deployment sampling at hourly intervals, but due to a faulty SD memory card, the data from 5/27 to 6/7 were lost and during the last 6 days of the deployment 122 values were flagged by the instrument as "low precision". Overall, the NOC-NO23 generated 375 accepted observations out of a possible 763 for a data completion result of 49.1%. The average and standard deviation of the measurement difference between instrument and reference NO_3 measurements for each matched pair (n=21 of a possible 51 observations) over the total deployment was -1.38 ± 1.29 mgN/L with a total range of -6.12 to 2.16 mgN/L. There was no significant trend in measurement difference over time as estimated by linear regression ($p= 0.48$; $r^2=0.027$). A linear regression of instrument versus reference measurement was highly significant ($p<0.001$; $r^2 = 0.77$) but with a slope of only 0.546 and intercept of 0.81.

An 84 day moored field test was conducted in Chesapeake Bay from July 18 to October 10, 2016. The NOC-NO23 malfunctioned during the first 3 days of the deployment, and the manufacturer was given permission to exchange the instrument with a new unit but keeping the same reagent and standards originally prepared. The replacement instrument operated from 7/21 to 8/21, measuring at hourly intervals, but then also failed. The instrument returned 603 data point out of a possible 2012 for the entire deployment period, with 1359 points missing and 50 flagged with no result calculated. While the unit was deployed it reported 603 of a possible 653 values for a data completion result of 92.3% (but only 33% of the scheduled total deployment was achieved). 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 103 observations) over the total deployment was -0.005 ±0.010 mgN/L, with the total range of differences between -0.027 to 0.031 mgN/L. There no significant trend in measurement difference over time ($p=0.85$; $r^2=0.001$). A linear regression of the data was highly significant ($p<0.0001$; $r^2 = 0.53$), but with a slope of only 0.54 and intercept of 0.0009.

A one month long moored field test was conducted in Kaneohe Bay from October 3, 2016 to November 2, 2016. The NOC-NO23 operated successfully for the entire 30 day deployment, sampling at hourly intervals, returning 720 measurements for a data completion result of 100%. The average and standard deviation of the differences between instrument and reference readings over the entire deployment (n=73 out of a possible 73) were -0.013 ± 0.007 mgN/L, with a total range in the differences of -0.0394 to -0.0029 mgN/L. There was a small but statistically significant trend in the measurement difference over time ($p=0.0009$; $r^2 = 0.182$) during the deployment, with a slope of 0.0003 mgN/L/d. The NOC-NO23 under-predicted all measurements and a linear regression of instrument versus reference concentrations was not significant ($p=0.13$; $r^2 = 0.04$).

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 the NOC Nitrite and Nitrate Analyzer 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

The NOC lab-on-chip nitrate sensor (denoted as NOC-NO23 throughout the report) is a submersible wet chemical analyzer that measures total nitrate + nitrite (NO_3^-+ NO_2^-) on a microfluidic chip using the Griess assay and cadmium reduction. The system can also be configured to measure just nitrite (NO_2^-). The system can perform hourly measurements for up to three months in both freshwater and saltwater environments (including the deep sea), but longer deployments at lower sampling frequencies are possible. Each sample measurement is compared directly to a subsequent on-board standard measurement, thus eliminating drift problems. The measurement range is 0.03 to 1000 μM (0.0004 to 14 mg/L-N).

The central component of the sensor is the lab-on-chip (LOC). The LOC is a circular multi-layer acrylic device incorporating an array of microfluidic channels (150 μm wide x 300 μm deep) for fluid handling and optical detection. The chip contains multiple length absorption cells to provide a large dynamic range. Each cell is configured for absorbance detection using 525 nm LEDs and photodiodes placed at opposite ends of the cells.

The reagents and analytical solutions (blank, sample, standard) are delivered to the chip using a custom built three-barrel syringe pump and miniaturized solenoid valves. All thee syringes are mechanically connected and operate simultaneously. The temperature of the reacting mixture is monitored using an on-chip thermistor. Each sample measurement is accompanied by a blank and on-board standard measurement, eliminating drift problems.

A custom on-board electronics package provides automation and data logging. Raw data are automatically stored on an 8 GB flash memory card. The raw data can be downloaded via USB

using a GUI, which also permits modification of the sensor configuration and manual operation. The sensor automatically processes data to provide the nitrate concentration of the sample in micromolar along with a time stamp and a quality flag. The processed data can be retrieved through the GUI or though interfacing with third-party loggers/platforms via RS-232 or RS-485. The system can operate in saltwater or freshwater, with the salinity of standard and blank solutions chosen to best match that of the deployment environment.

The sensor housing consists of two parts. The lower part comprises the LOC sensor and electronics, which are placed in an air-filed water-tight housing for shallow deployments. For deep deployments, the sensor uses an oil-filled pressure compensated housing. The top housing consists of a hollow PVC tube, where the fluid storage and waste collection bags are stored during deployment. The bags hang from a metal bar placed at the top of the tube and are connected to the LOC unit using ¼-28 connectors and 0.5 mm i.d Teflon tubing. The sample inlet, located at the bottom of the reagent housing, is fitted with a 0.45 μm 13 mm PES filter and connected to the chip using 0.5 mm id Teflon tubing.

A full set of reagents allows over 2000 measurements at a minimum sampling period of 15 minutes. Alternative smaller housing configurations are available for shorter deployments, or for deployments onboard AUVs/gliders (the main sensor housing can be deployed inside the payload bay of a Seaglider).

For each sample analyzed, the sensor automatically performs the following steps:

1. Blank measurement
2. Sample measurement
3. Standard measurement
6. Delay (optional, depends on required sampling frequency).

All waste generated by the sensor is collected on-board and is not expelled into the environment.

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 ^{o}C 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^{o}C.
- 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

NOC-NO23 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 -1.3061 to 0.0234 mgN/L, with an overall mean of -0.314 ±0.445 mgN/L. The means for each trial are given in Table 1. A plot of the absolute difference between NOC-NO23 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 ($p=0.0006$; $r^2=0.39$). More specifically, the magnitude of measurement offset and variability was substantially larger at C4 and C5 test concentrations which were both approximately 5 mgN/L. The measurement error approached 20% or 1.1 mgN/L against the 5.6 mgN/L reference concentration.

Table 1. Accuracy results for laboratory testing of the NOC-NO23 analyzer assessed by absolute difference (mgN/L) and percent error between instrument and reference measurements for the concentration range test.

Trial	Reference	NOC-NO_{23}	Absolute Diff	% Error
C0	0.0231	-0.0280	**-0.0516**	**223.2**
C1	0.0289	0.0114	**-0.0175**	**60.6**
C2	0.1337	0.1224	**-0.0113**	**8.4**
C3	1.101	1.104	**0.0035**	**0.3**
C4	5.663	4.538	**-1.1250**	**19.9**
C5	4.458	3.991	**-0.4669**	**10.5**

Precision

An assessment of precision was performed by computing the standard deviations and coefficients of variation of the five replicate measurements for each concentration challenge. The standard deviation of the mean ranged from 0.002 to 0.040 mgN/L across the five trials, and the coefficient of variation ranged from 0.36 to 12.9 % (Table 2).

Table 2. Precision assessment of the NOC-NO23 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_{23} (mgN/L)		Standard Deviation		Coefficient of Variation	
Trial	*Reference*	*NOC-NO_{23}*	*Reference*	*NOC-NO_{23}*	*Reference*	*NOC-NO_{23}*
C1	0.0289	0.0114	0.0032	0.0015	11.18	12.93
C2	0.1337	0.1224	0.0020	0.0022	1.51	1.80
C3	1.101	1.104	0.0087	0.0047	0.79	0.42
C4	5.663	4.538	0.1242	0.0165	2.19	0.36
C5	4.458	3.991	0.0195	0.0395	0.44	0.99

Lab Concentration Range Challenge

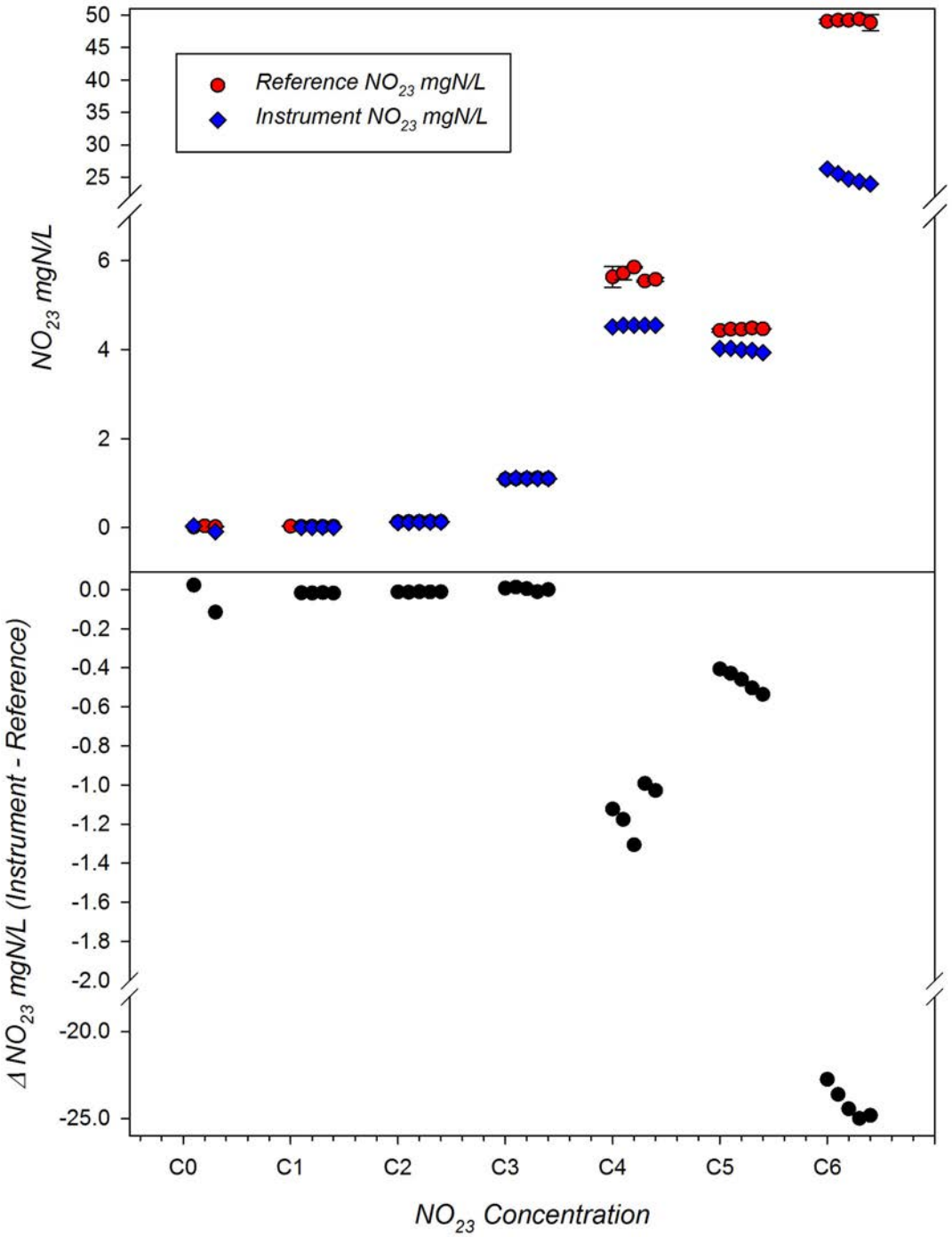

Figure 1. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO_{23} in the laboratory concentration range challenge covering ambient plus 6 concentration ranges. Five replicate measurements were made at each concentration level along with three measurements at ambient level. *Bottom Panel:* Plot of the absolute difference in mgN/L between NOC-NO23 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.

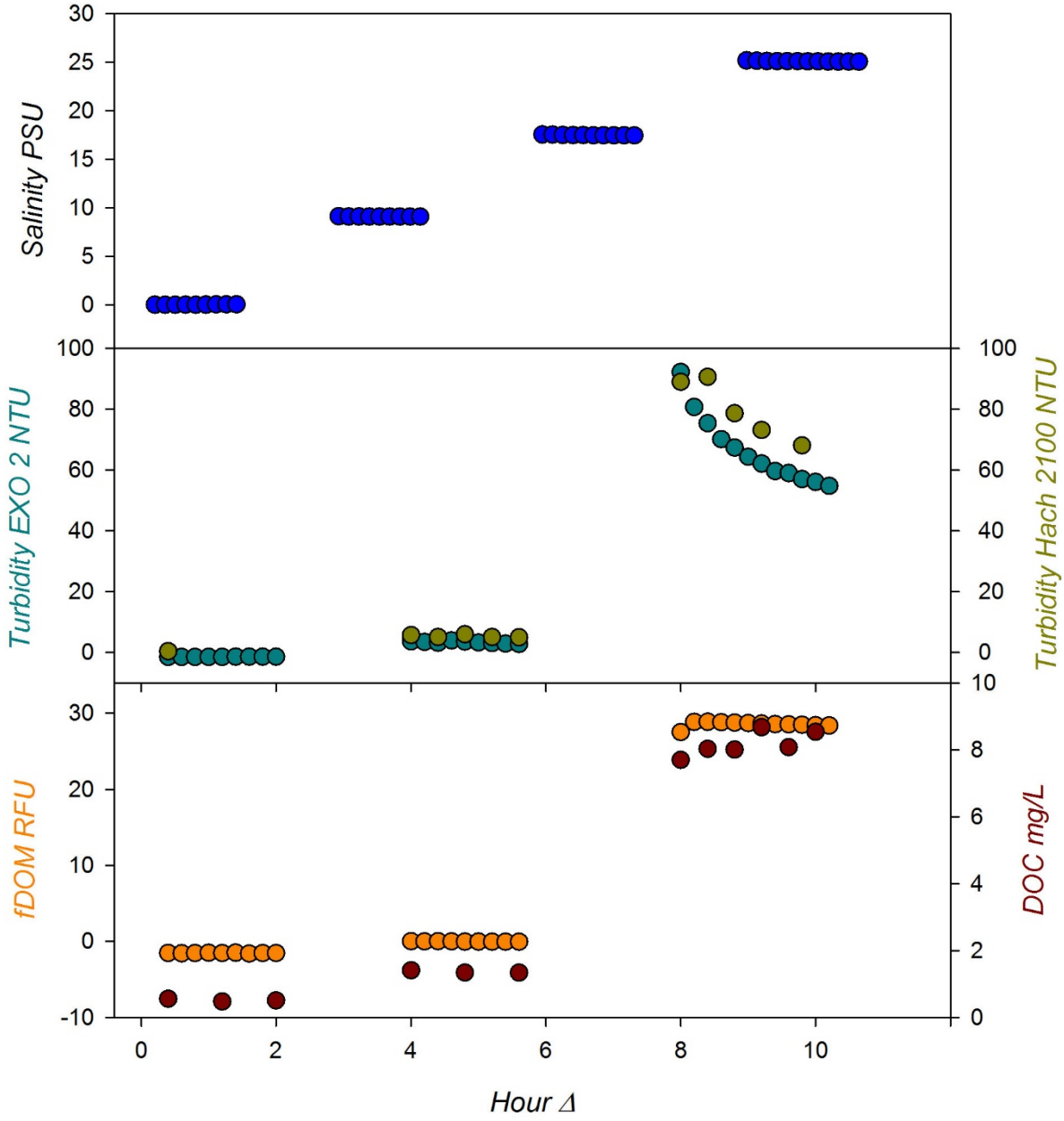

Figure 2. *Top Panel: In situ* salinity measured by EXO2 sonde in the laboratory salinity challenge covering ambient plus 3 salinity ranges. *Middle Panel: In situ* turbidity measured by EXO2 sonde (teal) and on grab samples by a Hach 2100 Turbidimeter (olive) during the laboratory turbidity challenge 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 -0.629 to 0.056 mgN/L, with a mean of -0.048 ±0.194 mgN/L. The means for each trial are given in Table 3. The measurement difference at C2 was not significantly different between temperatures; however, the offset at C3 was significant greater at 5 ^{o}C then at 20 ^{o}C (0.032 vs. 0.003 mgN/L). Only one timepoint comparison was generated for the C4 trial so no statistical comparison was possible, however the greater negative offset was similar to test results at 20 ^{o}C.

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	NOC-NO23	Absolute Diff	% Error
C2	0.1169	0.1050	**-0.0119**	**10.2**
C3	1.0634	1.0949	**0.0315**	**3.0**
C4	5.4637	4.9112	**-0.5525**	**10.1**

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 -0.281 to 0.021 mgN/L, with a mean of-0.155 ±0.086 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. A linear regression between salinity and measurement error was not significant ($p=0.17$; $r^2=0.11$), however, there was a noticeable increase in the variability and concentrations were consistently under-predicted at each added salinity level compared to zero.

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	NOC-NO_{23}	Absolute Diff	% Error
0	1.1013	1.1048	**0.0035**	**0.3**
10	0.9365	0.7835	**-0.1530**	**16.3**
20	1.0234	0.8094	**-0.2140**	**20.9**
30	0.9229	0.8393	**-0.0836**	**9.1**

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 0.010 to 0.050 mgN/L, with a mean of 0.030 ±0.016 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. A linear regression of

the measurement differences versus turbidity was not significant ($p=0.15$; $r^2=0.15$). Even though the measurement offset was greater with added turbidity than for zero, the measurement error at 100 NTU was actually less then at 10 NTU, so it unlikely that the turbidity additions directly affected measurement accuracy.

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	NOC-NO_{23}	Absolute Diff	% Error
0	1.1013	1.1048	**0.0035**	**0.3**
10	1.0009	1.0390	**0.0381**	**3.8**
100	0.9805	1.0032	**0.0227**	**2.3**

Results of the laboratory DOC challenge at the C3 concentration level are shown in figure 6. The absolute difference between instrument and reference measurement for the two added DOC levels ranged from -0.086 to 0.009 mgN/L, with a mean of -0.039 ±0.042 mgN/L, across all timepoints. 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. A linear regression of measurement differences versus DOC concentration was highly significant ($p<0.0001$; $r^2=0.79$), with a slope of -0.004 and intercept of 0.029. The measurement offset was approximately 0.08 more negative at 10 mg/L DOC compared to lab RO water which corresponded to a relative error of approximately 8%.

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	NOC-NO_{23}	Absolute Diff	% Error
0	1.1013	1.1048	**0.0035**	**0.3**
1	1.0020	1.0026	**0.0006**	**0.1**
10	0.9877	0.9102	**-0.0775**	**7.8**

Lab Temperature Challenge

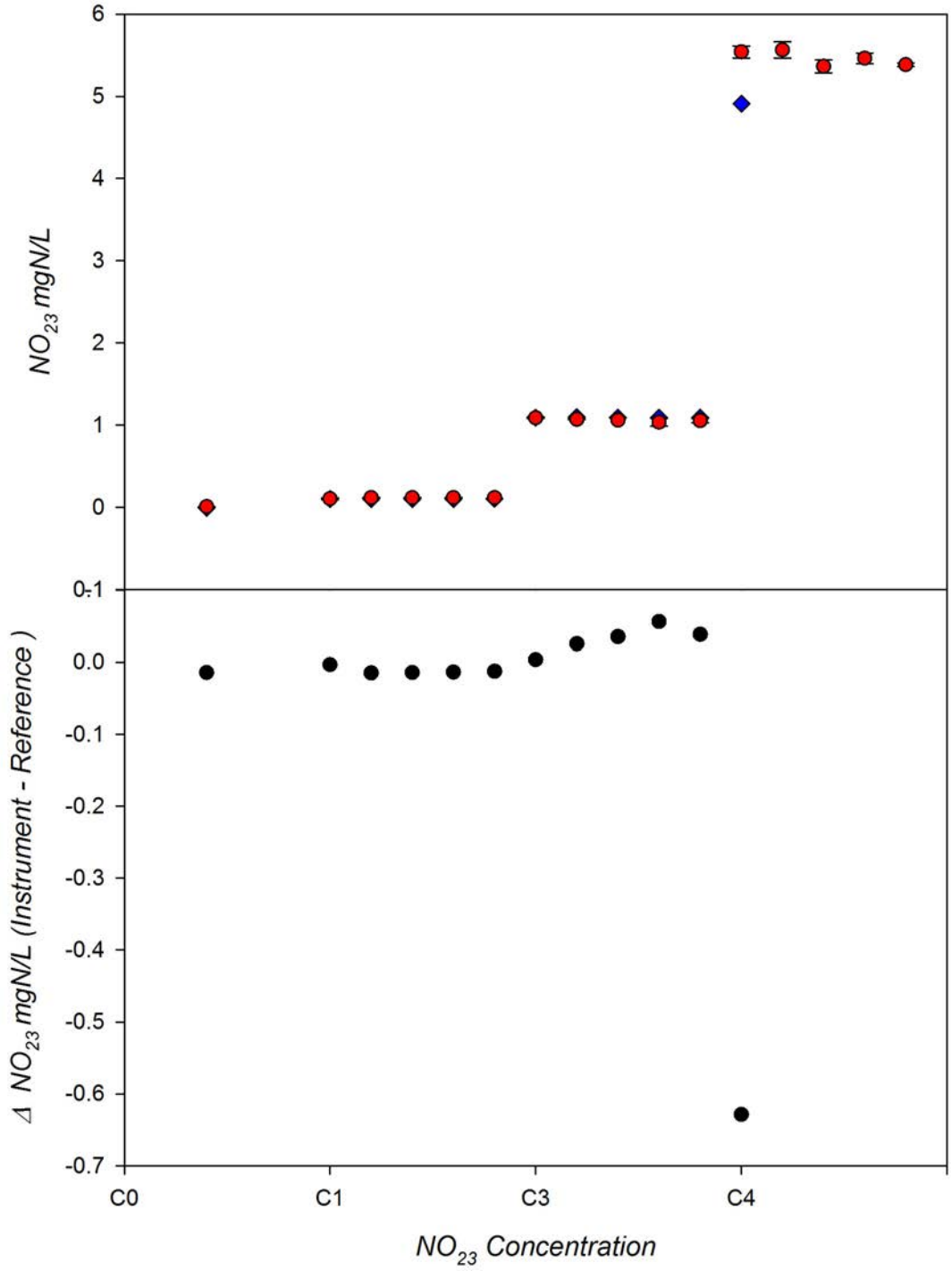

Figure 3. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO_{23} (mgN/L) in the temperature response challenge covering concentration ranges C2 – C4 measured at 5 ^{o}C test conditions. Five replicate measurements were made at each concentration level along with one measurement at ambient level. *Bottom Panel:* Plot of the absolute difference between NOC-NO23 and reference measurement.

Lab Salinity Challenge

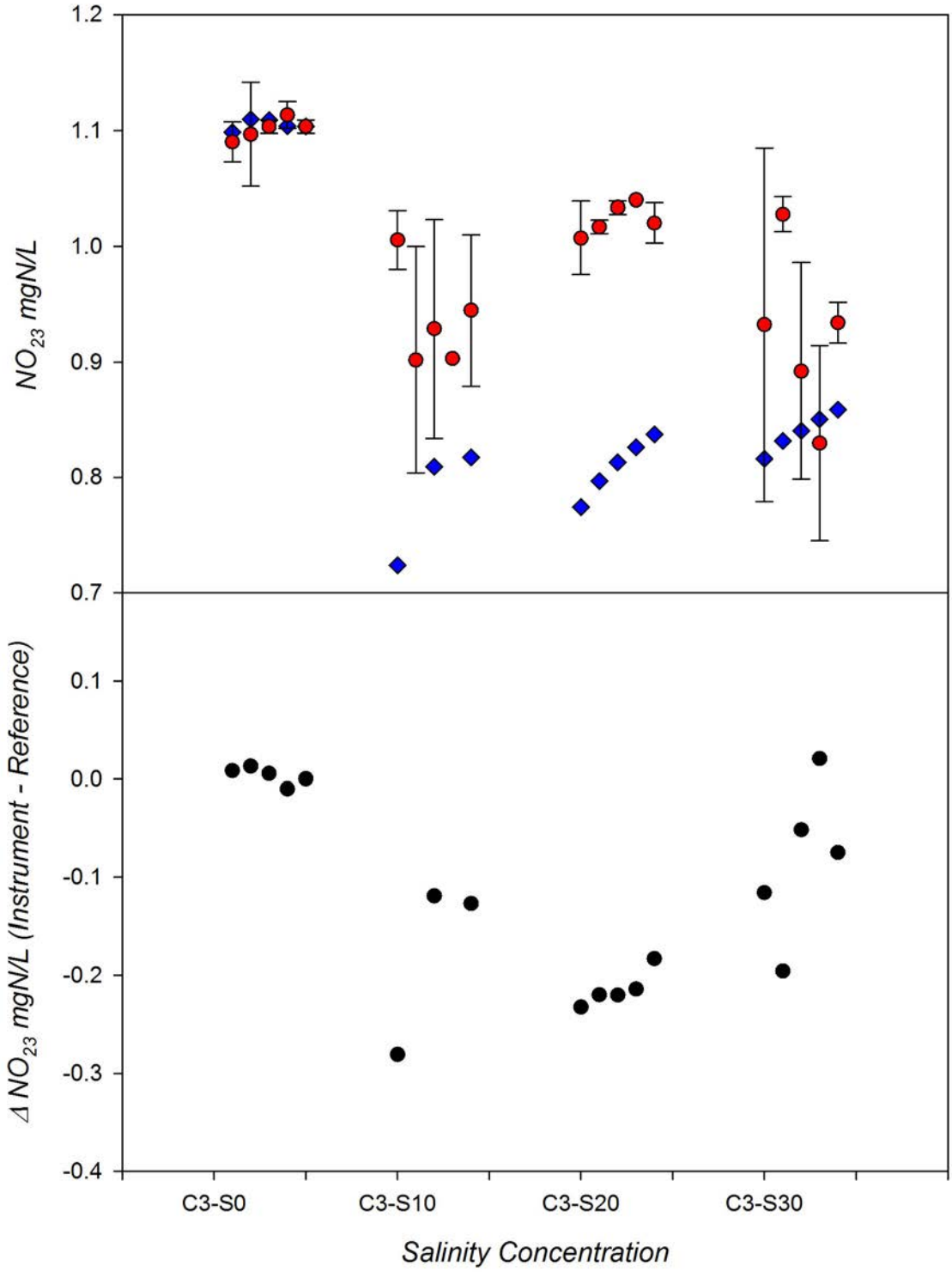

Figure 4. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO_{23} (mgN/L) at four salinity levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between NOC-NO23 and reference measurement.

Lab Turbidity Challenge

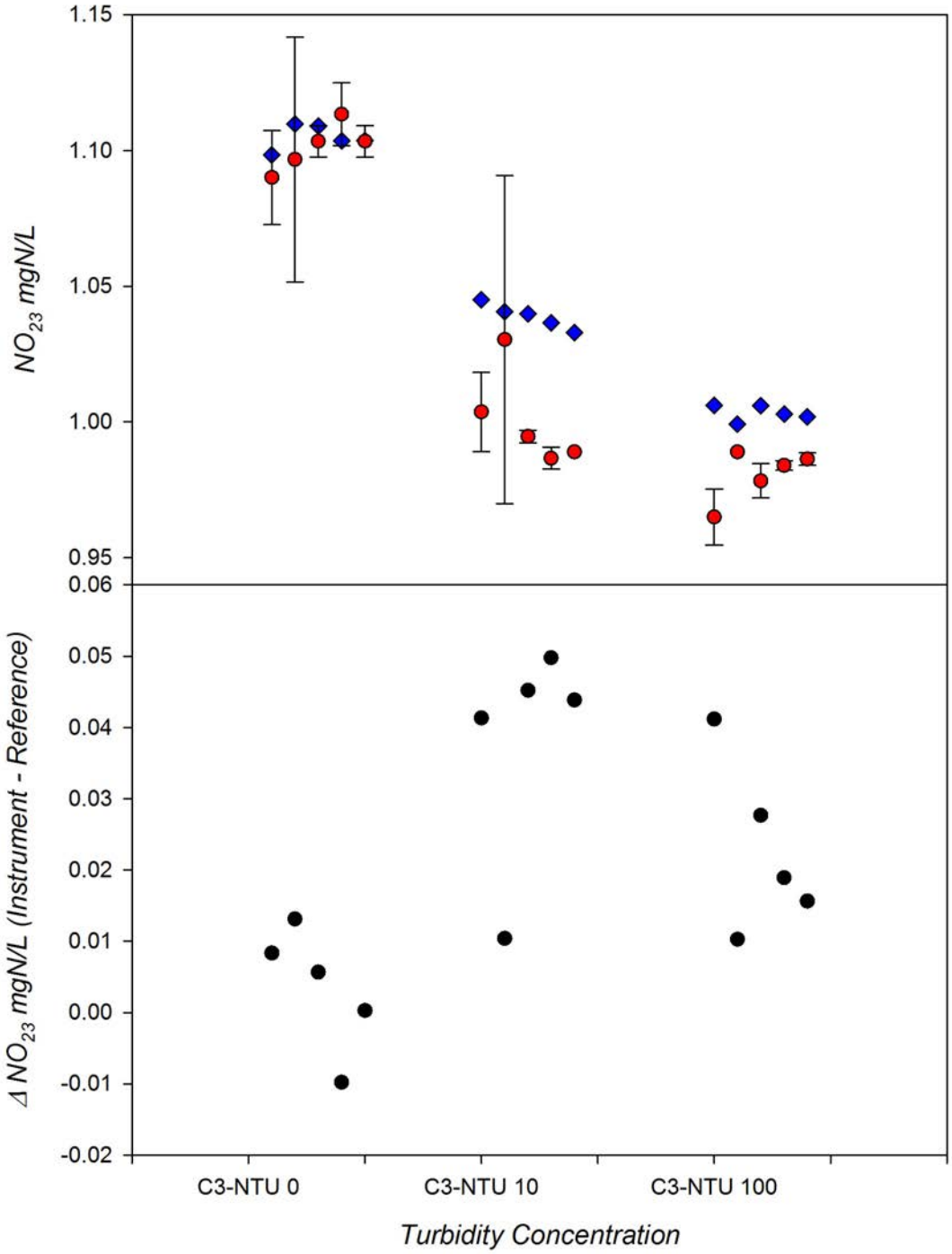

Figure 5. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO23 (mgN/L) at three turbidity levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between NOC-NO23 and reference measurement.

Lab DOC Challenge

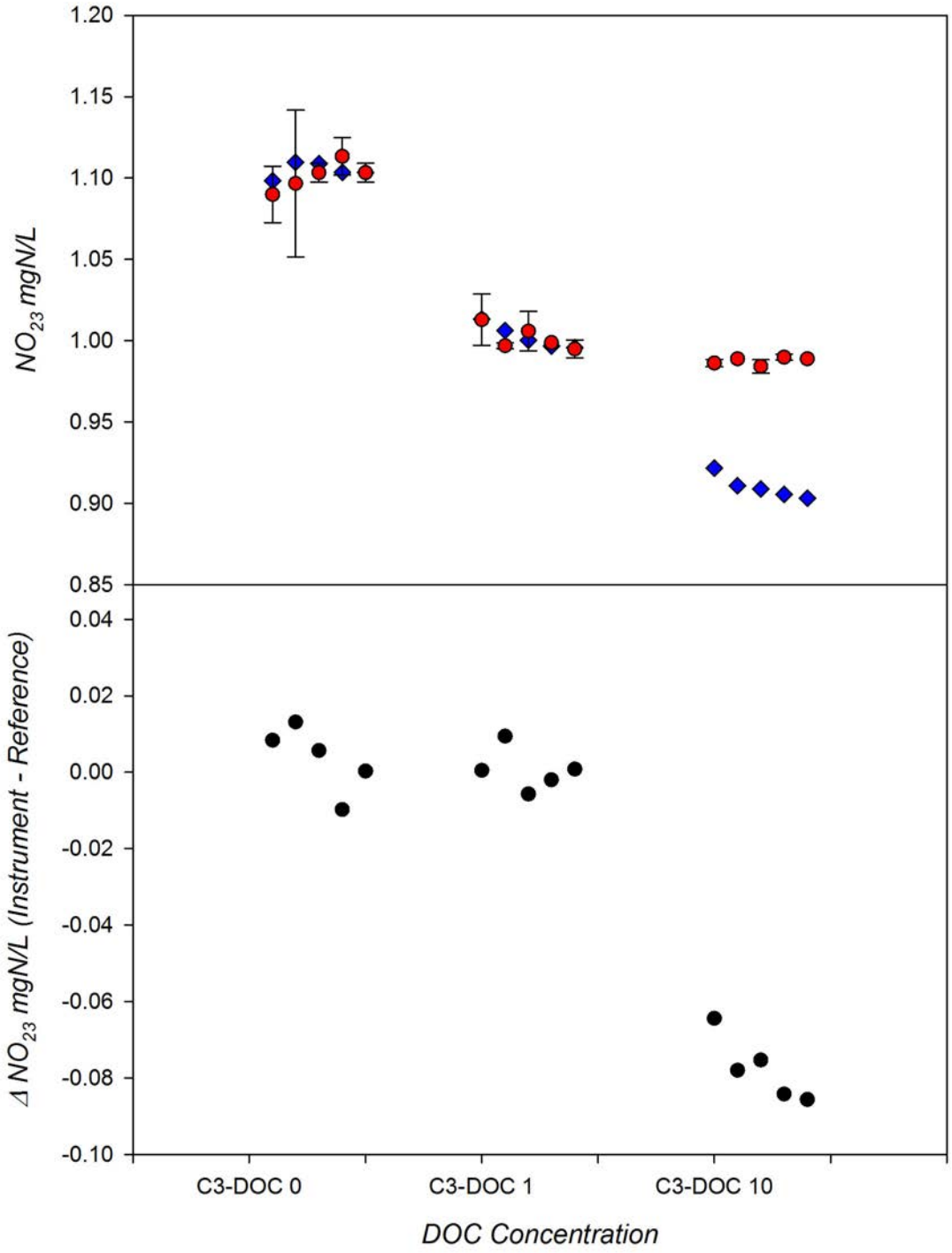

Figure 6. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of NO23 (mgN/L) at three DOC levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between NOC-NO23 and reference measurement.

A summary of measurement differences between the NOC-NO23 and reference sample for each trial of each laboratory challenge is presented in figure 7. It is not known why the instrument accuracy was worse at the 5.0 mgN/L concentration level for both the range and temperature challenges given this was within the expected measurement range of the instrument. Salinity had small but measurable impacts, with measurements being under-estimated at elevated levels compared to zero. Turbidity and DOC had minor and less predictable impacts. Results of measurement differences averaged across all trials within each of the challenge matrices are presented in Table 7.

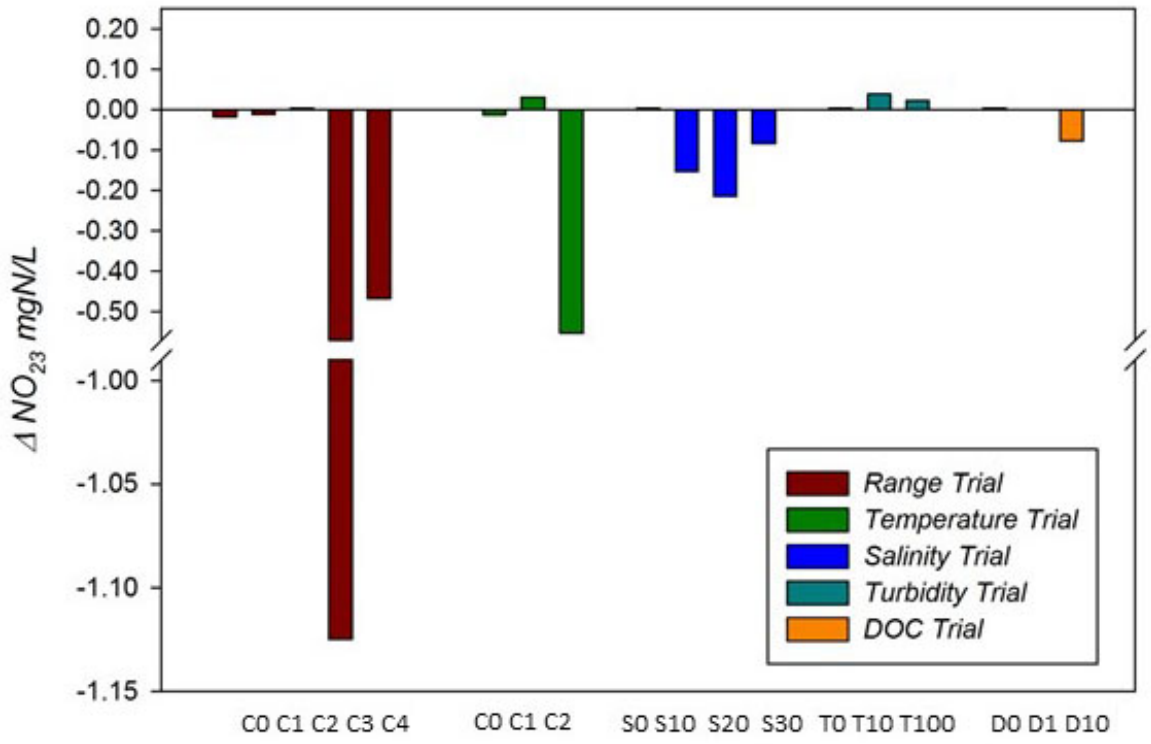

Figure 7. Global summary of difference between instrument and reference measurements for all laboratory tests at each trial conditions for the NOC-NO23 analyzer.

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

NOC-NO_{23}	Range	Temp	Salinity	Turbidity	DOC
min	-1.1250	-0.5525	-0.2140	0.0227	-0.0775
max	0.0035	0.0315	-0.0836	0.0381	0.0006
mean	**-0.3234**	**-0.1776**	**-0.1502**	**0.0304**	**-0.0385**
stdev	0.4902	0.3253	0.0653	0.0109	0.0552

RESULTS of FIELD TESTS

Moored field tests were conducted to examine the performance of the NOC-NO23 to consistently track natural changes in NO23 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 NOC-NO23 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 NOC-NO23 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** ($^{\circ}$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

Table 9. Results of the pre-deployment and post-deployment standard check for the NOC-NO23 for each deployment site. (n.d. denotes no data for that observation.)

Deployment Site	**Expected NO_{23} mgN/L**	**Pre NO_{23} mgN/L**	**Post NO_{23} mgN/L**
UM	4.198	4.350	3.930
CBL	0.0700	0.0635	n.d.
HIMB	0.0140	0.0225	0.0155

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 NOC-NO23 operated during the entire 32 day deployment sampling at hourly intervals, but due to a faulty SD memory card, the data from 5/27 to 6/7 were lost. During the last 6 days of the deployment 122 values were flagged by the instrument as "low precision" and were also omitted from direct comparison to reference samples given the observed variability (Fig. 10). Overall, the NOC-NO23 generated 375 accepted observations out of a possible 763 for a data completion result of 49.1%. Time series results of the NOC-NO23 measurements and corresponding reference NO_{23} results are given in figure 9 (top panel). NO_{23} measured by the NOC-NO23 ranged from 0.701 to 7.18 mgN/L compared to a range of 1.19 to 12.95 mgN/L within the reference samples.

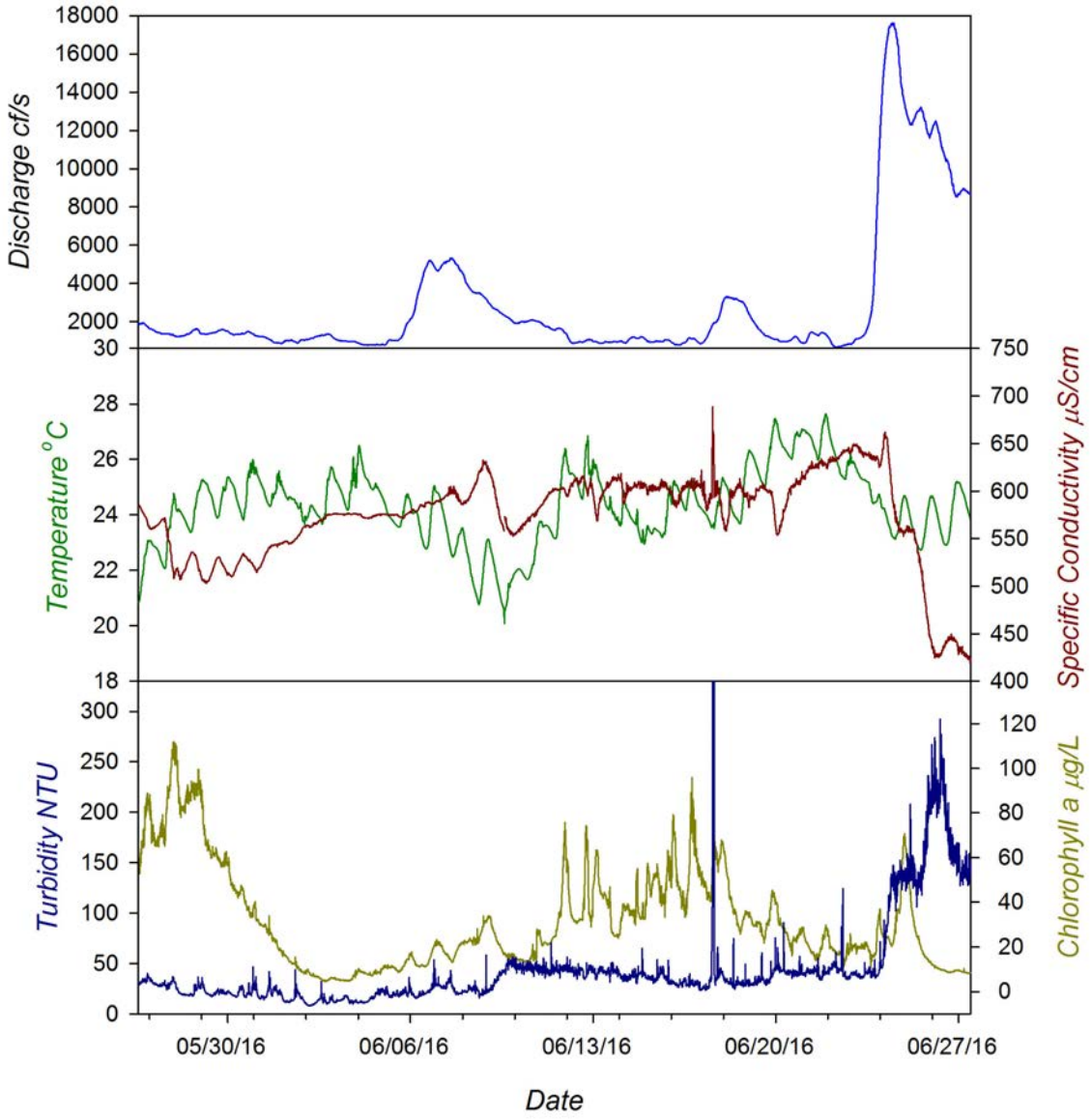

Figure 9. Environmental conditions encountered during the 32 day freshwater deployment in the Maumee River at Waterville, OH. *Top Panel:* Variation in river discharge over the term of the deployment. *Middle Panel:* Variation in temperature (green) and Conductivity (red) at the depth of the sensors, 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.

The time series of the difference between instrument and reference NO_{23} measurements for each matched pair (n=21 of a possible 51 observations) is given in the bottom panel of figure 9. For these direct comparisons, 23 were lost because of missing instrument data, and 7 were omitted when flagged by the instrument value as having low precision. The average and standard deviation of the measurement difference over the total deployment was -1.38 ± 1.29 mgN/L with a total range of -6.12 to 2.16 mgN/L. There was no significant trend in measurement difference over time as estimated by linear regression ($p= 0.48$; $r^2=0.027$).

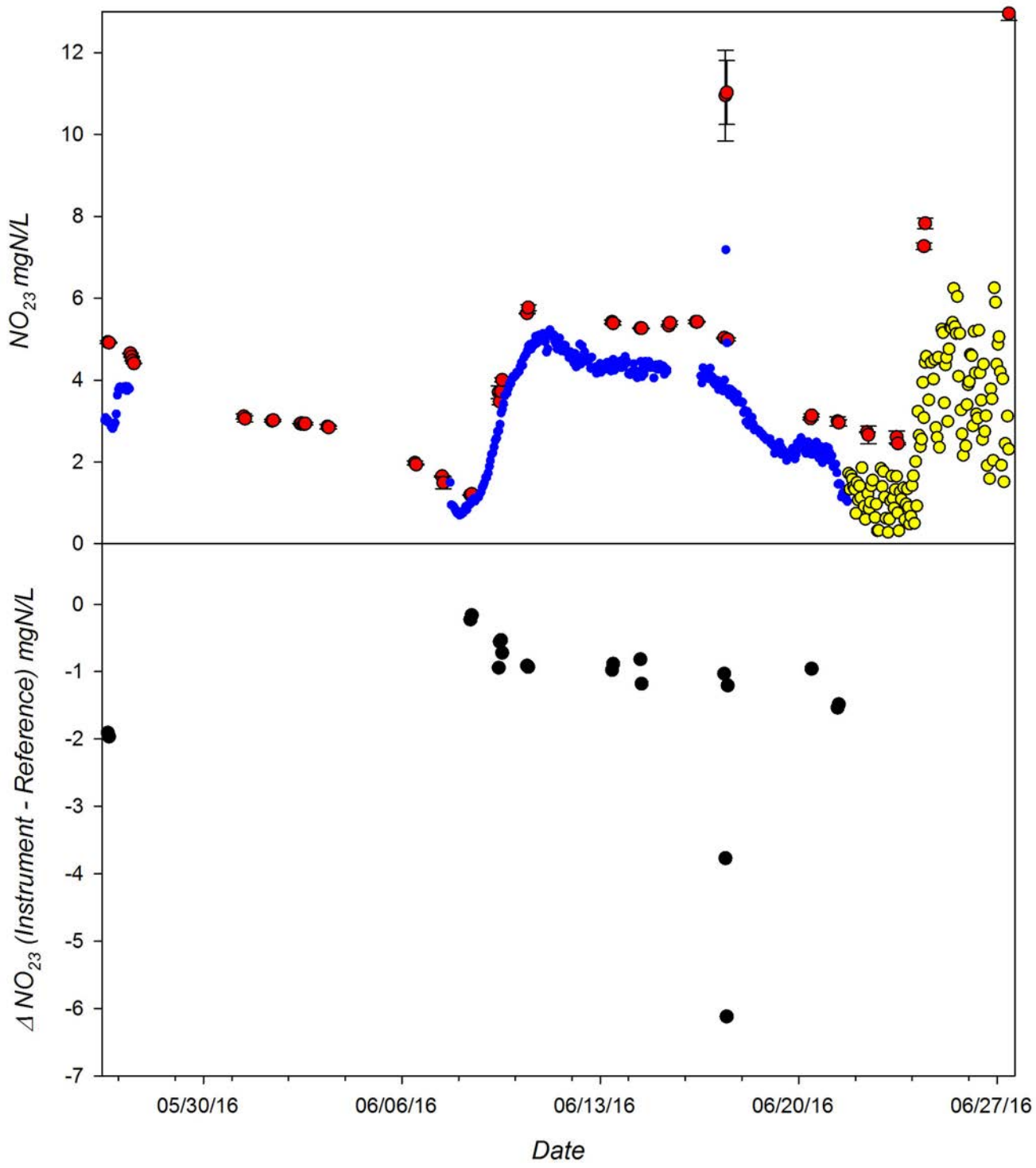

Figure 10. *Top Panel:* Time series plot of the NOC-NO23 measurement (blue dots) and reference measurements (red dots) of nitrate in mgN/L. The yellow dots represent data flagged as "low precision". *Bottom Panel:* Time series plot of the difference between the NOC-NO23 and reference measurements of nitrate in mgN/L (instrument – reference) during the freshwater deployment in the Maumee River at Waterville, OH.

A cross-plot of all accepted matched observations for the deployment is given in figure 11. The linear regression of instrument versus reference measurement was highly significant ($p<0.001$; $r^2 = 0.77$) but with a slope of only 0.546 and intercept of 0.81.

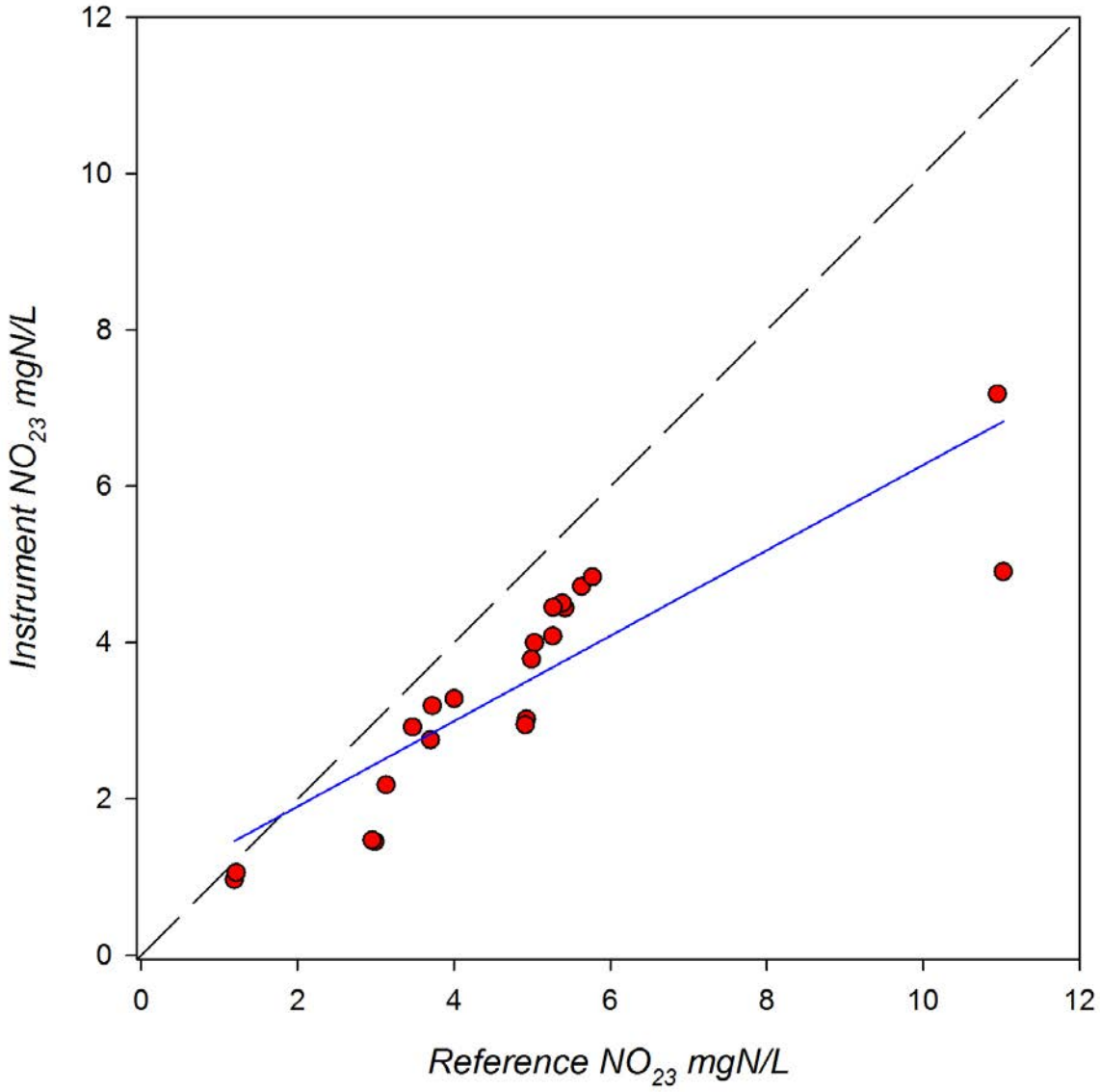

Figure 11. Maumee River field response plot for the 32 day deployment of the NOC-NO23 compared to reference NO_{23} samples. The yellow points represent data flagged "low precision". The plotted line represents a 1:1 correspondence.

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

Figure 12. Photographs of the NOC-NO23 prior to and 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 dock during during deployment.

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 NOC-NO23 malfunctioned during the first 3 days of the deployment, and the manufacturer was given permission to exchange the instrument with a new unit but keeping the same reagent and standards originally prepared. The replacement instrument operated from 7/21 to 8/21, measuring at hourly intervals, but then also failed. The instrument returned 603 data point out of a possible 2012 for the entire deployment period, with 1359 points missing and 50 flagged as NaN (no result calculated). While the unit was deployed it reported 603 of a possible 653 values for a data completion result of 92.3% (but only 33% of the scheduled total deployment was achieved). Time series results of the NOC-NO23 and corresponding reference NO_{23} results are given in figure 15 (top panel). For the interval deployed, the range of accepted values reported by the NOC-NO23 was -0.001 to 0.093 mgN/L, compared to 0.0014 to 0.1550 mgN/L within reference samples.

The bottom panel of figure 15 presents the time series of the difference between the NOC-NO23 and reference NO_{23} for each matched pair (n=34 comparisons out of a total of 103, (67 missing data points and 2 were flagged as NaN). The average and standard deviation of the measurement difference for the deployment was -0.005 ±0.010 mgN/L, with the total range of differences between -0.027 to 0.031 mgN/L. There no significant trend in measurement difference over time during the deployment ($p=0.85$; $r^2=0.001$).

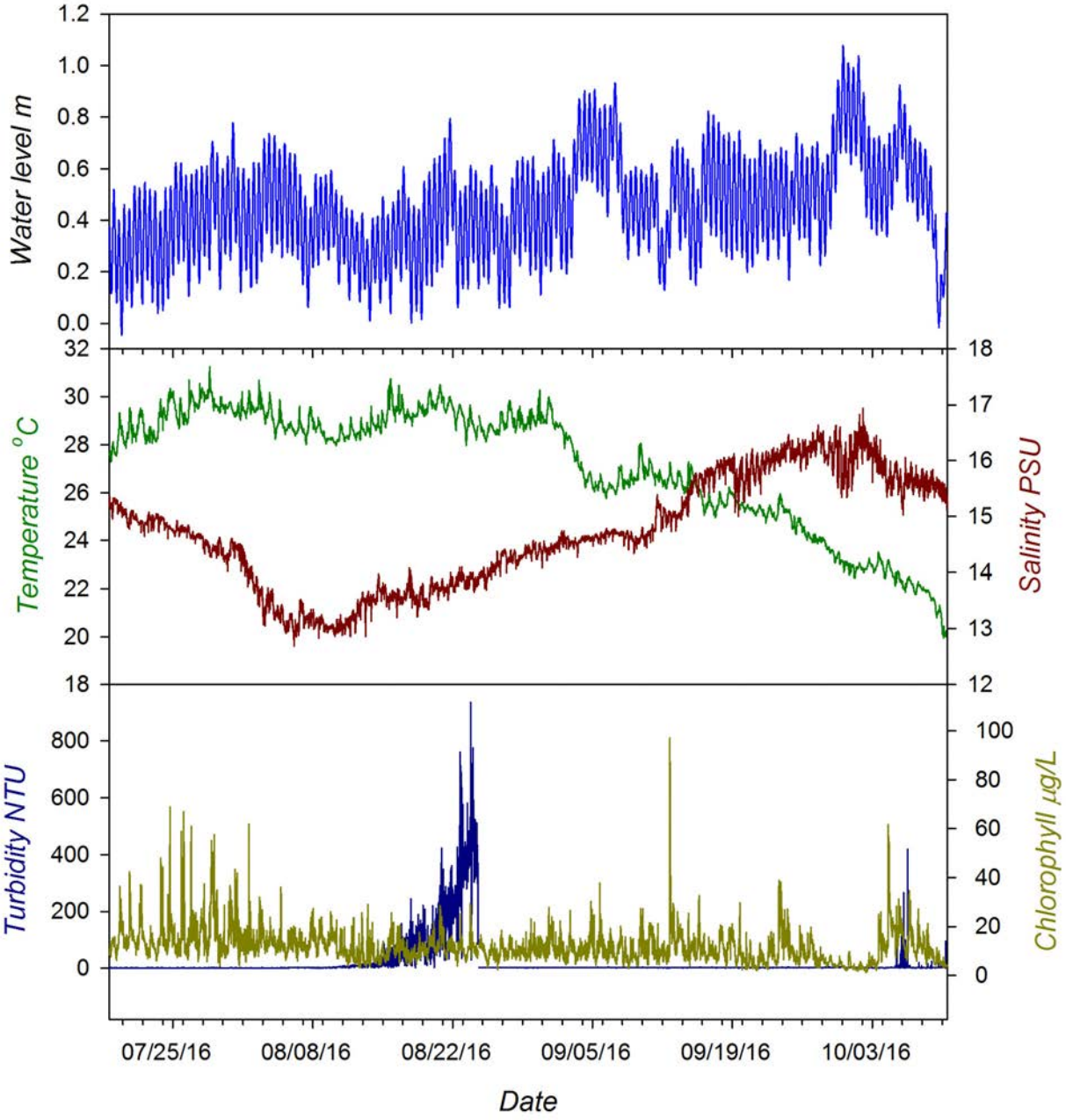

Figure 14. Environmental conditions encountered during the 84 day CBL floating dock deployment. Test sensor array deployed at 1 m fixed depth, variation in local tidal heights indicate active water flow around instrument (*Top Panel*). Variation in temperature (green) and salinity (red) at depth of instrument 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*).

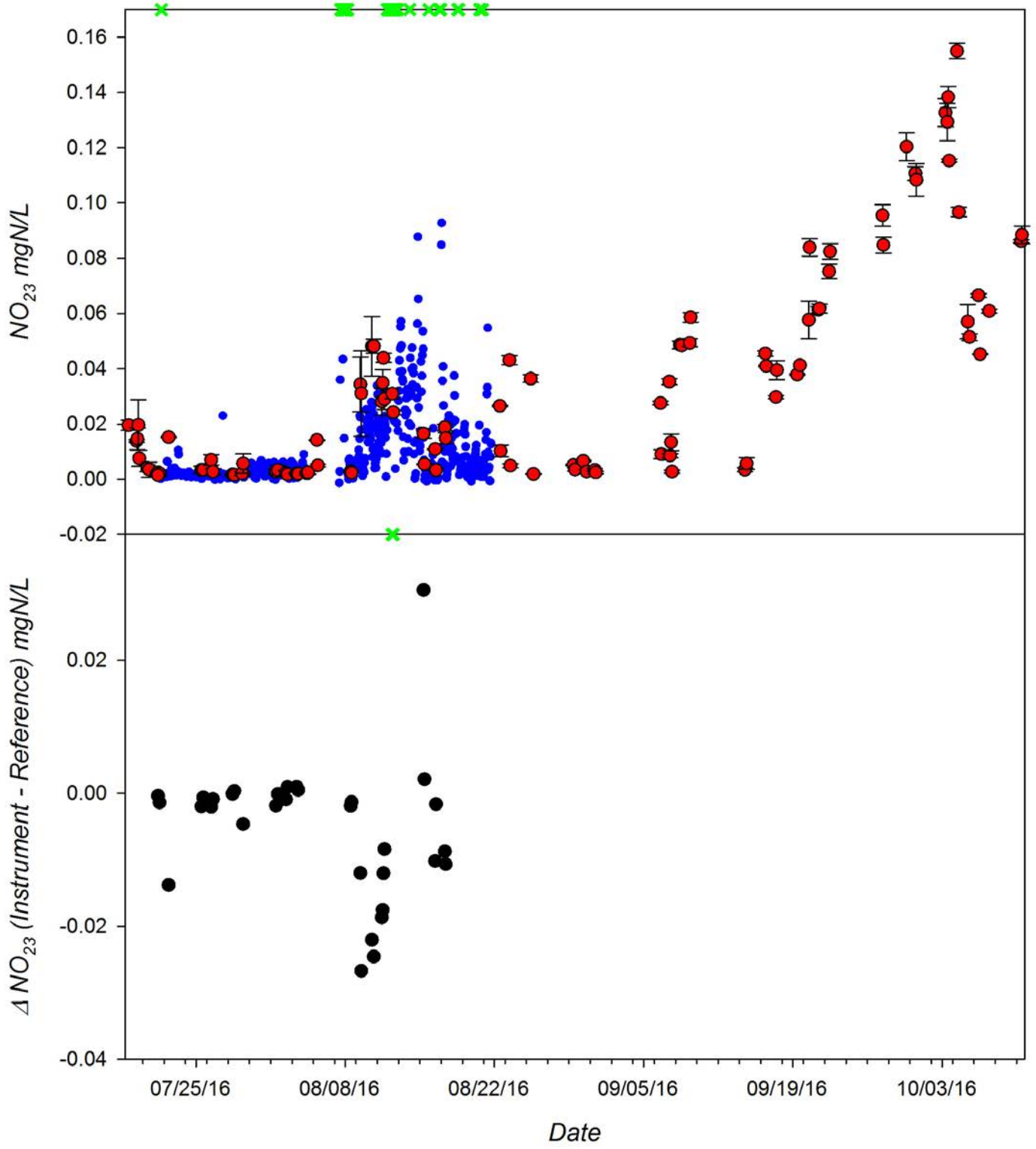

Figure 15. Time series of NO_{23} measured by the NOC-NO23 during the 84 day CBL field trial. *Top Panel*: Continuous NO_{23} recordings from instrument (blue circles) and NO_{23} of adjacent grab samples (red circles). The green crosses at the top of figure represent flagged data (not values) and are plotted on the date of occurrence. *Bottom Panel*: The difference in measured NO_{23} 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 highly significant ($p<0.0001$; $r^2 = 0.53$), but with a slope of only 0.54 and intercept of 0.0009.

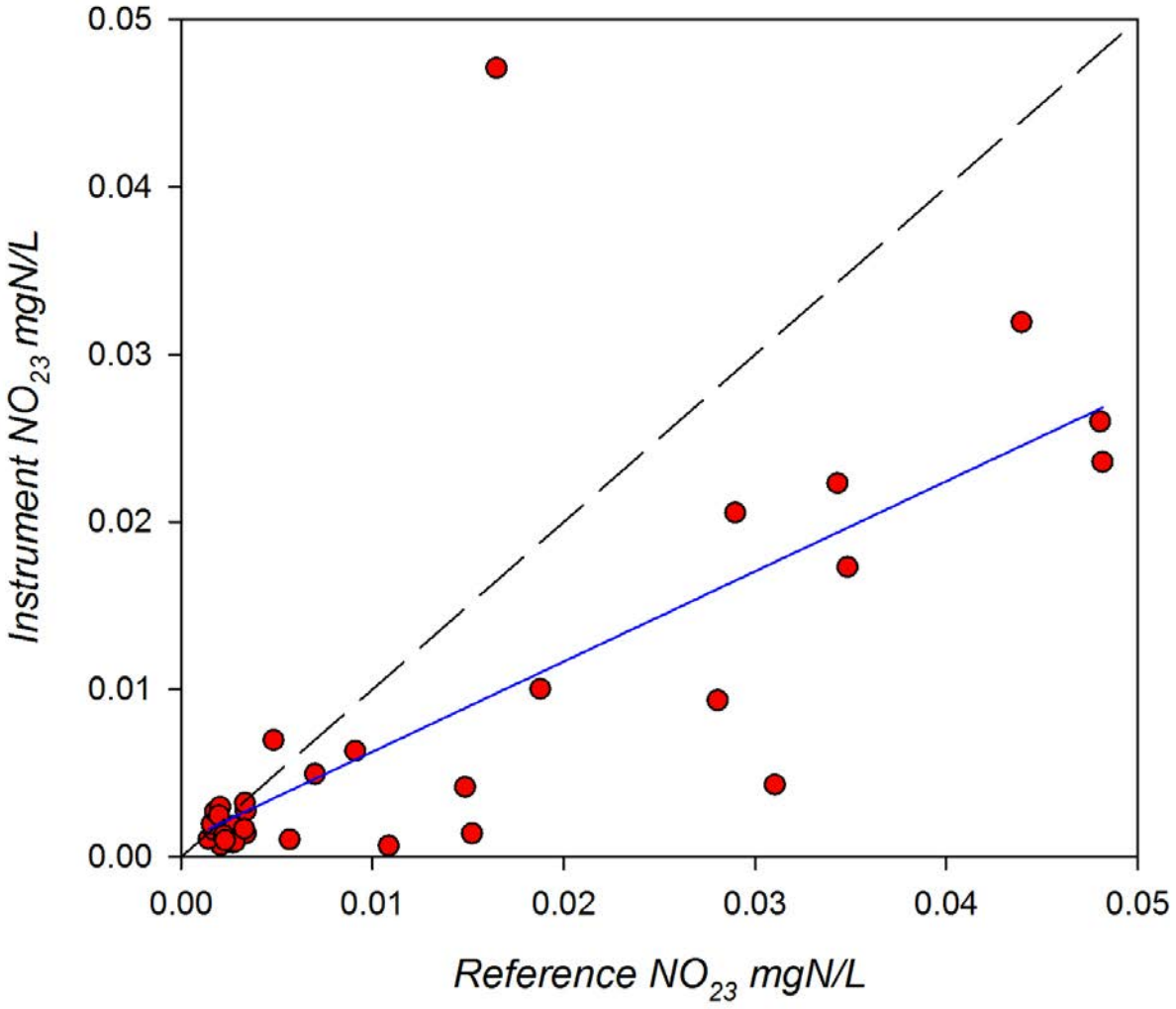

Figure 16. CBL field response plot for NOC-NO23 compared to reference NO_{23} samples. The plotted line represents a 1:1 correspondence.

Photographs of the NOC-NO23 before and after the field deployment to indicate potential impact of biofouling (Figure 17).

Figure 17. Photographs of the NOC-NO23 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 NOC-NO23 operated successfully for the entire 30 day deployment, sampling at hourly intervals. Time series results of the NOC-NO23 and corresponding reference NO_{23} results are given in figure 20 (top panel). The NOC-NO23 returned 720 instrument measurements of a possible 720 measurements for a data completion result of 100%. The range of values reported by the NOC-NO23 analyzer was 0.000 – 0.016 mgN/L, compared to the range within reference samples of 0.006 – 0.042 mgN/L. The bottom panel presents the time series of the measurement difference between the NOC-NO23 and reference NO23 for each matched pair. The average and standard deviation of the differences between instrument and reference readings (n=73 out of a possible 73) were -0.013 ± 0.007 mgN/L, with a total range in the differences of -0.0394 to -0.0029 mgN/L. There was a small but statistically significant trend in the measurement difference over time ($p=0.0009$; $r^2 = 0.182$) during the deployment, with a slope of 0.0003 mgN/L/d.

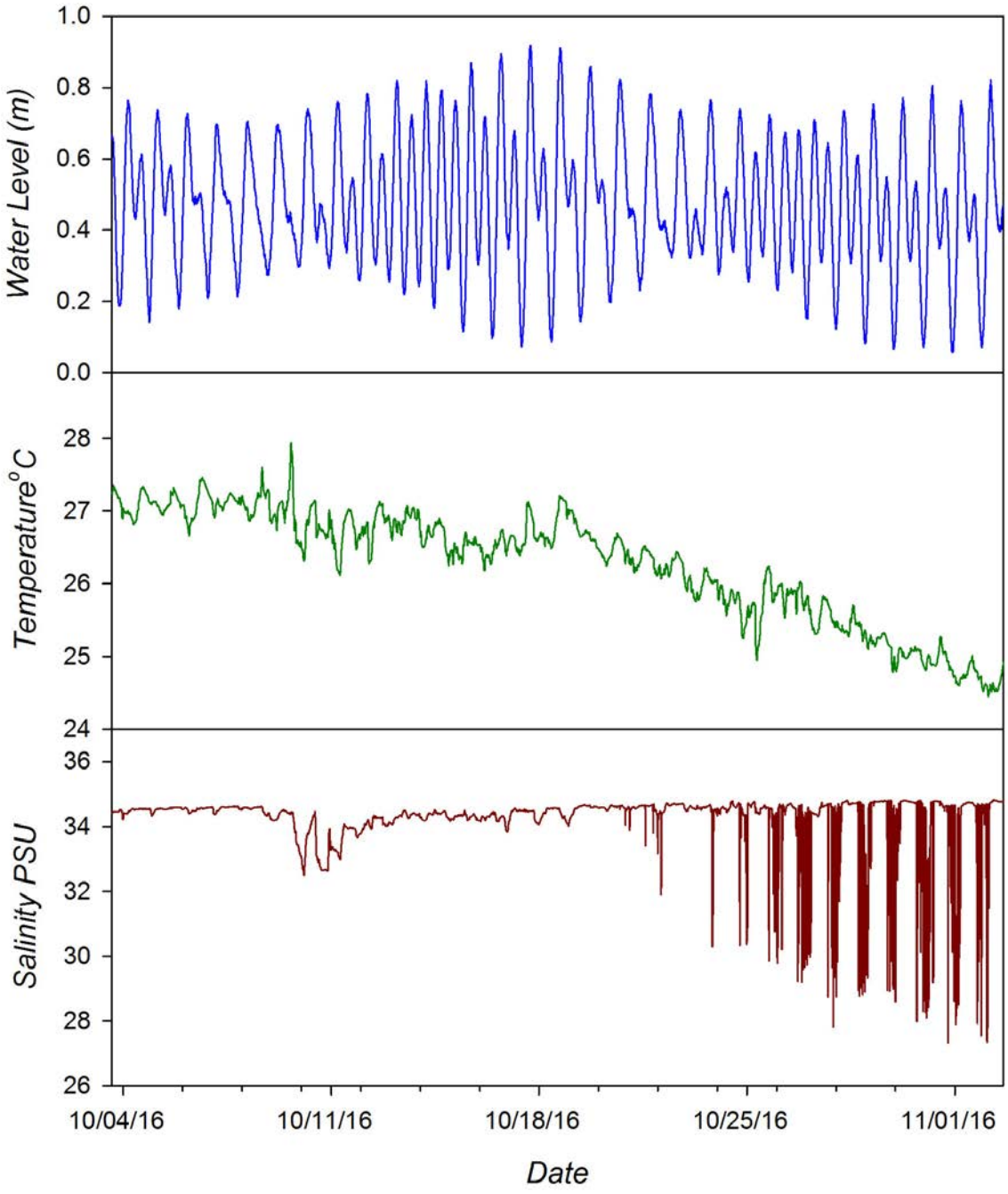

Figure 19. Environmental conditions encountered during the one month HIMB deployment on a floating dock off Coconut Island Test sensor array deployed at 1 m fixed depth, variation in local tidal heights indicate active water flow around instrument (*Top Panel*). Variation in temperature (green) and Salinity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

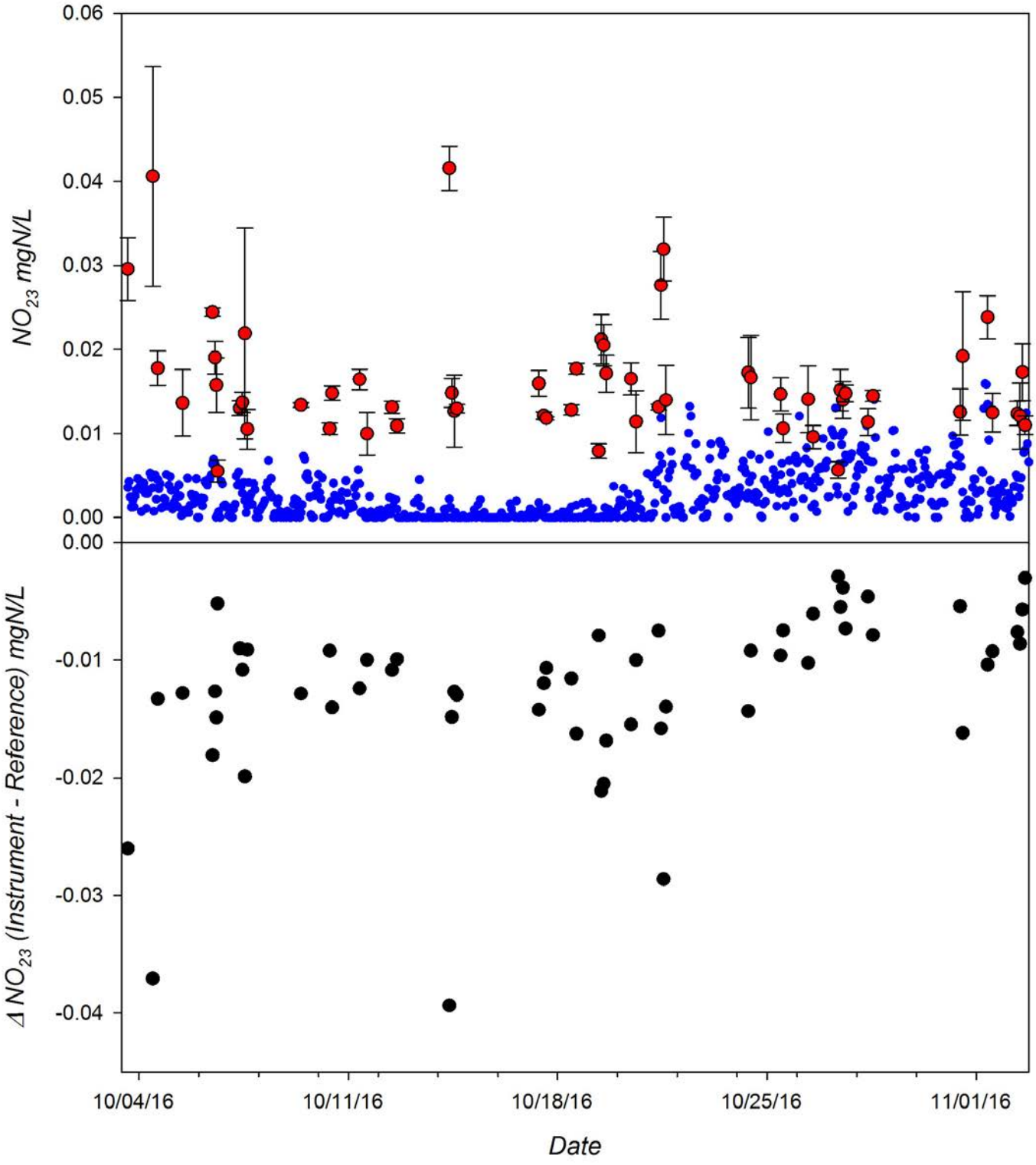

Figure 20. *Top panel:* Time series of NO23 measured by the NOC-NO23 deployed during the one month HIMB field trial. Continuous NO_{23} recordings from instrument (blue dots) and NO23 of adjacent grab samples (red circles.) *Bottom Panel:* Time series of the difference between the NOC-NO23 and reference measurements for each matched pair (Instrument mgN/L – Reference mgN/L).

A cross-plot of the matched observations for the deployment is given in figure 21. The NOC-NO23 under-predicted all measurements and a linear regression of the data was not significant ($p=0.13$; $r^2 = 0.04$).

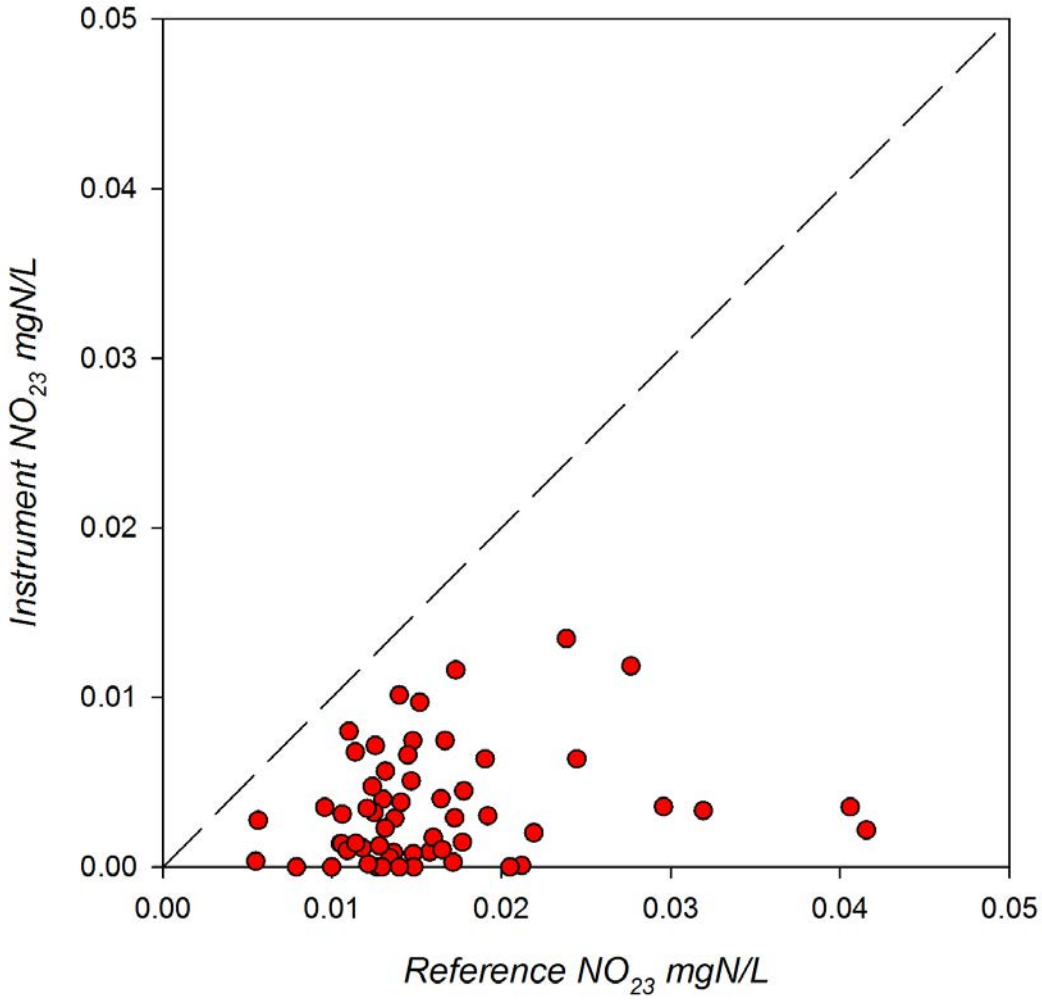

Figure 21. HIMB field response plot of NOC-NO23 compared to reference NO23 samples. The plotted line represents a 1:1 correspondence.

Photographs of and example of the test instrument prior to deployment and the test instrument after the HIMB field deployment to indicate potential impact of biofouling (Figure 22).

Figure 22. Photographs of the NOC-NO23 prior to and following the one month HIMB field trial.

A global summary of instrument versus reference readings for all three field deployment sites are plotted in figure 23. However, the scale of the Maumee River test is too different to allow a good overall comparison and an insert is provided for the lower ranged CBL and HI tests. The NOC-NO23 response showed good linearity for the Maumee River deployment up to levels around 4 mgN/L, but then under-predicted at higher levels. The NOC-NO23 also under-predicted NO_{23} at CBL and HI when concentrations were much lower, and was generally not responsive to concentration changes during the HI test. However, due to the spread generated within the Maumee River test, a linear regression of instrument and reference measurements for all field test results composited was highly significant ($p<0.0001$; $r^2 = 0.95$) with a slope of 0.676 and intercept of 0.030. The data comparison across all field tests covered a concentration range of 0.005 to 12.7 mgN/L.

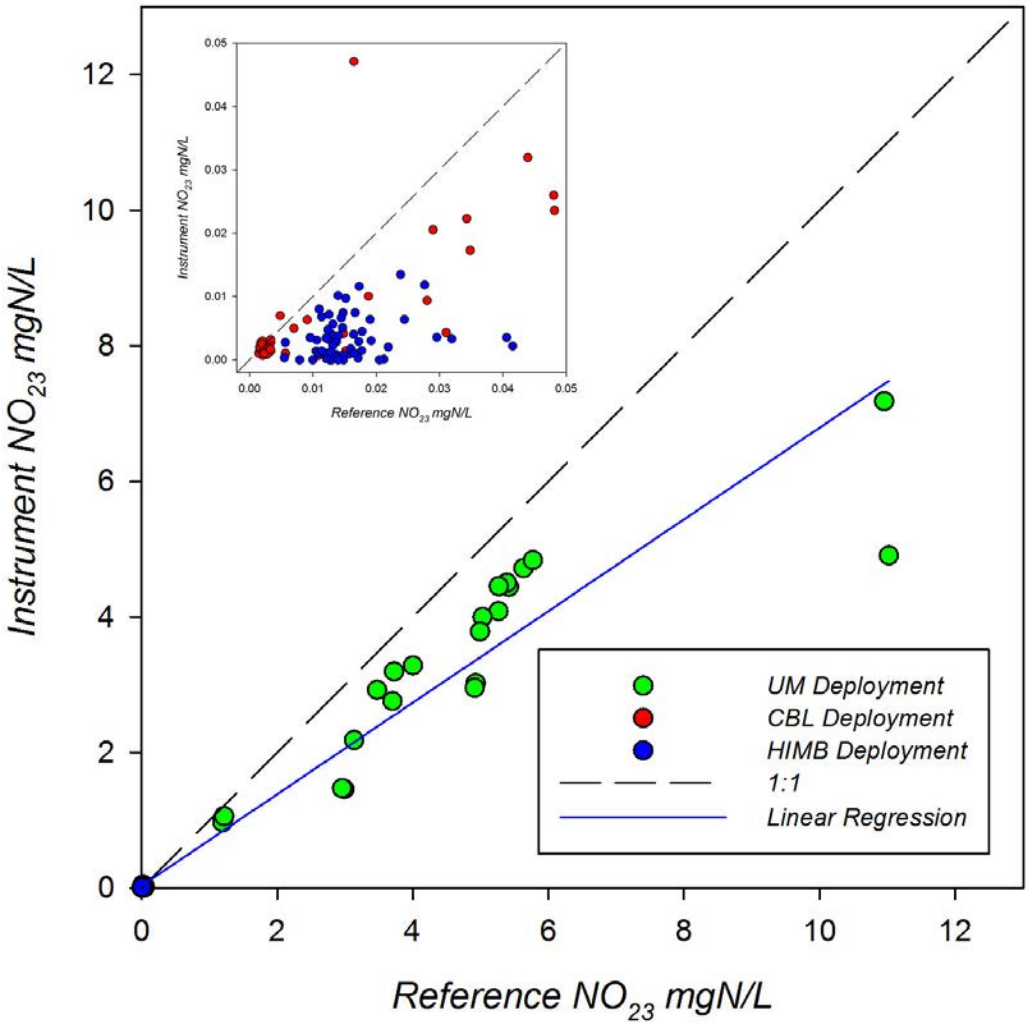

Figure 23. Global response plot for the NOC-NO23 observed during the three ACT field trials. Insert shows the CBL and HIMB deployments enlarged. Black dotted line represents a 1:1 correspondence, blue line is the linear regression.

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

Date/Time	Rep	NO_{23}	Mean	Std Dev	ABS Diff	CV%
6-16-16 9:00	FD1	5.423	5.420	0.005	0.007	0.09
	FD2	5.417				
6-17-16 12:00	FD1	4.990	4.928	0.087	0.123	1.77
	FD2	4.867				
6-20-16 10:00	FD1	3.060	3.072	0.017	0.023	0.54
	FD2	3.083				
6-23-16 11:00	FD1	2.607	2.520	0.123	0.173	4.86
	FD2	2.433				

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

Date/Time	Rep	NO_{23}	Mean	Std Dev	ABS Diff	CV%
7-20-16 10:00	FD1	0.0040	0.004	0.0000	0.0001	1.18
	FD2	0.0040				
7-26-16 14:00	FD1	0.0027	0.0021	0.0009	0.0013	43.3
	FD2	0.0014				
8-2-16 10:00	FD1	0.0022	0.0022	0.0000	0.0000	0.00
	FD2	0.0022				
8-10-16 16:00	FD1	0.0482	0.373	0.0153	0.0216	40.9
	FD2	0.0265				

8-23-16 12:00	FD1	0.0048	0.0045	0.0005	0.0007	10.5
	FD2	0.0042				
9-8-16 10:00	FD1	0.0486	0.0533	0.0066	0.0094	12.5
	FD2	0.0580				
9-16-16 12:00	FD1	0.0409	0.0412	0.0004	0.0006	0.97
	FD2	0.0415				
10-4-16 14:00	FD1	0.0967	0.1004	0.0052	0.0074	5.19
	FD2	0.1041				
10-10-16 10:00	FD1	0.0861	.0840	0.0030	0.0042	3.54
	FD2	0.0819				

Table 13. Results of Field Duplicates (FD) for the Kaneohe Bay, HI mooring test

Date/Time	**Rep**	**NO_{23}**	**Mean**	**Std Dev**	**ABS Diff**	**CV %**
10-6-16 14:00	FD1	0.016	0.0134	0.0034	0.0048	25.6
	FD2	0.011				
10-12-16 11:00	FD1	0.013	0.0123	0.0012	0.0017	9.79
	FD2	0.011				
10-17-16 9:00	FD1	0.016	0.0141	0.0026	0.0037	18.72
	FD2	0.012				
10-26-16 9:00	FD1	0.014	0.0134	0.0010	0.0014	7.22
	FD2	0.013				
11-1-16 9:00	FD1	0.024	0.0210	0.0040	0.0057	19.21
	FD2	0.018				

Table 14. Results of Field Trip Blanks all deployments.

Maumee River		**Chesapeake Bay**		**Kaneohe Bay**	
Field Blank ID	NO_{23} (Std Dev)	Field Blank ID	NO_{23} (Std Dev)	Field Blank ID	NO_{23} (Std Dev)
GLFB1	0.013 (0.004)	CBLFB1	0.0019 (0.0005)	HIFB1	0.005 (0.0028)
GLFB2	0.008 (0.003)	CBLFB2	0.0010 (0.0002)	HIFB2	0.002 (0.0010)
GLFB3	0.004 (0.001)	CBLFB3	0.0008 (0.0002)	HIFB3	0.004 (0.0038)
GLFB4	0.003 (0.001)	CBLFB4	0.0009 (0.0003)	HIFB4	0.013 (0.0034)
--	--	--	--	HIFB5	0.009 (0.0024)
Mean (Std Dev)	0.007 (0.005)	Mean (Std Dev)	0.001 (0.001)	Mean (Std Dev)	0.007 (0.0043)
Grand Mean (Std Dev)					**0.005 (0.0044)**

ACKNOWLEDGEMENTS:

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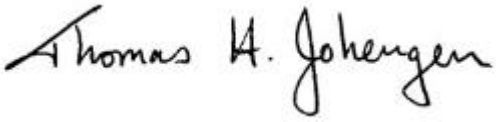
June 1, 2017

Date

Approved By: Dr. Mario Tamburri
ACT Executive Director

June 1, 2017

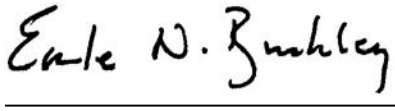
Date



Approved By: Dr. Tom Johengen
ACT Chief Scientist

June 1, 2017

Date



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Dear Dr. Johengen

Re: NOC Response to the ACT Nutrient Sensor Challenge NOC-NO3 report

Our team would like to thank the organizers of the Nutrient Sensor Challenge, who conducted the evaluations in a highly professional, friendly, thorough and fair manner. The NOC-NO3 nitrate sensor is a prototype instrument and at the time of writing is not yet commercially available. At this relatively early stage in its product lifetime, the Nutrient Sensor Challenge (NSC) presented an excellent opportunity for field testing (in collaboration with end-users) in a range of freshwater and coastal environments.

We consider the environments chosen for the field tests to be highly extreme in terms of biofouling (CBL), nutrient concentrations (high at Maumee River and low at HIMB) and sediment load (Maumee River). The challenge was therefore not only able to highlight a number of issues (most of which we were able to solve either during or after the challenge), it also allowed us to refine our on-board data-processing techniques to provide more accurate real-time processed values.

Therefore, while we were satisfied with the performance of the instrument during certain deployments and aspects of the lab tests, we do not feel that we were able to fully demonstrate the true potential of the instrument. The developments we have made since these tests mean we are satisfied that the instrument will be able to display superior performance and robustness when deployed again in these (or similar) scenarios. Below we discuss the issues that we encountered, and discuss how we addressed them both during and since the challenge in order to improve future performance.

Laboratory tests

Prior to the Nutrient Sensor Challenge, we introduced a new electronics package and software interface in order to increase user-friendliness (during setup and data download), and allow the instrument to calculate final concentration values on-board (rather than relying on post-processing). This package has significantly improved the user experience. The sensor can correct for optical changes in the sample water (caused by salinity, DOC and turbidity) automatically. Unfortunately, at the time of the NSC lab tests, this protocol had not been optimized and the sensor was over-correcting. This explains some of the inaccuracies at higher salinities and very high DOC levels. We have since optimized this protocol so that it does not over-correct.

Typically, the NOC-NO3 sensor is be deployed with one of three different onboard standard concentrations, depending on the expected concentation range. Under the rules of the NSC tests, switiching between standards to cover the very large range of concentrations was not possible (we used a mid-range standard as a compromise). This will have contributed to some inaccuracies at the very high and very low concentrations.

Maumee River deployment
Unfortunately a gap exists in the Maumee River dataset. This was due to an obscure firmware bug associated with our new electronics package (which eluded us during testing), which meant that under certain scenarios the sensor would not correctly write the datafile to the SD card when power was lost. This situation occurred during the Maumee River deployment when there was a power glitch midway through the deployment, meaning that we lost a section of data from this deployment. Not long after this issue was identified, our software team were able to identify the bug and fix it, meaning that the issue did not re-occur during subsequent NSC deployments.

Towards the end of the deployment, we flagged some of the nitrate data as poor quality. This was due to the filter starting to become clogged. The NOC LOC nitrate sensor sucks water in through an inline 0.45 µm syringe filter. While normally reagent consumption is the limiting factor on a deployment length, in environments with very high sediment load (e.g. Maumee River), filter blockage can be the limiting factor (although it is simple for the user to change the filter in the field). We have since optimised (i.e. reduced) the flushing volume of the filter in order to increase the sensor endurance in highly turbid waters. The filter flushing volume is now the same as that of the NOC-PO4 phosphate sensor (which did not become blocked during the Maumee River deployment), and we are therefore confident that the NOC nitrate sensor could endure 1-month of hourly measurements in a similarly highly turbid environment (or three-months if the filter is changed monthly).

CBL deployment
Unfortunately the LOC nitrate sensor failed just under halfway through the three-month deployment at the extremely high biofouling CBL site. Upon recovery it appears that crabs had severed some of the sensor inlet tubing. This was a highly unusual and unexpected failure, and prior to this we had never had a sensor failure due to any form of biofouling. We have since re-designed the reagent housing to prevent crabs (or other crustaceans) from causing damage to the inlet tubing.

The Nutrient Sensor Challenge was a challenging proving ground for NOC-NO3 sensor prototypes and a valuable learning experience for the NOC team. The laboratory and field data generated as part of the Challenge provided a unique opportunity to evaluate the NOC-NO3 performance against high-quality and high-resolution reference data in a diverse range of natural waters. These reference data are extremely beneficial as we continually seek to validate lab-on-chip (LOC) technology for long-term *in situ* monitoring. We are indebted to the ACT staff for giving us the opportinity to participate in the Challenge with a prototype instrument, for designing and implementing rigorous and laborious field/laboratory testing programs, and for their willingness to accommodate our needs. We thoroughly enjoyed collaborating with ACT staff and look forward to participate in future evaluations.

Yours Sincerely,

Alexander Beaton

Maxime Grand

Allison Schaap

Matthew Mowlem

On behalf of the NOC Ocean Technology and Engineering Group (OTEG)
Southampton, UK, June 1, 2017

Email: a.beaton@noc.ac.uk