Ref. No. [UMCES] CBL 2017-049
ACT VS17-04

PERFORMANCE VERIFICATION STATEMENT
For NOC Phosphate Analyzer

TECHNOLOGY TYPE: Nutrient Sensors

APPLICATION: In situ estimates of PO_4 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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Questions and comments should be directed to: Dr. Tom Johengen
ACT Chief Scientist
CILER- University of Michigan
4840 S. State Street
Ann Arbor, MI 48108 USA
Email: Johengen@umich.edu

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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 tank 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-PO4 and reference measurement across all timepoints for trials C0 – C5 ranged from -0.0153 to 0.0025 mgP/L, with a mean of -0.0027 ±0.0043 mgP/L. There was a small but significant increase in the measurement difference with increasing concentration as determined by linear regression ($p=0.008$; $r^2=0.27$). However, the change in accuracy mostly occurred at the highest test concentration (0.406 mgP/L) with measurement difference of -0.0103 mgP/L. 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 0.0002 to 0.0050 mgP/L across the five trials, and the coefficient of variation ranged from 1.25 to 5.25 percent. 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 -0.0095 to -0.0004 mgP/L, with a mean of -0.0045 ±0.0031 mgP/L. There was no significant difference in measurement accuracy at the C2 concentration level. However, measurement differences were significantly more negative (under-predicted) for the C3 and C4 concentration trials at 5 ^{o}C then at 20 ^{o}C, with offsets of -0.0050 and -0.0071, respectively. 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 -0.0021 to 0.0125 mgP/L, with a mean of 0.004 ±0.0051 mgP/L. There was no statistically significant response between measurement accuracy and salinity across all trials ($p=0.32$; $r^2=.08$). 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 -0.0033 to 0.0014 mgP/L, with a mean of -0.0007 ±0.0017 mgP/L. A linear regression of the measurement differences versus turbidity was not significant ($p<0.12$; $r^2=0.20$). 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 -0.0006 to 0.0098 mgP/L, with a mean of 0.0015 ±0.0034 mgP/L. A linear regression of the measurement differences versus DOC concentration was barely non-significant ($p=0.056$; $r^2=0.27$). Measurement offset was 0.004 mgP/L more positive at 10 versus 1 mgC/L.

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 NOC-PO4 operated

successfully during the entire 32 day deployment sampling at hourly intervals, but lost 12 days of data between 5/27 – 6/8 due to a problem writing results to the SD memory card. The NOC-PO4 generated 461 observations out of a possible 763 for a data completion result of 60.4%. The average and standard deviation of the measurement difference between instrument and reference PO_4 measurements for each matched pair (n=28 of a possible 51 observations) over the total deployment was 0.034 ± 0.024 mgP/L with a total range of -0.033 to 0.079 mgP/L. There was a small but significant trend in measurement difference over time as estimated by linear regression ($p= 0.03$; $r^2=0.17$) with a slope of 0.001 mgP/L/d. A linear regression of instrument versus reference measurement was highly significant ($p<0.0001$; $r^2 = 0.49$) but with a slope of only 0.65 and intercept of 0.045. The NOC-PO4 handled the measurement range equally well, but was generally over-predicting concentrations as noted by the positive intercept of 0.045 mgP/L.

An 84 day moored field test was conducted in Chesapeake Bay from July 18 to October 10, 2016. The NOC-PO4 operated continuously for the first 8 days of the deployment sampling at hourly intervals but stopped reporting on 7/31 when it appears to have fallen off the mooring when an attachment bolt was corroded away. A new instrument was deployed on 9/16 and operated until the end of the deployment reporting 874 of a possible 883 accepted values for a data completion result of 99%, but this represented only 43% of the total possible record. During the second unit's operation, 9 values were flagged by the instrument as bad data. The average and standard deviation of the measurement difference between instrument and reference PO_4 measurements for each matched pair (n=48 of a possible 103 observations) over the total deployment was 0.006 ±0.005 mgP/L, with the total range of differences between -0.003 to 0.015 mgP/L. There was a similar range of measurement offset during the two deployment periods; however the sharp rise in instrument values and offset during the initial 8 days may have indicated somethings was malfunctioning within the instrument, leading to the corrosion problem. A linear regression of NOC-PO4 versus reference measurements was highly significant ($p<0001$; $r^2 = 0.743$), with a slope of 0.933 and intercept of 0.006. NOC-PO4 covered the range equally well, but in general over-predicted concentrations.

A one month long moored field test was conducted in Kaneohe Bay from October 3, 2016 to November 2, 2016. The NOC-PO4 operated successfully for the entire 30 days of the deployment, sampling at hourly intervals returning 718 of a possible 720 measurements for a data completion result of 99.7%. The average and standard deviation of the differences between instrument and reference readings over the entire deployment (n=73 out of a possible 73) was -0.0014 ± 0.0009 mgP/L, with a total range in the differences of -0.0034 to -0.0001 mgP/L. There was a small but statistically significant trend in the measurement difference over time ($p=0.0001$; $r^2 = 0.233$) during the deployment, with a slope of -0.00004 mgP/L/d. A linear regression of instrument versus reference measurements was significant ($p=0.014$; $r^2 = 0.103$), but with a slope of only 0.149 and intercept of 0.002. The NOC-PO4 under-predicted throughout the measurement range and was marginally responsive to concentrations above 0.004 mgP/L.

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 Systea WIZ Phosphate 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 phosphate sensor (denoted as NOC-PO4 throughout the report) is a submersible wet chemical analyzer that measures Soluble Reactive Phosphorus (SRP) on a microfluidic chip using a modified version of the phosphomolybdenum blue method. The chemical method involves the reaction between phosphate and acidified molybdate (reagent 1) to form a heteropoly acid, which is reacted with a reducing agent (reagent 2) to produce a blue colored compound. The intensity of the blue color is proportional to the concentration of phosphate present in the solution analyzed and is measured by absorbance spectrophotometry at 700 nm.

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 which provide a large dynamic range (0.02 to 40 μM). Each cell is configured for absorbance detection using 700 nm LEDs and photodiodes placed at opposite ends of the cells.

The reagents and analytical solutions (blank, sample, standards) 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 onboard standard measurement, eliminating drift problems.

A custom onboard 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 phosphate 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 13mm PES filter and connected to the chip using 0.5 mm id Teflon tubing.

A full set of reagents allows over 1500 measurements at a minimum sampling period of 20 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 1 measurement
4. Standard 2 measurement (optional)
5. Flush with cleaning solution
6. Delay (optional, depends on required sampling frequency).

Each step is performed by merging the analytical solution (blank, sample or standards) with the two reagents into the measurements cells. Once the cells are filled with the reacting solution, the flow is stopped for 150-300 seconds to allow color development. After the blank, sample and standards have been analyzed; a cleaning solution is flushed through the chip before waiting for the next measurement. All waste generated by the sensor is collected onboard 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 the 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 ^{o}C 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 ^{o}C versus the temperature of 20 ^{o}C 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-PO4 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 -0.0153 to 0.0025 mgP/L, with a mean of -0.0027 ±0.0043 mgP/L. The means for each trial are given in Table 1. A plot of the absolute difference between NOC-PO4 and reference measurement is shown in the bottom panel of figure 1. There was a small but significant increase in the measurement difference with increasing concentration as determined by linear regression ($p=0.008$; $r^2=0.27$). However, the change in accuracy mostly occurred at the highest test concentration (0.406 mgP/L) with measurement difference of -0.0103 mgP/L.

Table 1. Accuracy results for laboratory testing of the NOC-PO4 analyzer assessed by absolute difference (mgP/L) and percent error between instrument and reference measurements for the concentration range test.

Trial	Reference	NOC-PO4	Absolute Diff	% Error
C0	0.0059	0.0033	**-0.0026**	**44.2**
C1	0.0105	0.0096	**-0.0009**	**8.4**
C2	0.0189	0.0182	**-0.0007**	**3.8**
C3	0.0621	0.0624	**0.0002**	**0.4**
C4	0.1159	0.1155	**-0.0005**	**0.4**
C5	0.4059	0.3957	**-0.0103**	**2.5**

Precision

An assessment of precision was performed by computing the standard deviations and coefficients of variation of the five replicate measurements within each of C1 – C5 concentration trials. The standard deviation of the mean ranged from 0.0002 to 0.0050 mgP/L across the five trials, and the coefficient of variation ranged from 1.25 to 5.25 percent (Table 2).

Table 2. Precision assessment of the NOC-PO4 analyzer during the laboratory concentration range testing. 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 PO_4 (mgP/L)		Standard Deviation		Coefficient of Variation	
Trial	*Reference*	*NOC-PO4*	*Reference*	*NOC-PO4*	*Reference*	*NOC-PO4*
C1	0.0105	0.0096	0.0004	0.0002	4.21	1.94
C2	0.0189	0.0182	0.0001	0.0010	0.66	5.25
C3	0.0621	0.0624	0.0005	0.0012	0.75	1.91
C4	0.1159	0.1155	0.0003	0.0016	0.23	1.40
C5	0.4059	0.3957	0.0023	0.0050	0.56	1.25

Lab Concentration Range Challenge

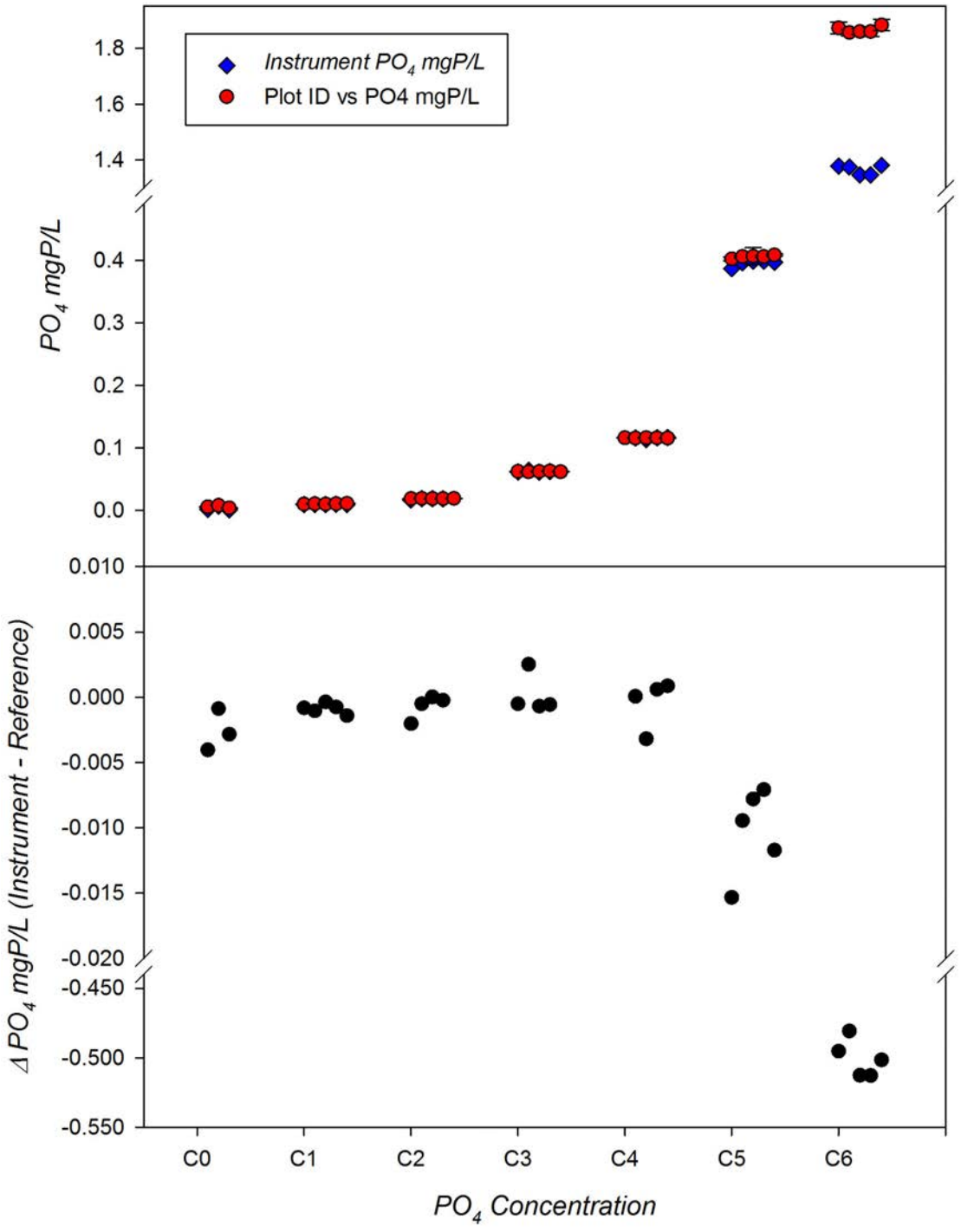


Figure 1. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of PO_4 in the laboratory concentration range challenge covering ambient plus 6 concentration ranges. Five replicate measurements were made at each concentration level along with three measurements at ambient level. *Bottom Panel:* Plot of the absolute difference in mgP/L between NOC-PO4 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.

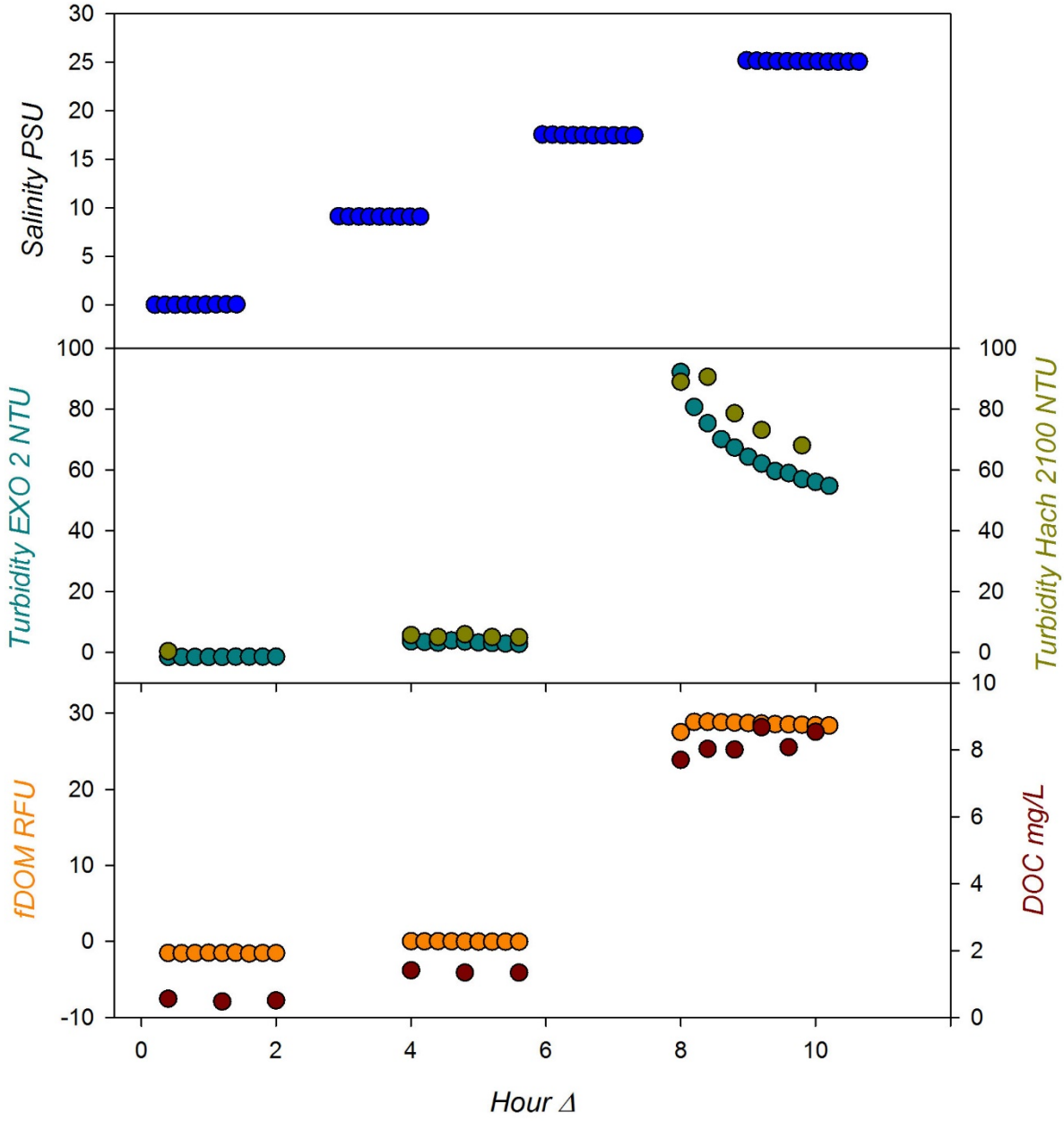


Figure 2. *Top Panel: In situ* salinity measured by EXO2 sonde in the laboratory salinity challenge covering ambient plus 3 salinity ranges. *Middle Panel: In situ* turbidity measured by EXO2 sonde (teal) and on grab samples by a Hach 2100 Turbidimeter (olive) during the laboratory turbidity challenge 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 -0.0095 to -0.0004 mgP/L, with a mean of -0.0045 ±0.0031 mgP/L. The means for each trial are given in Table 3. There was no significant difference in measurement accuracy at the C2 concentration level. However, measurement differences were significantly more negative (under-predicted) for the C3 and C4 concentration trials at 5 ^{o}C then at 20 ^{o}C, with offsets of -0.0050 and -0.0071, respectively.

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

Trial	Reference	NOC-PO4	Absolute Diff	% Error
C2	0.0109	0.0103	**-0.0006**	**5.7**
C3	0.0547	0.0497	**-0.0050**	**9.1**
C4	0.1040	0.0969	**-0.0071**	**6.8**

Results of the laboratory salinity challenge, performed 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 -0.0021 to 0.0125 mgP/L, with a mean of 0.004 ±0.0051 mgP/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 no statistically significant response between measurement accuracy and salinity across all trials ($p=0.32$; $r^2=.08$). Measurement differences were greater during the first salinity addition to 10 psu, but then were lower at each of the next two salinity additions. It is noted that the reference data indicate that phosphate concentration in the tank were declining as salt was added. The NOC-PO4 generally tracked this pattern as well.

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

Trial	Reference	NOC-PO4	Absolute Diff	% Error
0	0.0621	0.0624	**0.0002**	**0.4**
10	0.0443	0.0500	**0.0057**	**12.9**
20	0.0385	0.0416	**0.0031**	**8.1**
30	0.0297	0.0299	**0.0001**	**0.4**

Results of the laboratory turbidity challenge, performed 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 -0.0033 to 0.0014 mgP/L, with a mean of -0.0007 ±0.0017 mgP/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 ($p<0.12$; $r^2=0.20$), however the offset was more negative (-0.0015 mgP/L) at the 100 NTU level compared to the 10 NTU level.

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

Trial	Reference	NOC-PO4	Absolute Diff	% Error
0	0.0621	0.0624	**0.0002**	**0.4**
10	0.0525	0.0525	**0.0000**	**0.0**
100	0.0520	0.0505	**-0.0015**	**2.8**

Results of the laboratory DOC challenge, performed 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 -0.0006 to 0.0098 mgP/L, with a mean of 0.0015 ±0.0034 mgP/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. A linear regression of the measurement differences versus DOC concentration was barely non-significant ($p=0.056$; $r^2=0.27$). Measurement offset was 0.004 mgP/L more positive at 10 versus 1 mgC/L.

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

Trial	Reference	NOC-PO4	Absolute Diff	% Error
0	0.0621	0.0624	**0.0002**	**0.4**
1	0.0560	0.0555	**-0.0005**	**0.9**
10	0.0762	0.0796	**0.0035**	**4.6**

Lab Temperature Challenge

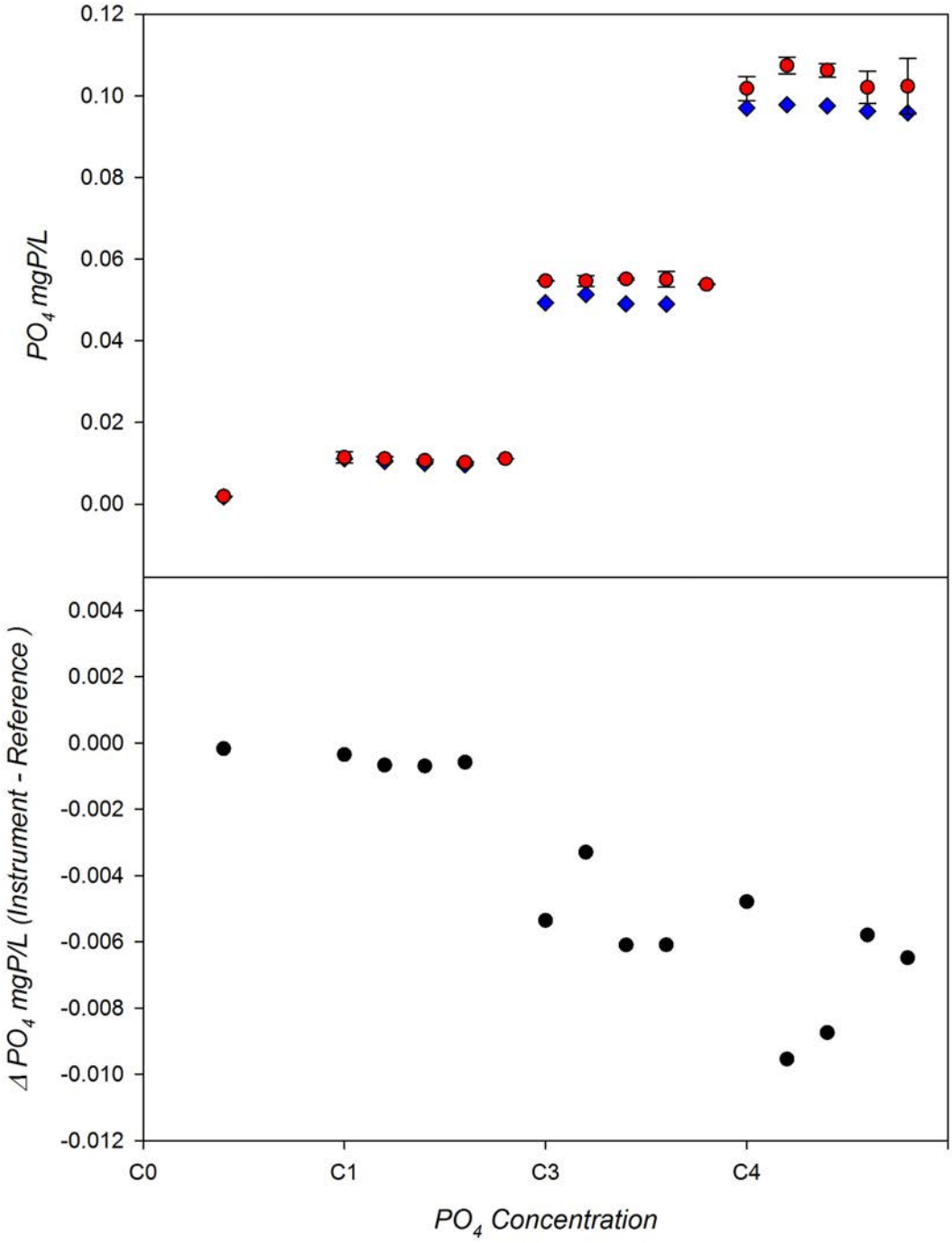


Figure 3. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of PO_4 (mgP/L) in the temperature response challenge covering concentration ranges C2 – C4 measured at 5 ^{o}C test conditions. Five replicate measurements were made at each concentration level along with one measurement at ambient level. *Bottom Panel:* Plot of the absolute difference between NOC-PO4 and reference measurement.

Lab Salinity Challenge

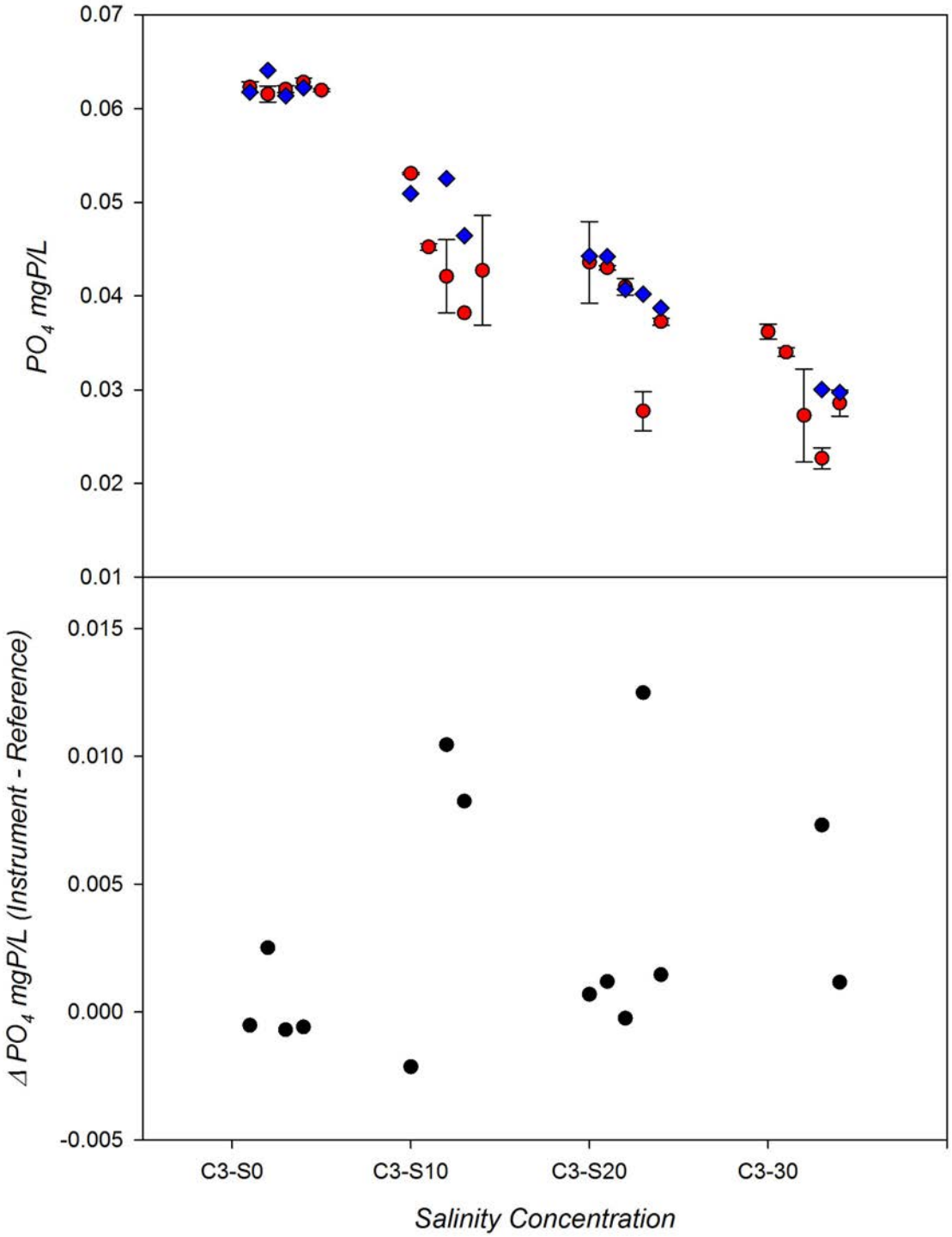


Figure 4. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of PO_4 (mgP/L) at four salinity levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between NOC-PO4 and reference measurement.

Lab Turbidity Challenge

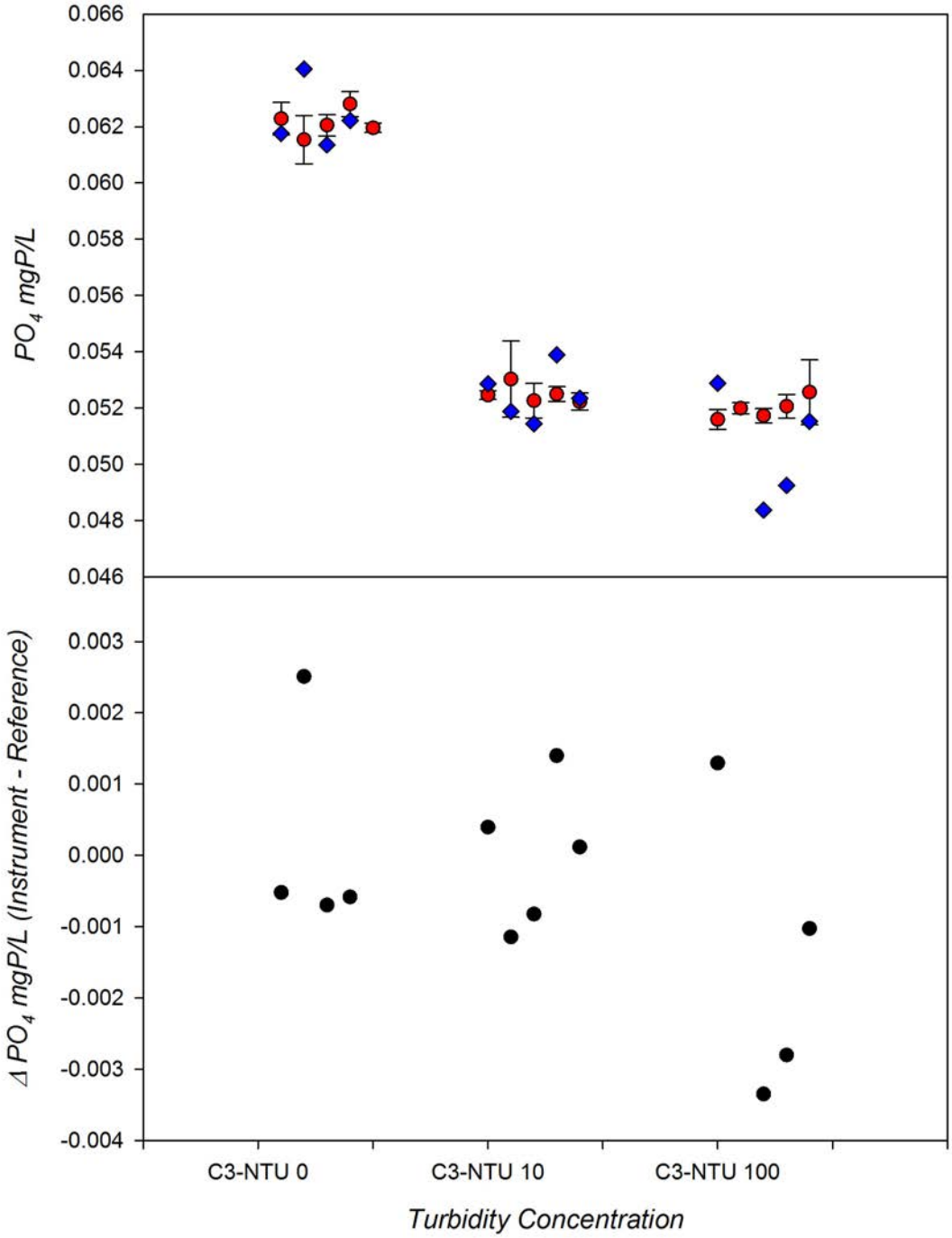


Figure 5. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of PO_4 (mgP/L) at three turbidity levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between NOC-PO4 and reference measurement.

Lab DOC Challenge

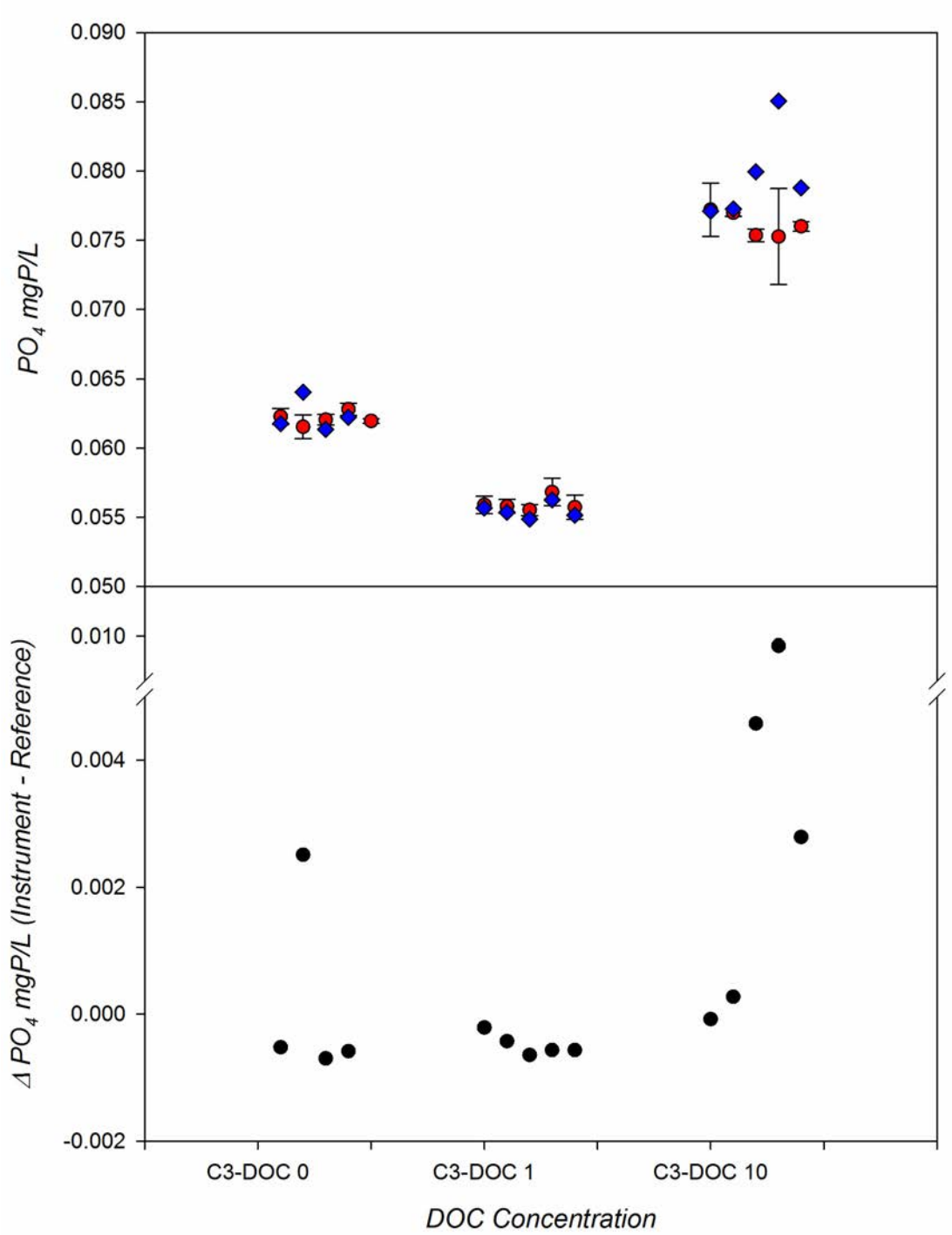


Figure 6. *Top Panel:* Plot of instrument (blue dots) and reference (red dots) measurements of PO_4 (mgP/L) at three DOC levels for the C3 concentration. Five replicate measurements were made at each concentration level. *Bottom Panel:* Plot of the absolute difference between NOC-PO4 and reference measurement.

A summary of measurement differences between the NOC-PO4 and reference sample for each trial of each laboratory challenge is presented in figure 7. It is not known why the measurement offset greatly increased for the C5 concentration range trial, which corresponded to a test level of 0.41 mgP/L. There was a significant temperature effect in measurement accuracy for two of three trials with the NOC-PO4 under-predicting concentrations at 5 ^{o}C compared to 20 ^{o}C. There was noticeable salinity effect during two trials but the effect was not mono-directional and accuracy actually improved during sequential salt additions after a large initial over-prediction. The effects of added turbidity or DOC were less clear, with no real predictable pattern. Results of measurement differences averaged across all trials within each of the challenge matrices are presented in Table 7.

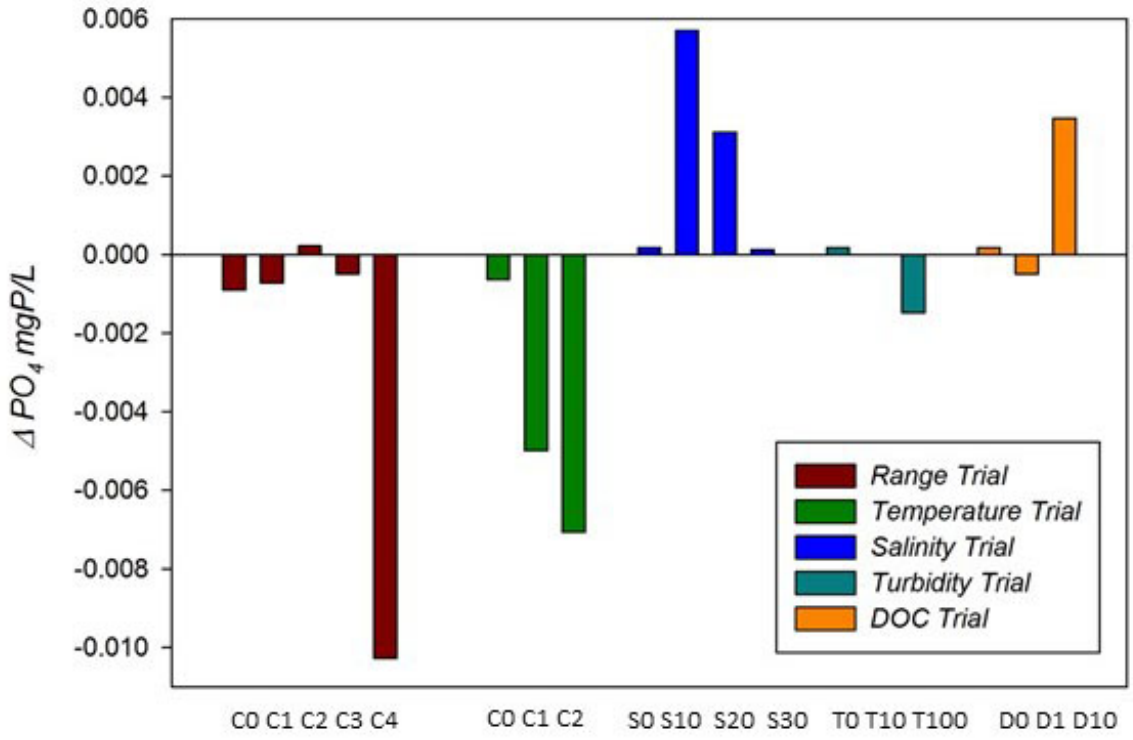


Figure 7. Global summary of difference between instrument and reference measurements for all laboratory tests at each trial conditions for the NOC-PO4 analyzer.

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

NOC-PO4	Range	Temp	Salinity	Turbidity	DOC
min	-0.0103	-0.0071	0.0001	-0.0015	-0.0005
max	0.0002	-0.0006	0.0057	0.0000	0.0035
mean	**-0.0024**	**-0.0042**	**0.0030**	**-0.0007**	**0.0015**
stdev	0.0044	0.0033	0.0028	0.0010	0.0028

RESULTS of FIELD TESTS

Moored field tests were conducted to examine the performance of the NOC-PO4 to consistently track natural changes in PO_4 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-PO4 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-PO4 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-PO4 for each deployment site. (n.d. denotes no data for that observation.) Two on board standards were used for the CBL and HIMB deployments but only a single higher one for the Maumee River.

Deployment Site	**Expected PO_4 mgN/L**	**Pre PO_4 mgN/L**	**Post PO_4 mgN/L**
UM	0.0465	0.0489	0.0482
CBL	0.0031 0.0155	0.0037 0.0165	0.0031 0.0157
HIMB	0.0031 0.0155	0.0041 0.0155	0.0033 n.d.

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^{o}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-PO4 operated successfully during the entire 32 day deployment sampling at hourly intervals, but lost 12 days of data between 5/27 – 6/8 due to a problem writing results to the SD memory card. The NOC-PO4 generated 461 observations out of a possible 763 for a data completion result of 60.4%. During the deployment 289 data points were not reported by the instrument due to the SD memory card problem and 13 data points were flagged by the instrument as bad. Time series results of the NOC-PO4 measurements and corresponding reference PO_4 results are given in figure 10 (top panel). PO_4 measured by the NOC-PO4 ranged from 0.008 to 0.157 mgP/L compared to a range of 0.001 to 0.118 mgP/L within the reference samples.

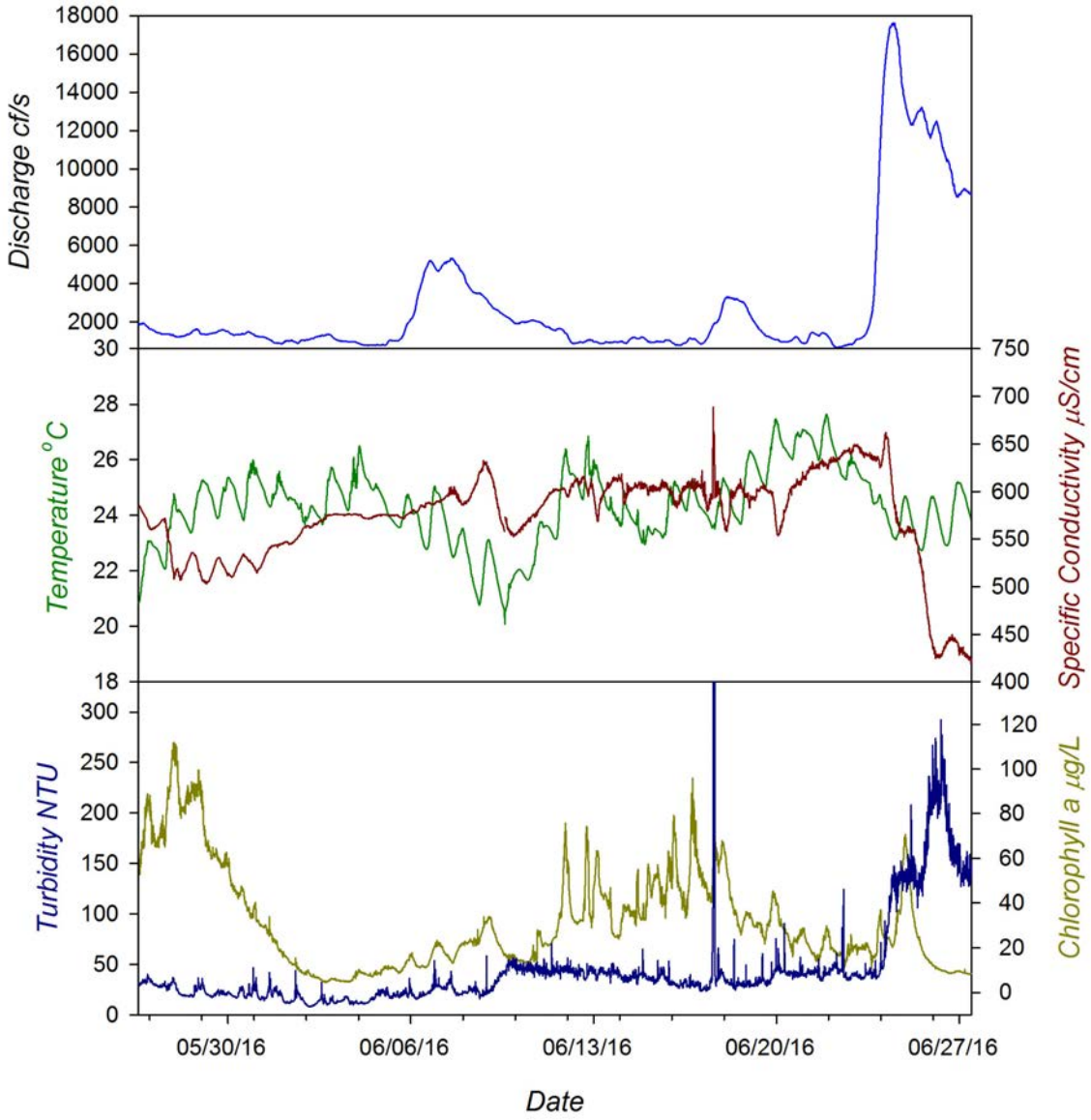


Figure 9. Environmental conditions encountered during the 32 day freshwater deployment in the Maumee River at Waterville, OH. *Top Panel:* Variation in river discharge over the term of the deployment. *Middle Panel:* Variation in temperature (green) and Conductivity (red) at the depth of the sensors, 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 PO_4 measurements for each matched pair (n=28 of a possible 51 observations) is given in the bottom panel of figure 10. 22 of the 51 possible comparisons were lost because of missing instrument data and 1 measurement was flagged as bad. The average and standard deviation of the measurement difference over the total deployment was 0.034 ± 0.024 mgP/L with a total range of -0.033 to 0.079 mgP/L. There was a small but significant trend in measurement difference over time as estimated by linear regression ($p= 0.03$; $r^2=0.17$) with a slope of 0.001 mgP/L/d.

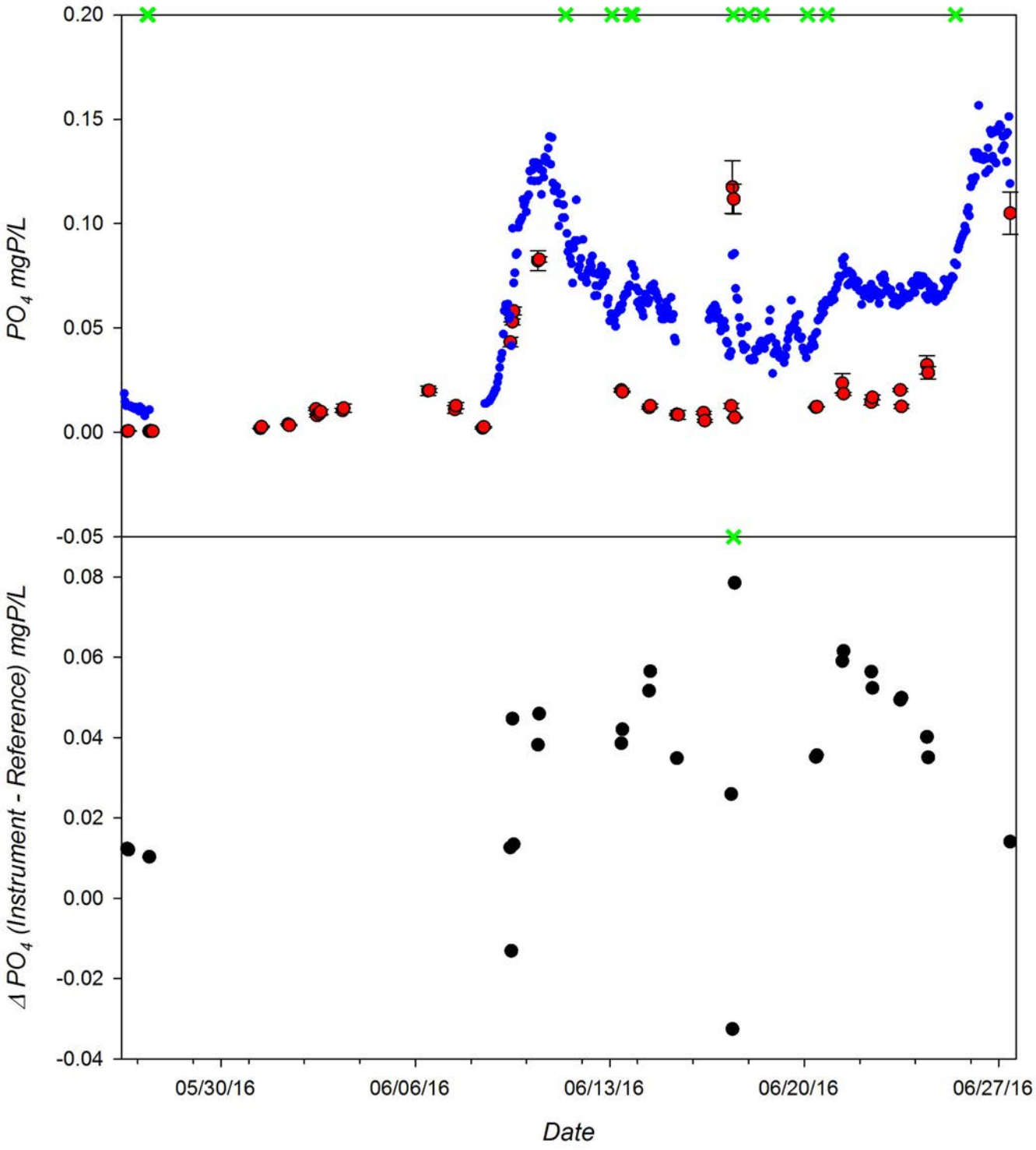


Figure 10. *Top Panel:* Time series plot of the NOC-PO4 measurement (blue dots) and reference measurements (red dots) of phosphate in mgP/L. The green crosses at the top of figure represent flagged data (not values) and are plotted on the date of occurrence. *Bottom Panel:* Time series plot of the difference between the NOC-PO4 and reference measurements of phosphate in mgP/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 ($p<0.0001$; $r^2 = 0.49$) but with a slope of only 0.65 and intercept of 0.045. The NOC-PO4 handled the measurement range equally well, but was generally over-predicting concentrations as noted by the positive intercept of 0.045 mgP/L.

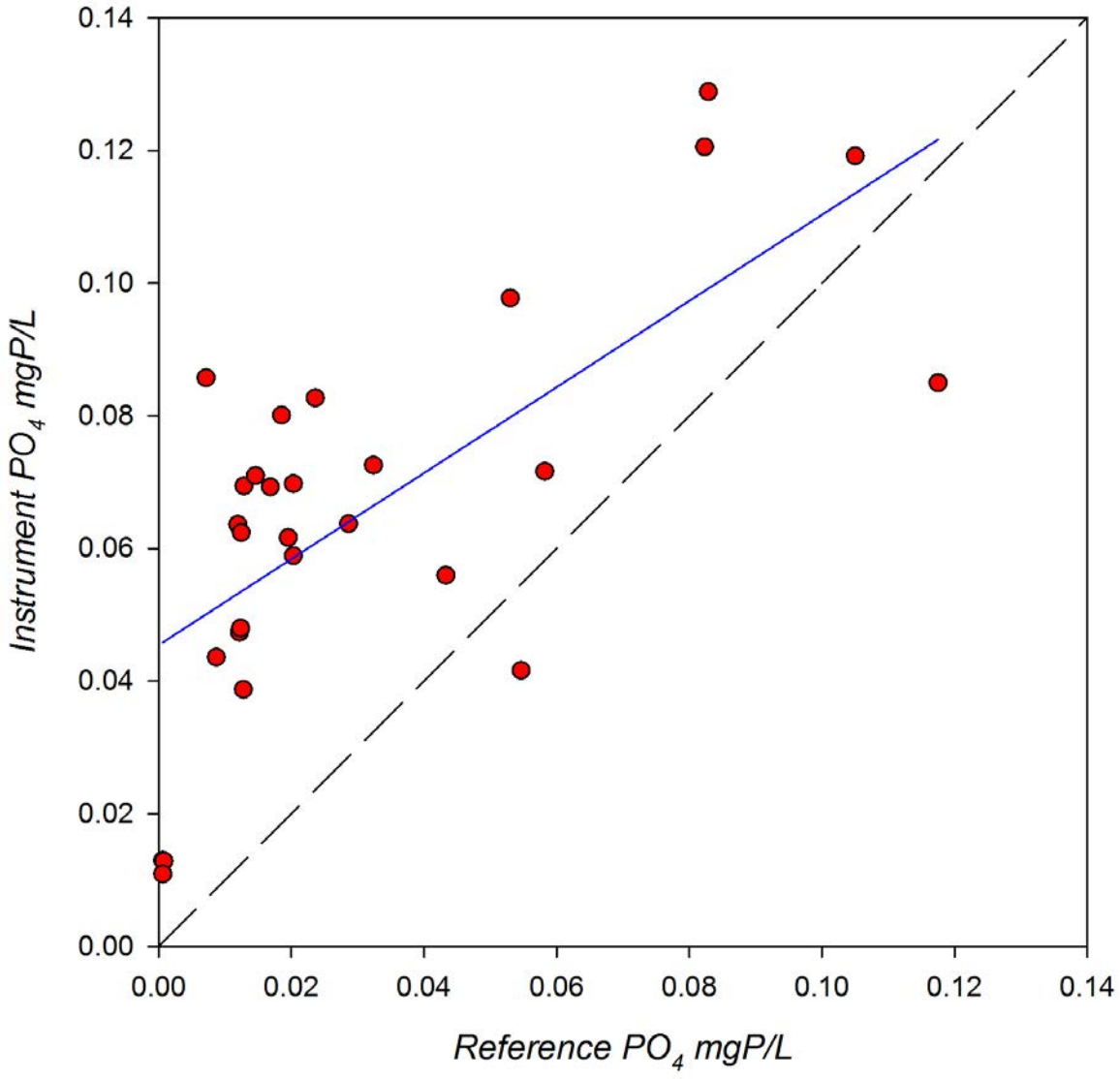


Figure 11. Maumee River field response plot for the 32 day deployment of the NOC-PO4 compared to reference PO_4 samples. The black dashed line represents a 1:1 correspondence, the blue line represents the linear regression.

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

Figure 12. Photographs of the NOC-PO4 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 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 NOC-PO4 operated continuously for the first 8 days of the deployment sampling at hourly intervals but stopped reporting on 7/31 when it appears to have fallen off the mooring when an attachment bolt was corroded away. The instrument was retrieved from the bottom on 8/1 and returned to the manufacturer for repair. A new instrument was re-deployed on 9/16 and operated until the end of the deployment. While the unit was deployed it reported 874 of a possible 883 accepted values for a data completion result of 99%, but this represented only 43% of the total possible record. During its operation, 9 values were flagged by the instrument as bad data. Time series results of the NOC-PO4 and corresponding reference PO_4 results are given in figure 15 (top panel). For the interval deployed, the range of accepted values reported by the NOC-PO4 was 0.002 to 0.041 mgP/L, compared to 0.003 to 0.034 mgP/L for reference samples.

The bottom panel of figure 15 presents the time series of the difference between the NOC-PO4 and reference PO_4 for each matched pair (n=48 comparisons out of a total of 103, (54 missing data points from retrieval/repair period, and 1 data points were flagged). The average and standard deviation of the measurement difference for the deployment was 0.006 ±0.005 mgP/L, with the total range of differences between -0.003 to 0.015 mgP/L. There was a similar range of measurement offset during the two deployment periods; however the sharp rise in predicted values

and offset during the initial 8 days may indicate somethings was malfunctioning within the instrument.

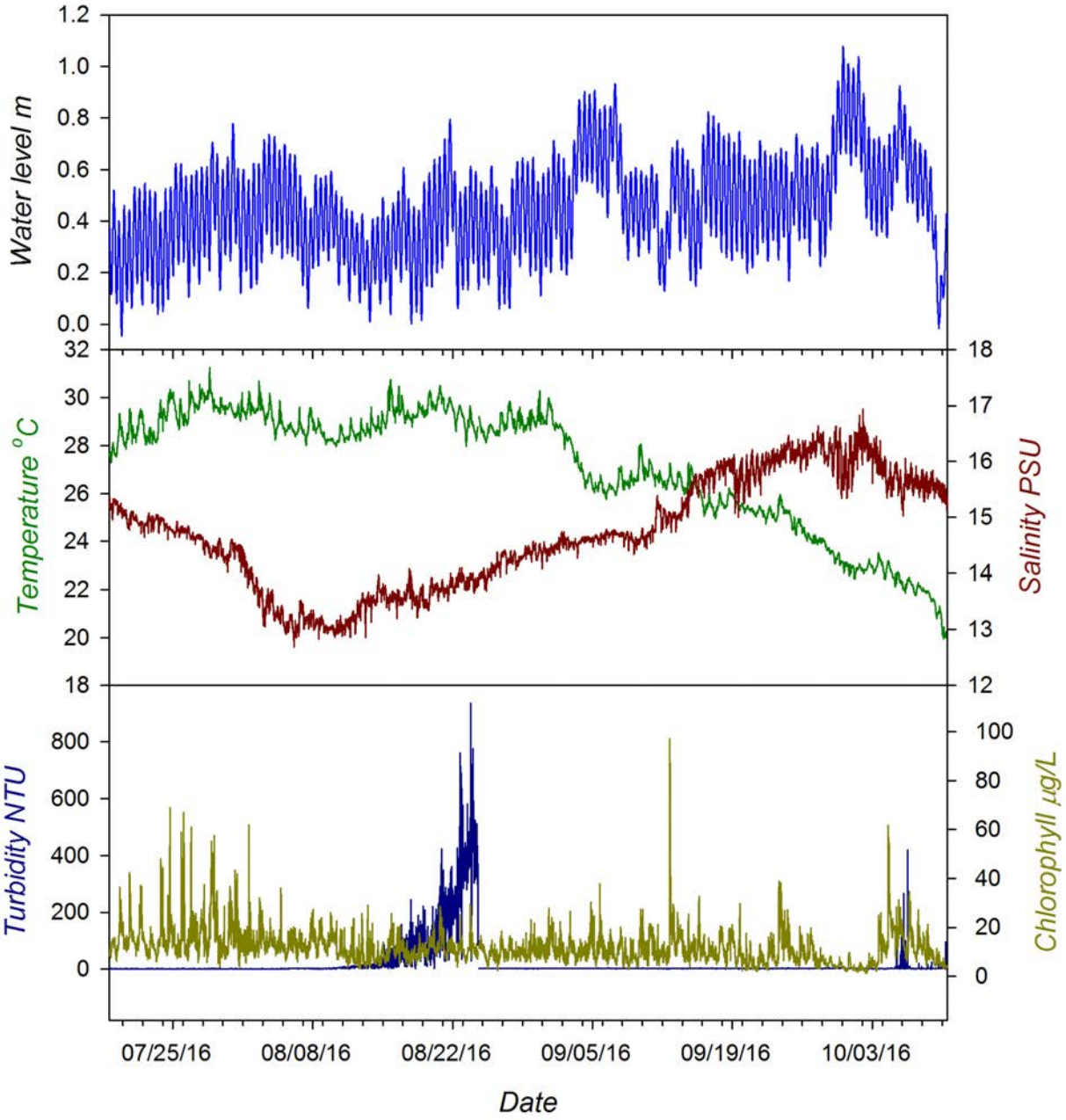


Figure 14. Environmental conditions encountered during the 84 day CBL floating dock deployment. Test sensor array deployed at 1 m fixed depth, variation in local tidal heights indicate active water flow around instrument (*Top Panel*). Variation in temperature (green) and salinity (red) at depth of instrument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

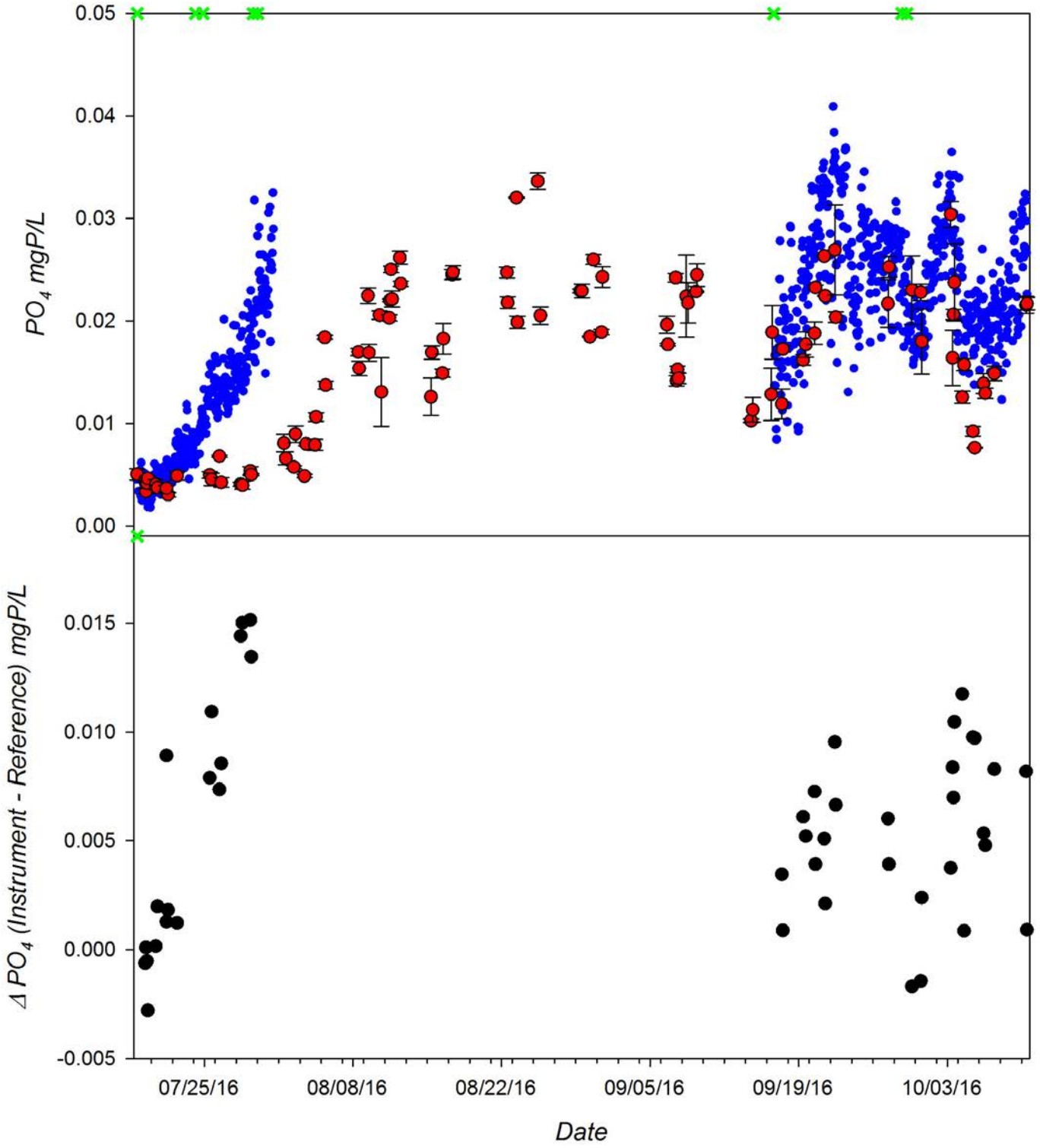


Figure 15. Time series of PO_4 measured by the NOC-PO4 during the 84 day CBL field trial. *Top Panel*: Continuous PO_4 recordings from instrument (blue circles) and PO_4 of adjacent grab samples (red circles). The green crosses at the top of figure represent flagged data (not values) and are plotted on the date of occurrence. *Bottom Panel*: The difference in measured PO_4 relative to reference samples (Instrument mgP/L – Reference mgP/L) observed during deployment.

A cross-plot of the matched observations for the deployment is given in figure 16. A linear regression of NOC-PO4 versus reference measurements was highly significant ($p<0001$; $r^2 = 0.743$), with a slope of 0.933 and intercept of 0.006. NOC-PO4 covered the range equally well but in general over-predicted concentrations.

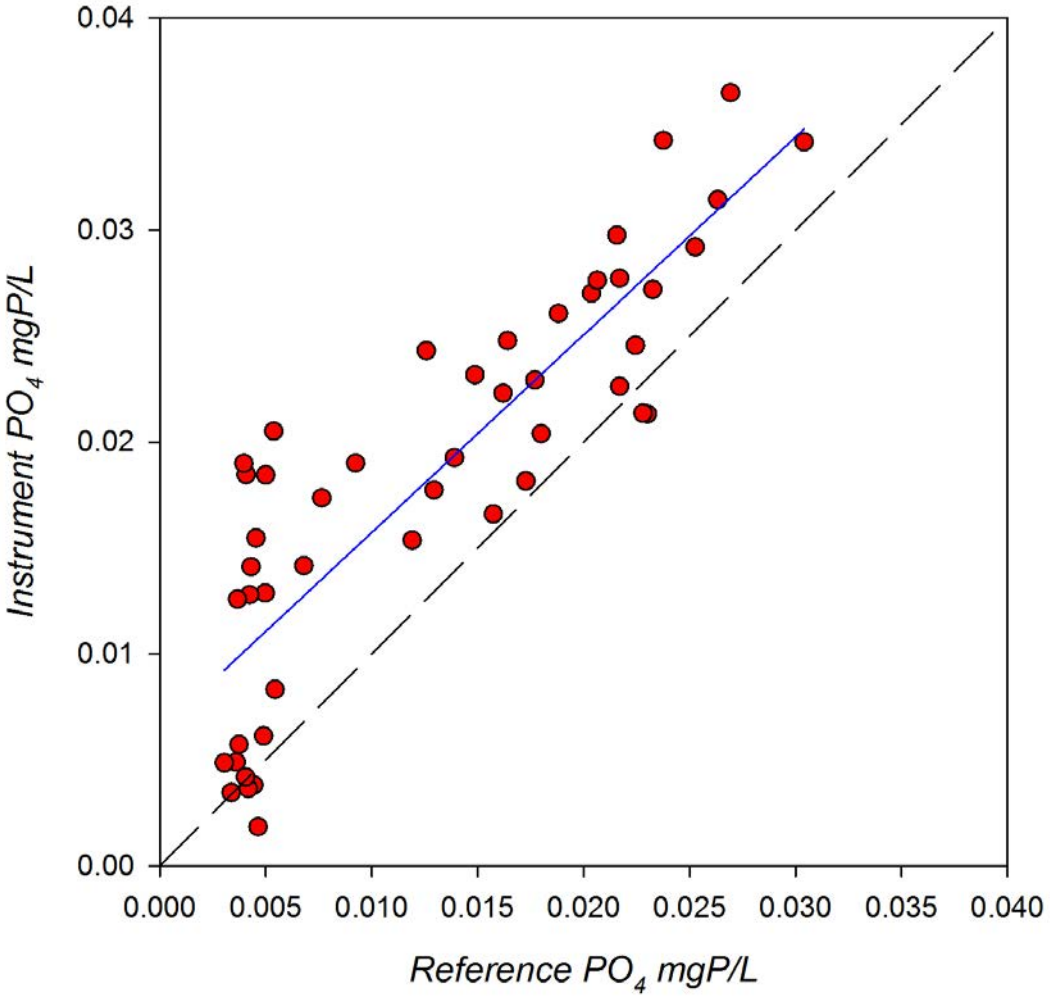


Figure 16. CBL field response plot for NOC-PO4 compared to reference PO_4 samples. The black dashed line represents a 1:1 correspondence, the blue represents the linear regression.

Photographs of the NOC-PO4 before and after the field deployment to indicate potential impact of biofouling (Figure 17).

Figure 17. Photographs of the NOC-PO4 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-PO4 operated successfully for the entire 30 days of the deployment, sampling at hourly intervals. Time series results of the NOC-PO4 and corresponding reference PO_4 results are given in figure 20 (top panel). During the deployment the NOC-PO4 returned 718 instrument measurements of a possible 720 measurements for a data completion result of 99.7%. During the deployment 2 data points were flagged as bad data. The range of values reported by the NOC-PO4 analyzer was 0.002 to 0.005 mgP/L, compared to the range within reference samples of 0.0024 to 0.0061 mgP/L.

The bottom panel of figure 20 presents the time series of the measurement difference between the NOC-PO4 and reference PO4 for each matched pair. The average and standard deviation of the differences between instrument and reference readings (n=73 out of a possible 73) was -0.0014 ± 0.0009 mgP/L, with a total range in the differences of -0.0034 to -0.0001 mgP/L. There was a small but statistically significant trend in the measurement difference over time ($p=0.0001$; $r^2 = 0.233$) during the deployment, with a slope of -0.00004 mgP/L/d.

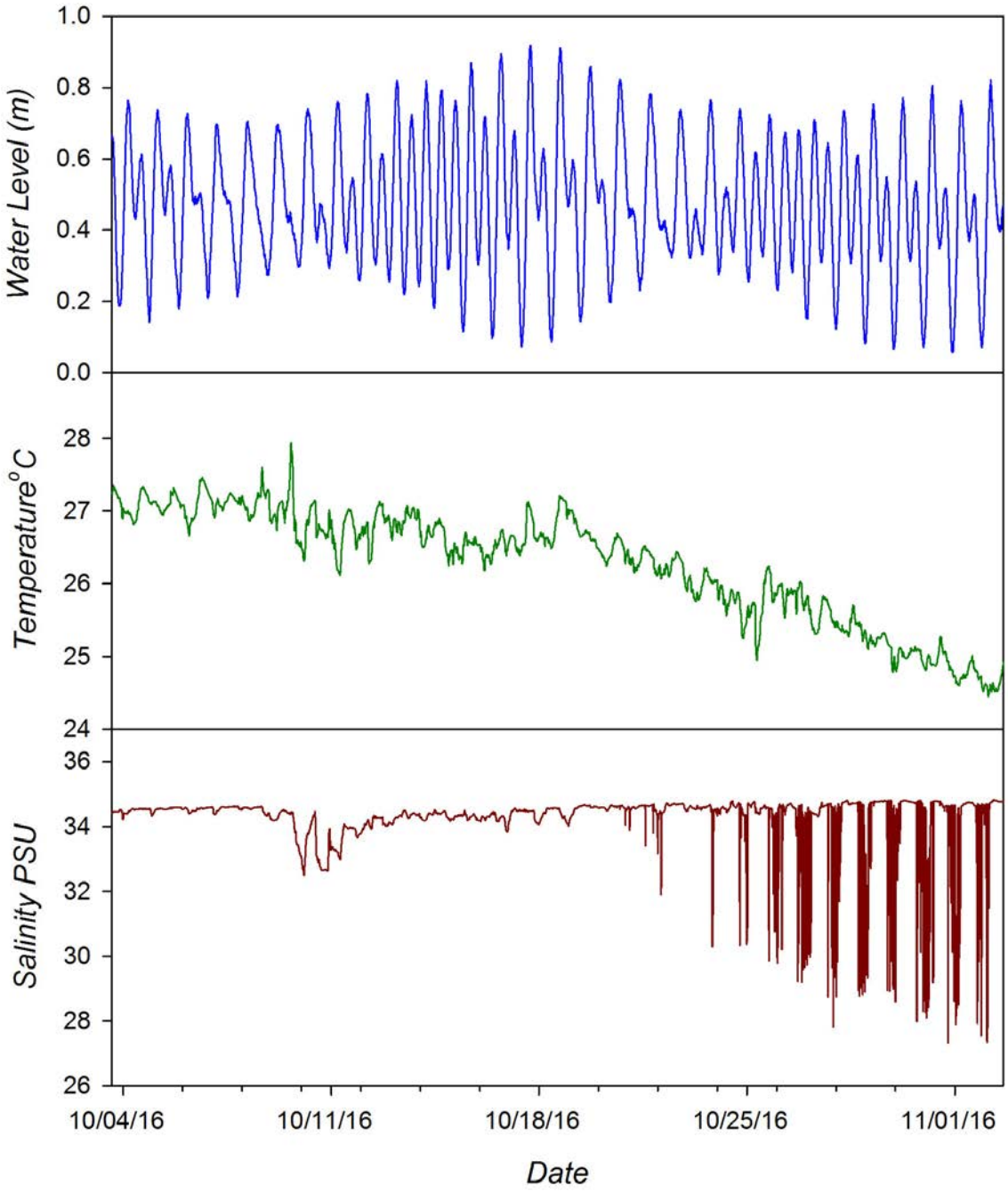


Figure 19. Environmental conditions encountered during the one month HIMB deployment on a floating dock off Coconut Island Test sensor array deployed at 1 m fixed depth, variation in local tidal heights indicate active water flow around instrument (*Top Panel*). Variation in temperature (green) and Salinity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

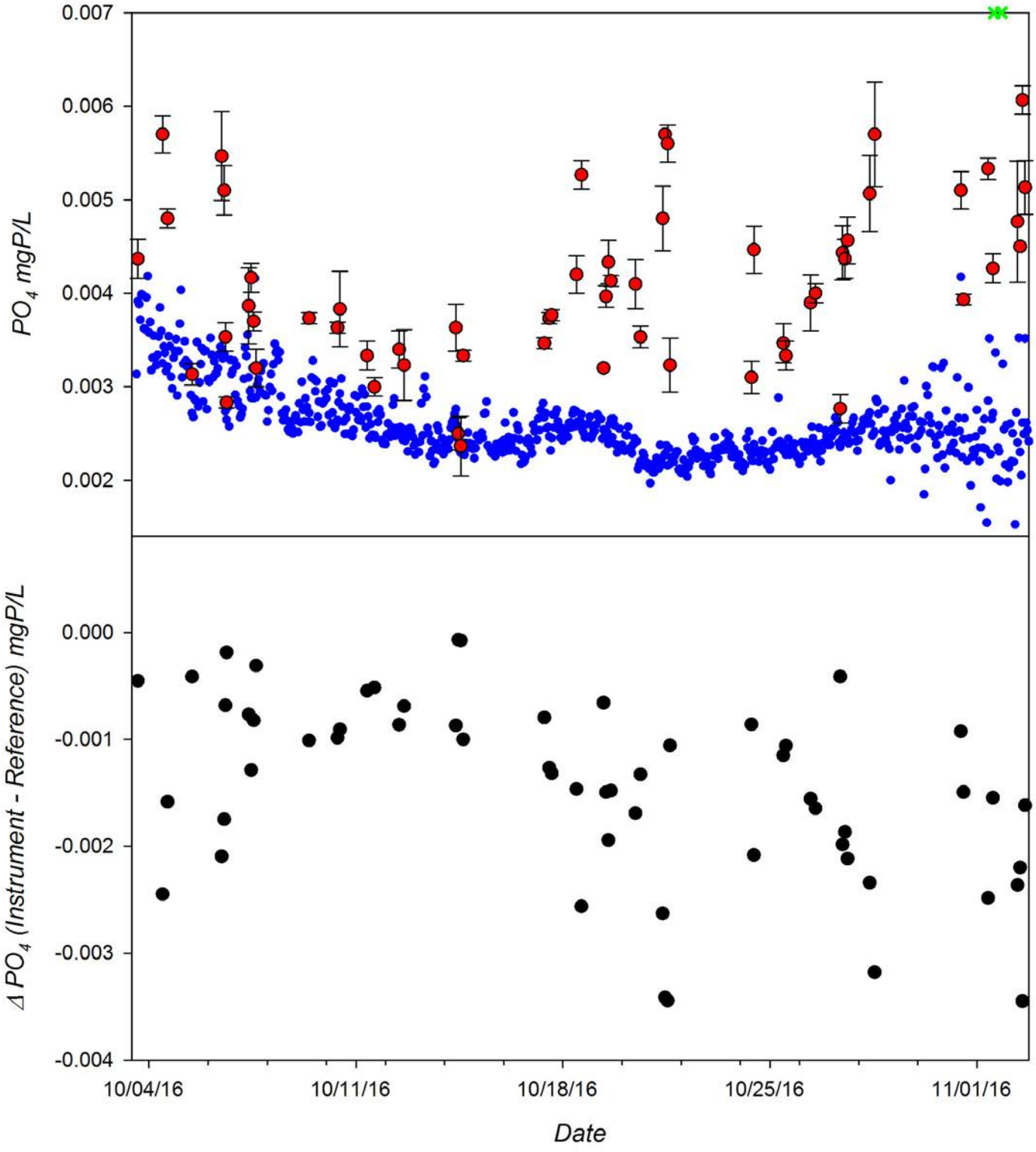


Figure 20. *Top panel:* Time series of PO_4 measured by the NOC-PO4 deployed during the one month HIMB field trial. Continuous PO_4 recordings from instrument (blue dots) and PO_4 of adjacent grab samples (red circles.) *Bottom Panel:* Time series of the difference between the NOC-PO4 and reference measurements for each matched pair (Instrument mgP/L – Reference mgP/L).

A cross-plot of the matched observations for the deployment is given in figure 21. A linear regression of instrument versus reference measurements was significant ($p=0.014$; $r^2 = 0.103$), but with a slope of only 0.149 and intercept of 0.002. The NOC-PO4 under-predicted throughout the measurement range and was marginally responsive to concentrations above 0.004 mgP/L.

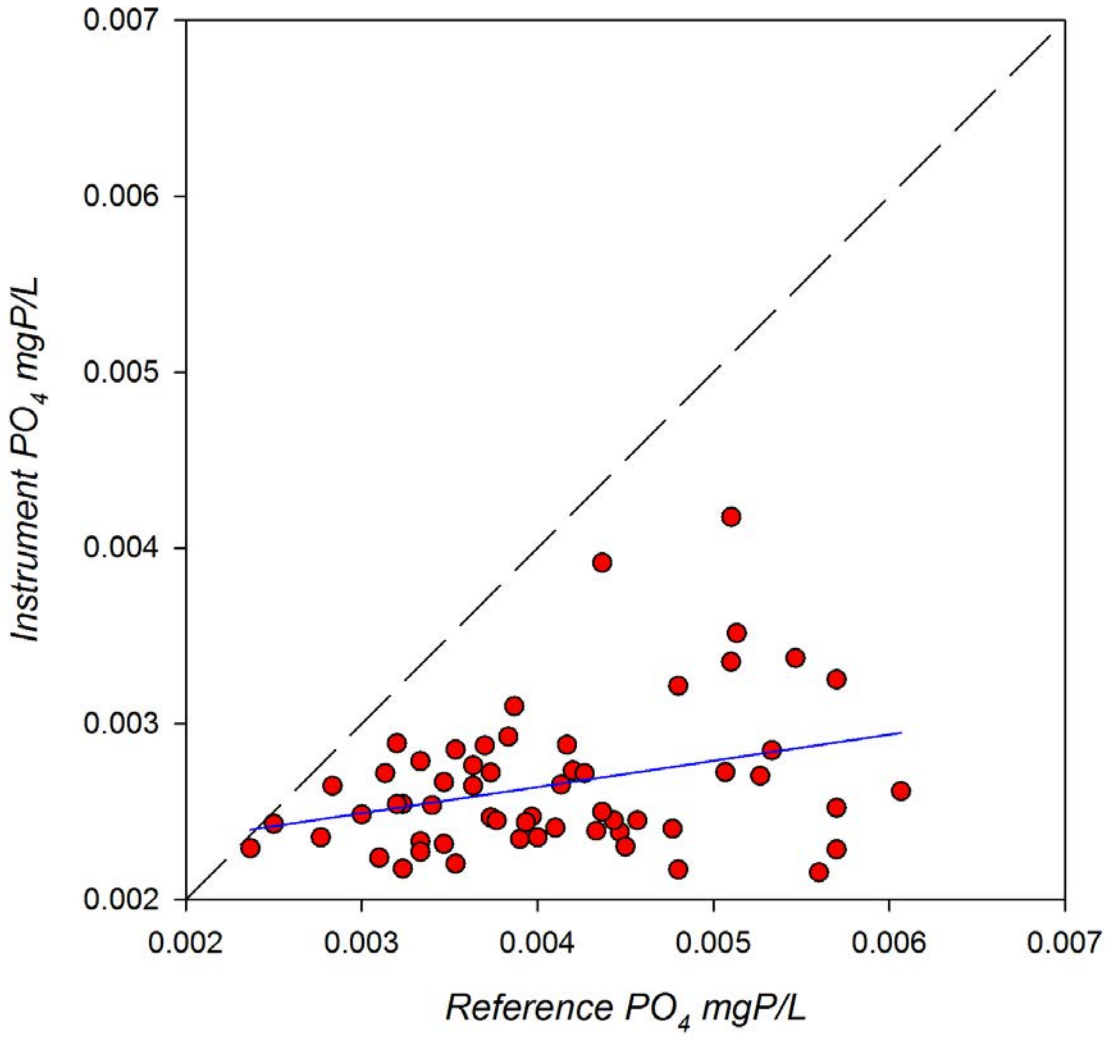


Figure 21. HIMB field response plot of NOC-PO4 compared to reference PO_4 samples. The plotted line represents a 1:1 correspondence, the blue line represents the linear regression.

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-PO4 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. The NOC-PO4 had a different specific response for each of the field tests (see above), but in general showed a good linearity with reference measurements over the entire range tested. A linear regression of all the data was highly significant ($p<0.0001$; $r^2 = 0.62$) with a slope of 1.196 and intercept of 0.006. The data comparison covered a field concentration range of 0.001 to 0.118 mgP/L.

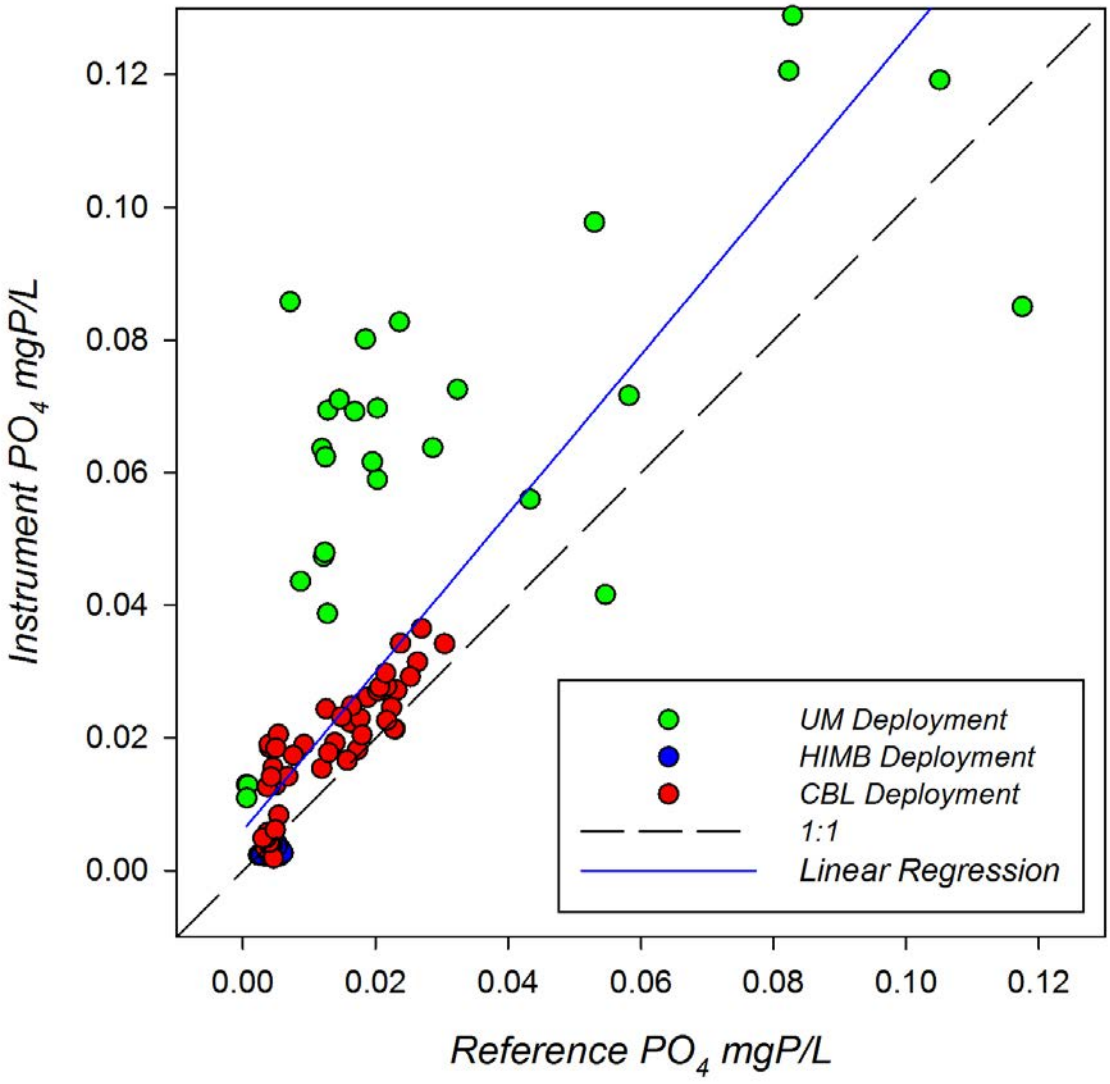


Figure 23. Global response plot for the NOC-PO4 observed during the three ACT field trials. Insert shows the CBL and HIMB deployments enlarged. Black dotted line represents a 1:1 correspondence, the blue line represents the linear regression.

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 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	PO_4	Mean	Std Dev	ABS Diff	CV%
6-16-16 9:00	FD1	0.0094	0.008	0.0016	0.002	19.2
	FD2	0.0072				
6-17-16 12:00	FD1	0.0071	0.008	0.0005	0.001	7.11
	FD2	0.0079				
6-20-16 10:00	FD1	0.0122	0.012	0.0003	0.0005	2.59
	FD2	0.0117				
6-23-16 11:00	FD1	0.0203	0.020	0.0001	0.0001	0.489
	FD2	0.0202				

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

Date/Time	Rep	PO_4	Mean	Std Dev	ABS Diff	CV%
7-20-16 10:00	FD1	0.0040	0.0039	0.0002	0.0003	6.1
	FD2	0.0037				
7-26-16 14:00	FD1	0.0042	0.0039	0.0005	0.0007	12.09
	FD2	0.0036				
8-2-16 10:00	FD1	0.0057	0.0057	0.0000	0.0001	0.827
	FD2	0.0057				
8-10-16 16:00	FD1	0.0131	0.0148	0.0024	0.0034	16.14
	FD2	0.0164				

8-23-16 12:00	FD1	0.0199	0.0197	0.0003	0.0004	1.56
	FD2	0.0194				
9-8-16 10:00	FD1	0.0224	0.0249	0.0035	0.0050	14.11
	FD2	0.0274				
9-16-16 12:00	FD1	0.0189	0.0195	0.0008	0.0011	4.00
	FD2	0.0200				
10-4-16 14:00	FD1	0.0157	0.0144	0.0019	0.0027	13.27
	FD2	0.0130				
10-10-16 10:00	FD1	0.0216	0.0221	0.0007	0.0010	3.10
	FD2	0.0225				

Table 13. 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	0.0035	0.0036	0.000	0.0001	.664
	FD2	0.0036				
10-12-16 11:00	FD1	0.0034	0.0033	0.0002	0.0003	5.77
	FD2	0.0031				
10-17-16 9:00	FD1	0.0035	0.0034	0.0001	0.0001	2.07
	FD2	0.0034				
10-26-16 9:00	FD1	0.0039	0.0040	0.0002	0.0003	4.68
	FD2	0.0042				
11-1-16 9:00	FD1	0.0053	0.0053	0.0001	0.0002	2.25
	FD2	0.0052				

Table 14. Results of Field Trip Blanks all deployments.

Maumee River		**Chesapeake Bay**		**Kaneohe Bay**	
Field Blank ID	PO_4 (Std Dev)	Field Blank ID	PO_4 (Std Dev)	Field Blank ID	PO_4 (Std Dev)
GLFB1	0.0008 (0.0001)	CBLFB1	0.0027 (0.0001)	HIFB1	0.0017 (0.0000)
GLFB2	0.0012 (0.0003)	CBLFB2	0.0026 (0.0001)	HIFB2	0.0016 (0.0002)
GLFB3	0.0021 (0.0001)	CBLFB3	0.0014 (0.0001)	HIFB3	0.0013 (0.0002)
GLFB4	0.0027 (0.0004)	CBLFB4	0.0011 (0.0003)	HIFB4	0.0013 (0.0002)
--	--	--	--	HIFB5	0.0010 (0.0001)
Mean (Std Dev)	0.0017 (0.001)	Mean (Std Dev)	0.002 (0.001)	Mean (Std Dev)	0.001 (0.0003)
Grand Mean (Std Dev)					0.002 (0.0007)

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.

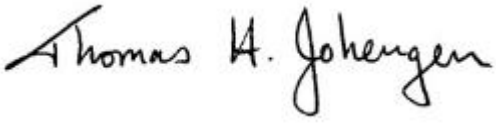
June 1 , 2017

Date

Approved By: Dr. Mario Tamburri
ACT Executive Director

June 1 , 2017

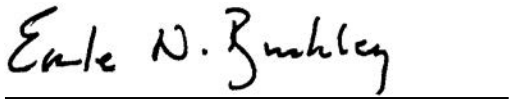
Date



Approved By: Dr. Tom Johengen
ACT Chief Scientist

June 1 , 2017

Date



Approved By: Dr. Earle Buckley
Quality Assurance Supervisor

National Oceanography Centre
NATURAL ENVIRONMENT RESEARCH COUNCIL

National Oceanography Centre
European Way
Southampton
SO14 3ZH
United Kingdom

Tel: +44 (0)23 8059 666

Dear Dr. Johengen,

Re: NOC Response to the ACT Nutrient Sensor Challenge NOC-PO4 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-PO4 phosphate 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 extreme in terms of biofouling (CBL), nutrient concentrations (high at Maumee River and low at HIMB) and sediment load (Maumee River). The challenge therefore not only highlighted 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 accurate real-time processed values.

The Challenge was the first time that we operated the NOC-PO4 sensor in the field with a newly designed electronics package, Graphical User Interface, and longer path-length absorbance cells. We also modified some of our standard operating protocols throughout the challenge in order to achieve the targets set forth in the Challenge. As such, given the novelty of some of the sensor features and our willingness to modify our proven standard operating protocols, some issues were to be expected.

We are therefore pleased with several aspects of the performance of the instrument (particularly its performance during the lab tests, the second part of the CBL deployment and the re-processed HIMB data – see below). In addition, the developments that we made during and since the Challenge mean that we are satisfied that the instrument will display improved performance and robustness when deployed again in these (or similar) scenarios. Below we highlight some of the challenges that we encountered, and discuss how we have addressed them both during and since the challenge.

LABORATORY TESTS. Generally we were satisfied with the performance of the sensor during the laboratory test. However, for optimal performance, it is best to operate the NOC-PO4 sensor with onboard standards that bracket the range of concentrations and best match the salinity of the deployed environment. Such fine tuning was not possible for the Challenge laboratory tests given the extreme range of phosphate concentrations and matrix conditions tested. To match the Challenge 30 min sampling intervals, we had to operate the sensor with only one standard (prepared in deionized water) and halve the color development time. Under such operating circumstances, we had anticipated that the performance of the sensor at the most extreme conditions (for e.g., C0 and C6) would not have been optimal.

During the laboratory tests we optimized the NOC-PO4 sensor for deployment in natural waters with phosphate concentrations up to ~ 0.31 mgP/L. The under-measurement of the extremely high C6

concentration (>1.5 mgP/L, Figure 1, and not included in the actual evaluation) was likely due to the limited range of the currently used reagent formulation. The slightly under-predicted values reported by the NOC-PO4 sensor at low temperatures is due to the fact that we had to halve the usual color development time to match the 30 min sampling interval requirement of the Challenge. At lower temperatures, a longer measurement interval would allow superior performance.

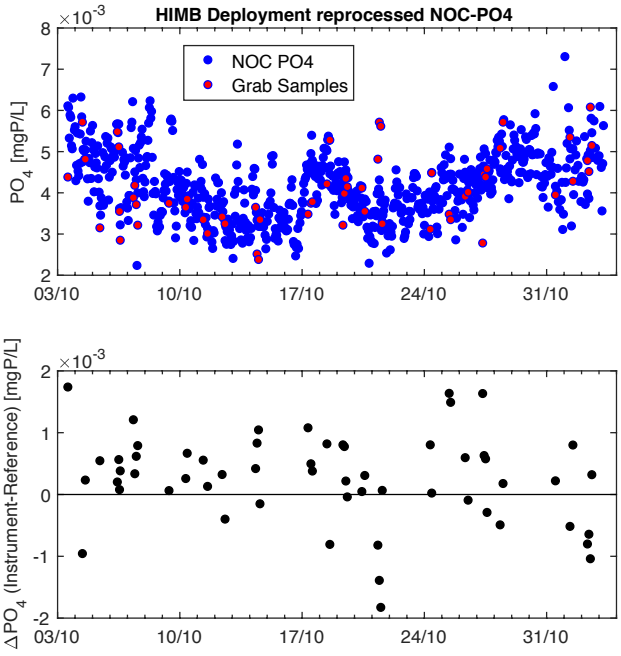
MAUMEE RIVER. Unfortunately some gaps exists in the Maumee River dataset. Prior to the Nutrient Sensor Challenge, we introduced a new electronics package and software interface in order to increase user-friendliness (during setup and data download), and allow the instrument to calculate final concentration values on-board (rather than relying on post-processing). Unfortunately, an obscure firmware bug (which eluded us during testing) 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 (the first NSC deployment) when there was a power glitch midway through th deployment, meaning that we lost a section of data from this deployment. Not long after this issue was identified, our software team was able to identify the bug and fix it, meaning that the issue did not re-occur during subsequent NSC deployments.

In addition, for this deployment we did not preserve the onboard calibration standard using our now established method. We think that this may have contributed to an observed offset in the later part of the deployment. Note that we have operated the NOC-PO4 sensor in UK rivers using our standard operating protocol and have obtained excellent agreement with discrete data. The interested reader is referred to our published works for more details.

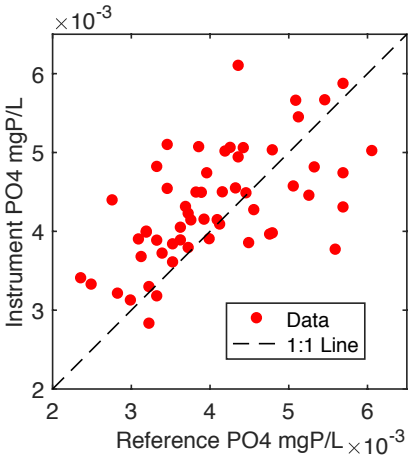
CHESAPEAKE BIOLOGICAL LABORATORY (CBL). The sensor came detached from its mount partway through the deployment. This event was unrelated to the design or performance of the sensor, but it did result in damage to the sensor that could not be repaired in the field. The sensor was therefore sent back to NOC for repairs. While the sensor was being repaired at NOC, the sensor reagents and standards were stored at CBL, and were later redeployed along with the sensor by ACT staff. The storage of the reagents and standards did not impair the performance of the sensor upon redeployment. After this re-deployment, the sensor operated well and showed good agreement with reference samples.

Prior to the sensor becoming damaged, there appears to be an offset between the sensor and discrete data from July 22 to July 31. The fact that this offset occurred prior to the breaking of the bolt that secured the sensor to the ACT mounting rack is simply a coincidence. Since the onboard standards showed stable readings during the first 8 days of deployment, the most plausible explanation is that the sample inlet line became compromised.

HIMB. Since submitting the data from the HIMB deployment to ACT, we have re-evaluated how the onboard data processing is performed by the NOC-PO4 sensor. A closer inspection of the sensor raw data revealed some drift in the intensity of the LED light sources used for the spectrophotometric measurements. This drift can be automatically corrected for *in situ* using the monitoring photodiodes (one of the new sensor features implemented for the Nutrient Challenge). However, the importance of this correction scheme in tropical environments was unknown when the data was submitted to the ACT. The figure to the right shows the updated NOC-PO4 data from the HIMB deployment with the LED intensity correction along with the difference between the paired NOC-PO4 and reference values. The NOC-PO4



sensor data generally mirrored the discrete data. The updated range of values reported by the NOC-PO4 analyzer was 0.0022 to 0.0073 mg P/L. The mean and standard deviation of the differences between the updated NOC-PO4 and reference readings (n=57 out of a possible 73) were 0.0024 ± 0.0073 mg P/L. An updated regression plot of the matched HIMB observations is shown below.



The Nutrient Sensor Challenge was a challenging proving ground for the NOC-PO4 sensor prototype 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-PO4 performance against high-quality and high-resolution reference data in a variety of natural waters. These reference data are extremely beneficial as we continually seek to improve the Lab-On-Chip (LOC) technology for long-term *in situ* monitoring. We are indebted to the ACT staff for giving us the opportunity 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