# PERFORMANCE DEMONSTRATION STATEMENT
# *WET Labs Cycle-P Nutrient Analyzer*

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

**NOTICE:**

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

This document has been peer reviewed by ACT Partner Institutions and a technology-specific advisory committee and was recommended for public release. Mention of trade names or commercial products does not constitute endorsement or recommendation by ACT for use.

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## EXECUTIVE SUMMARY:

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

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

During this Performance Demonstration the WET Labs Cycle-P was successfully tested in a fixed, surface mooring deployment at three test sites including freshwater, brackish water, and saltwater coastal environments. With the exception of an initial instrument exchange on day 2 of the first field test, the test instrument functioned continuously for all 4 weeks of each test. Data recovery was 84, 79, and 99% of the total potential for MD, MI, and AK test sites respectively. Several logistical problems prevented complete execution of all of the quality control testing, but based on the completed blank and standard test exposures the instrument appeared capable of accurately measuring a wide range of phosphate concentrations and functioning consistently over a four week interval. There was no apparent degradation of the on-board reagents or standards over the deployment period.

Extremely low phosphate concentrations at the MD test site did not allow for meaningful data analysis and there appeared to be a significant impact from biofouling at this site after several weeks. At the AK test site direct comparisons of instrument to reference sample data were quite variable, ranging from 0.2 to 4.1 but with an overall mean of 1.5. The offset and variability seemed to improve over time with much better accuracy during the last two weeks of the deployment. Part of the variability may have been due to a mismatch in sample timing intervals and resulting averaging of timepoints. At the Michigan test site, the in situ measured concentrations were much more consistent and accurate. The instrument to reference sample ratio ranged from 0.3 – 1.9 with an overall mean of 1.04.

Because of the complexity of the tests conducted and the number of variables examined, a concise summary is not possible. We encourage readers to review the entire document for a comprehensive understanding of instrument performance and to discuss results with the instrument manufacturer. The application of any post-deployment corrections, and manufacturer's interpretation of the test results are presented in Appendix 1. In general, however, it appears that the fundamental technology has the capability to successfully measure phosphate in situ under a variety of field conditions.

## BACKGROUND:

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

A key to the successful adoption and transition to operational use of new technologies is broad community awareness and confidence. To this end, the NOAA-funded Alliance for Coastal Technologies (ACT) serves as an unbiased, third party testbed for evaluating sensors and sensor platforms for use in coastal environments. ACT also serves as a comprehensive data and information clearinghouse on coastal technologies and a forum for capacity building through workshops on specific technology topics (visit www.act-us.info).

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

## TECHNOLOGY TYPE:

The WET Lab’s Cycle-P measures in-situ dissolved phosphate concentration in natural waters using standard wet chemistry. The Cycle-P combines precision micro-fluidics with state of the art optics to provide a high level of precision and accuracy in operational in-situ nutrient monitoring. Designed for unattended long term moored operations, the Cycle-P features on-board calibration against a standard solution to assure quality data over the entire deployment period. While specifications have not been finalized, laboratory and field testing results have measurement resolution in the nanomolar range. WET Labs is currently conducting extensive field testing of the Cycle-P and has beta units in the field with independent researchers.

## OBJECTIVES OF THE NUTRIENT ANALYZER PERFORMANCE DEMONSTRATION:

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

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

## PARAMETERS INVESTIGATED:

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

- **Accuracy** – a measure of the closeness of an estimated value to the true value (see below). For this demonstration, the accuracy of the test instruments was determined in field tests by comparing the difference between the in situ instrument's determined nutrient concentrations and laboratory measured concentrations of collected reference water samples using approved analytical methods. Laboratory analyses followed approved standard operating procedures and were checked against external certified reference standards to ensure they represented the best possible measure of the nutrient concentration. All laboratory analyses were run in triplicate to assess the precision of these reference measurements.
- **Reliability** – the ability to maintain integrity or stability of the instrument and data collections over time. Reliability of instruments was determined in two ways. In field tests, comparisons were made of the percent of data recovered versus percent of data expected. In addition, instrument stability was determined by pre and post measurement of blanks and reference standards to quantify drift during deployment periods. Comments on the physical condition of the instruments (e.g., physical damage, flooding, corrosion, battery failure, etc.) were also recorded.

## SUMMARY OF DEMONSTRATION PROTOCOLS:

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

### Laboratory Based Nutrient Analysis

All nutrient concentrations for lab and field samples were determined by the Nutrient Analytical Services Laboratory (NASL) at the Chesapeake Biological Laboratory following their Standard Operating Procedures Manual (CEES, UMD, Publication Series No. SS-80-04-CBL). Phosphate concentrations were analyzed using the standard 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). All laboratory nutrient analyses were conducted on an Aquakem 250. A statistically determined method of detection limit has been established at 0.023 $\mu$M $PO_4$-P by prior laboratory studies. The typical working concentration range for the method and SOP is between 0.113 – 47.8 $\mu$M $PO_4$-P. A sample reagent blank was analyzed in conjunction with every sample and all internal standards were verified and calibrated using certified external nutrient standards. Additional internal QAQC samples including laboratory duplicates and nutrient recovery spikes were analyzed with each analytical batch.

### Moored Deployment

Field demonstration tests of instrument performance in a moored application were conducted at three ACT Partner Institution sites including Chesapeake Bay, Solomons, MD; Resurrection Bay, Seward, AK; and Clinton River, Mt. Clemens, MI. The same model instrument was tested at all three sites. At each test site the instrument was deployed at a fixed depth of 1 m over four weeks. Prior to deployment, the instrument was set up and calibrated as required at the field sites by a manufacturer representative. The manufacturer was allowed to select a sampling interval of up to a maximum of 2 hours based on instrument settings needed to allow continued operation over a 30 day deployment. The WET Labs Cycle-P was set to operate at either a 1.5 or 2 h interval for these tests. The Cycle-P was provided with a laboratory blank (type 1 deionized water, DIW) and reference standard (ca. 1.0 $\mu$M) both before and after deployment in order to estimate any drift in response over time. A photograph of each individual instrument and instrument rack was taken before and after deployment to provide a qualitative estimate of biofouling during the field test. Finally, a sub-sample of the on-board standard solution was collected immediately before and after deployment for independent analysis by NASL to help account for any possible accuracy offset and degradation of the standard over time.

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

### Ancillary Environmental Data

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

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

## Quality Assurance / Quality Control

The ACT Nutrient Demonstration was implemented according to the test protocols and technical documents (e.g. Standard Operating Procedures) prepared during the planning stages of the test. Prescribed procedures and a sequence for the work were defined and all work performed during the Demonstration followed those procedures and sequence. All implementation activities were documented and are traceable to the test/QA plan, SOPs and to test personnel.

Four levels of QAQC were applied to the sampling and analytical procedures for each field test. First, ACT provided the companies with a laboratory blank (type 1 deionized water, DIW) and reference standard (ca. 1.0 μM) both before and after the field test deployment. All concentrations were confirmed by analysis at NASL. Secondly, ACT sub-sampled an aliquot of the on board standard that was present in the nutrient analyzer at the beginning and end of the test to verify that it matched with its stated value and to assess whether there was any degradation during the deployment. Thirdly, field trip blanks were collected once a week during mooring tests to test for any measurable contamination resulting from sampling and analytical protocols. Field trip blanks consisted of carrying DIW through all of the collection, processing, storage and analysis steps. Lastly nutrient spikes of field reference samples were performed once a week during mooring tests. Spikes were created by adding a known amount of certified standard to a known volume of filtrate of an existing field reference sample.

## DEMONSTRATION RESULTS:

The Cycle-P was successfully tested at fixed locations and a depth of 1m for 4 week durations at three different field sites, Chesapeake Bay, Solomons, MD; Resurrection Bay, Seward, AK; and Clinton River, Mt. Clemens, MI (Table 1). Results are reported by individual site and summarize the complete time series of nutrient concentrations predicted by the Cycle-P during the deployment as well as direct comparisons of the reference sample concentrations determined by NASL. In addition we report the results for blanks and reference standards provided immediately before and after the deployment.

**Table 1.** Physical/chemical conditions observed during the moored deployment field tests for the WET Lab's Cycle-P nutrient analyzer at three ACT sites. Temperature and Salinity (or Conductivity for the MI test) were determined by a CTD, relative fluorescence was measured with a SeaPoint fluorometer and transmissivity was measured with a 25cm path length transmissometer.

| SITES | | Temp. (ºC) | Salinity/ Conductivity (μS/cm) | Fluorescence (mV) | % Transmission |
|---|---|---|---|---|---|
| **Chesapeake Bay, MD (5/16/07 - 6/12/07)** | Min | 17.0 | 9.8 | 31 | 6.2 |
| | Max | 25.8 | 11.9 | 2549 | 52.1 |
| | Mean | 21.3 | 10.9 | 713 | 27.5 |
| | | | | | |
| **Resurrection Bay, AK ( 7/30/07 - 8/29/07)** | Min | 11.5 | No data | 4.1 | 63.0 |
| | Max | 16.4 | No data | 511 | 93.6 |
| | Mean | 13.8 | No data | 146 | 85.5 |
| | | | | | |
| **Clinton River, MI (10/01/07 - 10/26/07)** | Min | 11.5 | 259 | No data | No data |
| | Max | 24.7 | 895 | No data | No data |
| | Mean | 17.2 | 496 | No data | No data |

## Moored Deployment Results in Chesapeake Bay, Solomons, MD

The mooring test in Chesapeake Bay took place at the end of a fixed pier located at the mouth of the Patuxent River on the campus on the Chesapeake Biological Laboratory. The water depth of the test site was 2.2 m (Fig.1). The site was brackish with salinity ranging from 9.8 - 11.9, and water temperature ranged from 177 - 25.8. (Fig. 2). The site water was quite turbid (mean % beam transmission = 27) with significant algal concentration (mean fluorescence = 713 mV) (Table 1 and Fig. 2).

**Figure 1.** Site Photos from the field deployment in Chesapeake Bay, MD. Left - fixed pier at mouth of Patuxent River on the campus of the Chesapeake Biological Laboratory. Right - ACT staff collecting a reference sample next to the instrument sampling inlet through a well on the sampling platform.

Phosphate concentrations at the Chesapeake Bay test site were very low throughout the deployment (range = 0.039 – 0.092 $\mu$M; Fig. 3), despite a significant contribution of river water to the site (mean salinity = 10.9). Field trip blanks consistently low (0.058 ±0.001 $\mu$M) for this site, however, represented approximately 93% of the average phosphate signal (0.062 ±0.014 $\mu$M) within the field reference samples. The lack of analyte signal strength presented a significant challenge for evaluating instrument performance for field samples. We show the regressed in situ versus field reference sample data (Fig. 4) for completeness (i.e. common analysis at the other test sites) but caution that the field sample concentrations were mostly near detection level and that the magnitude of offset (Fig. 4a) is easily skewed by the near zero reference data. We do not know the reason for the increased discrepancy that occurred in the later half of the deployment (Fig 3 and Fig. 4b). The post-deployment test of a laboratory reference standard indicated that the instrument was still functioning with good accuracy (see below).

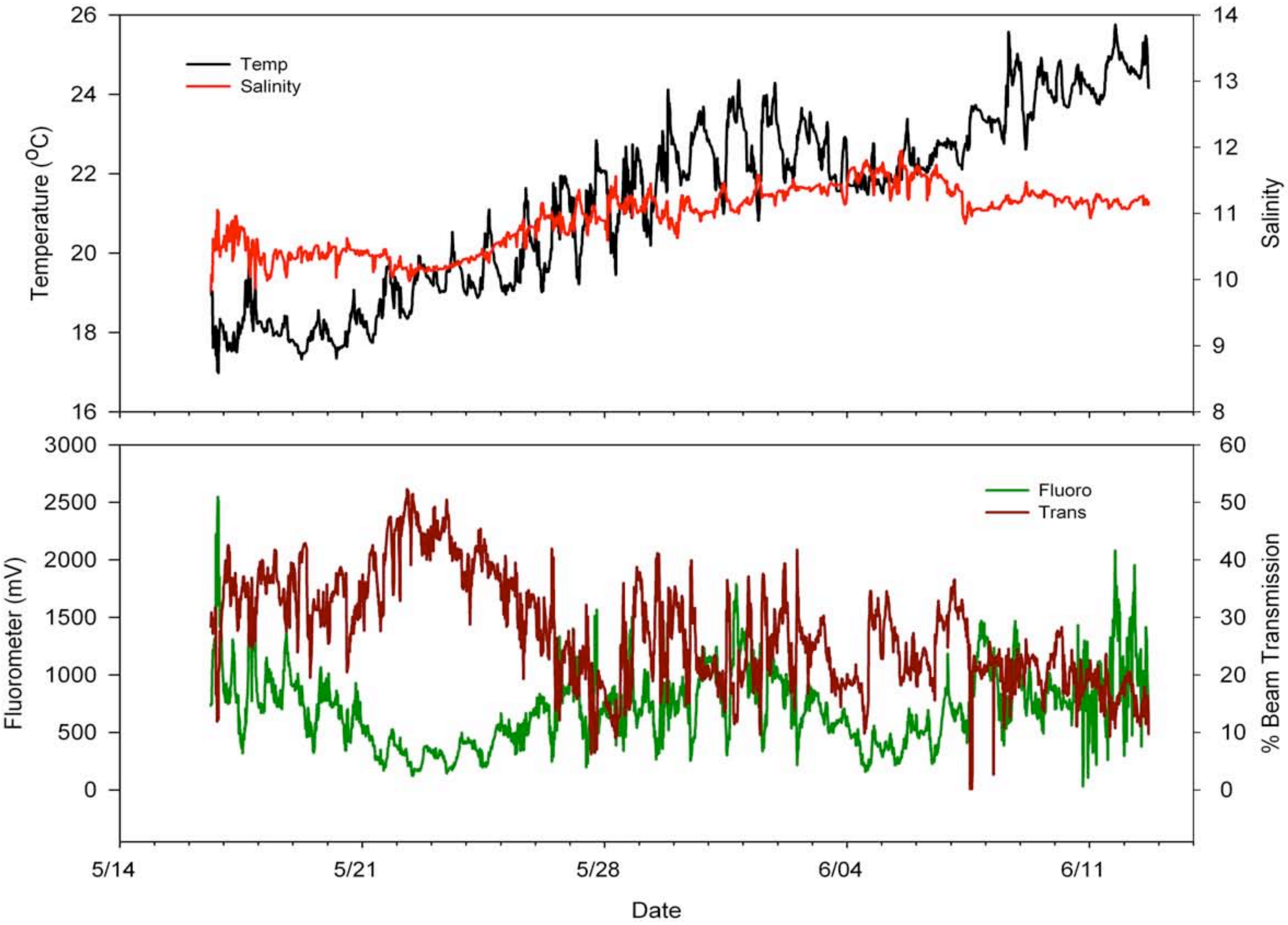


**Figure 2.** Ancillary data collected at the Chesapeake Bay, MD field test site describing conditions of temperature, salinity, algal fluorescence and water transparency.

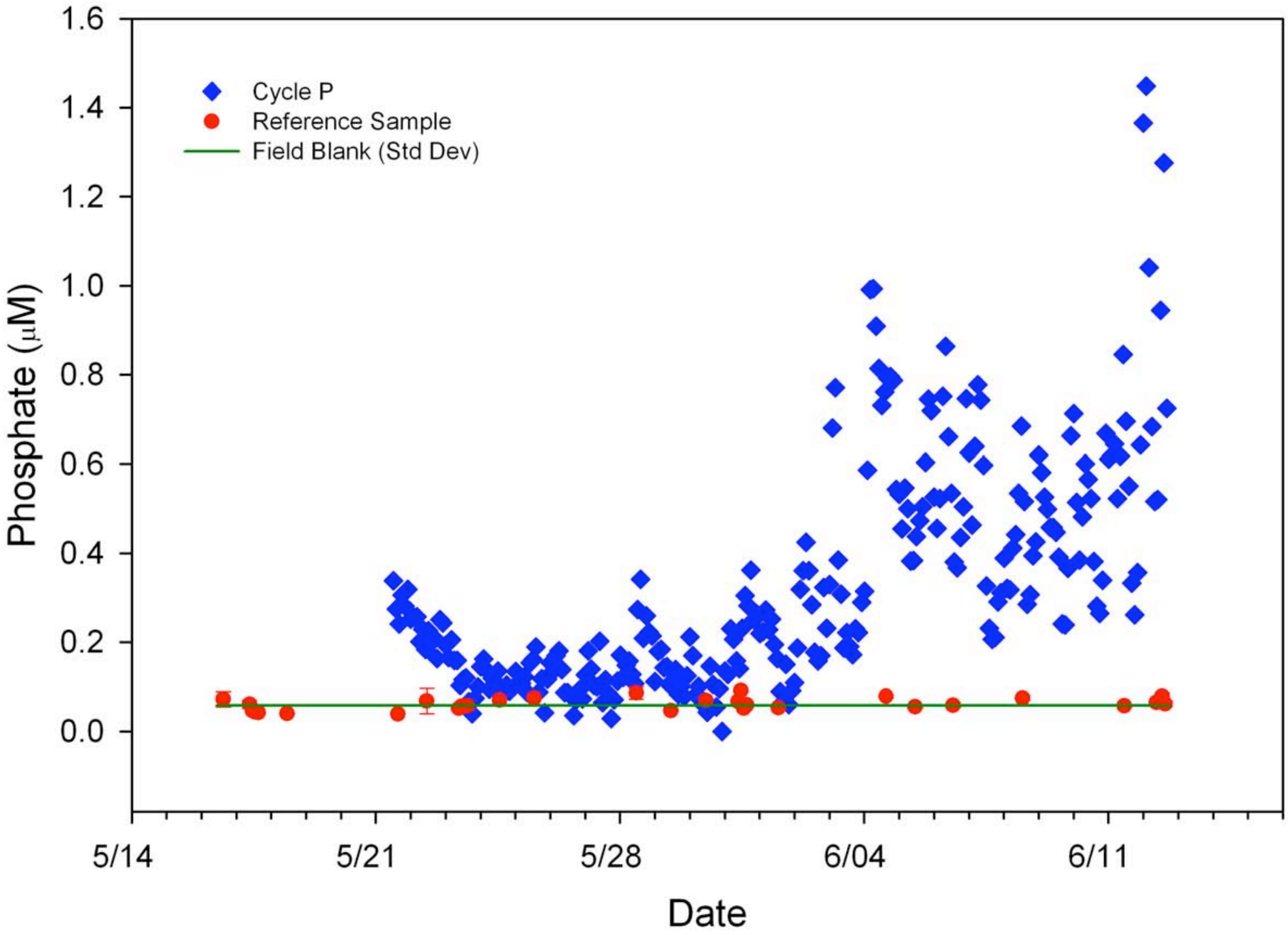


**Figure 3.** Time series comparison of WET Labs Cycle-P measured phosphate concentrations against laboratory measured reference samples and field trip blanks for the Chesapeake Bay, MD moored deployment test. (Laboratory measured concentrations represent mean ± sd., n=3).

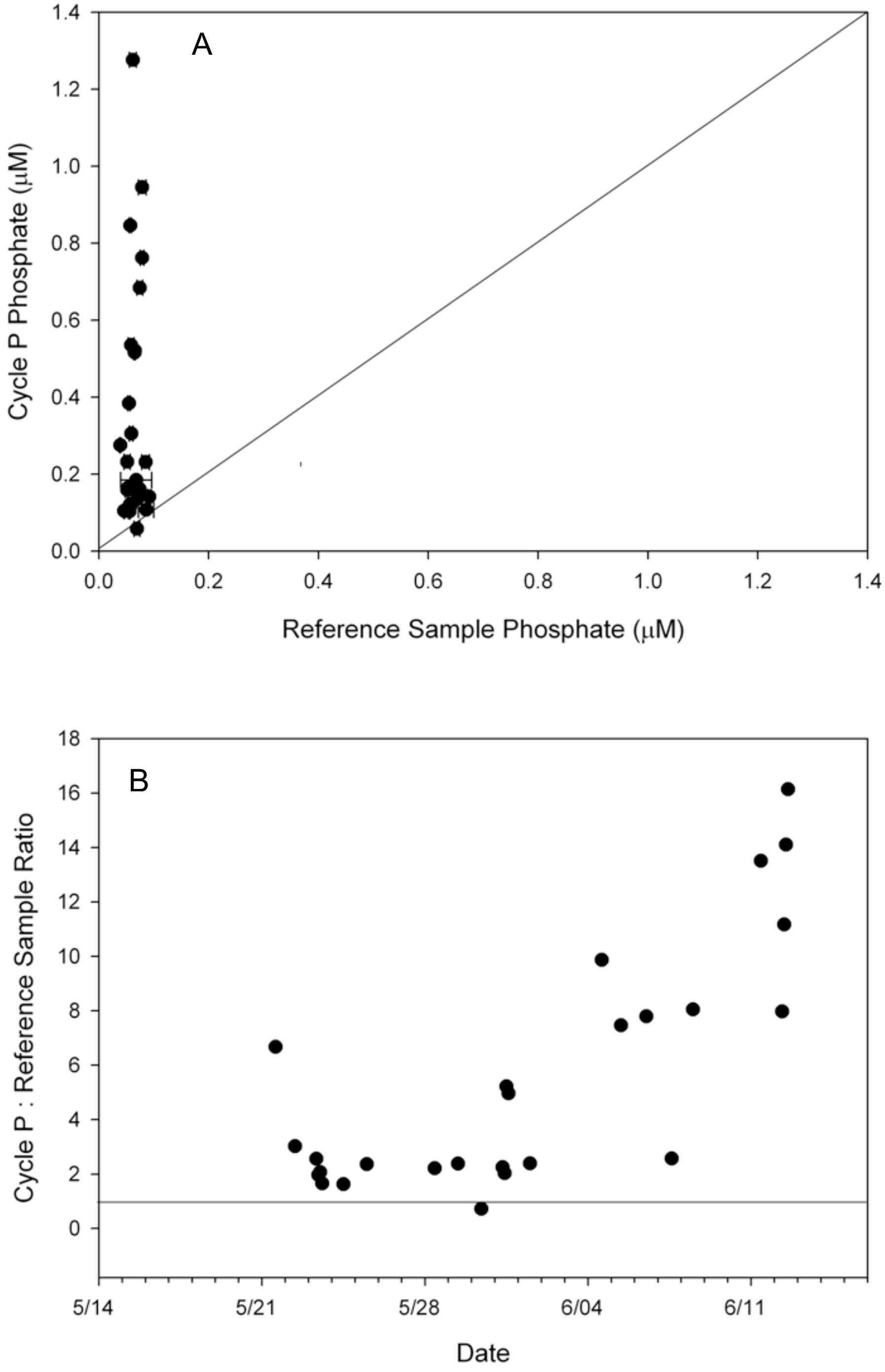


**Figure 4.** Analysis of test results from Chesapeake Bay, MD test site. (A) One to one comparison of Cycle P in situ phosphate determinations versus laboratory determined concentrations for matching field reference samples. (B) Time series of the ratio of Cycle P versus laboratory determined phosphate concentrations for matching field reference samples.

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

A set of pre- and post- exposures to blanks (DIW) and reference standards where completed in the laboratory as part of each deployment test (Table 2). The Cycle-P instrument initially deployed for the test stopped reporting after 2 days and was replaced with a new instrument. Initial exposure readings were lost in this exchange. Additionally, the final blank reading was not reported by the manufacturer. The measured concentration of the final exposure standard by the Cycle-P (1.23 $\mu$M) was approximately 20% higher than the laboratory determined value (1.02 $\mu$M). The relatively close agreement for this standard indicates that the instrument was still functioning properly after the four week deployment and that the disparity in field measurements may have resulted from the extensive bio-fouling around the sampling inlet. The instrument may have been picking up excretion signals from the bi-valve fouling community.

**Table 2.** Comparison of phosphate values for pre-test and post-test exposure to blanks and reference standards. (Lab results represent mean and sd. for n=3 replicates).

| | **Lab Result ($\mu$M)** | **Cycle-P Result ($\mu$M)** |
|---|---|---|
| **Lab Blank-pre** | 0.032 (0.002) | No data |
| **$PO_4$-P Standard - pre** | 1.004 (0.028) | No data |
| | | |
| **Lab Blank-post** | 0.042 (0.002) | No data |
| **$PO_4$-P Standard - post** | 1.022 (0.002) | 1.23 (0.60) |

ACT also collected a sub-sample of the “on board” nutrient standard that was used by the instrument before and after deployment. The sample was handled and analyzed identically to all of the reference samples, with the exception that it was not filtered. This value was compared against the reported concentration used by the Cycle-P to estimate in situ concentrations. The laboratory determined concentration of the on-board standard was 8.691 ± 0.119 $\mu$M at the beginning of the test and 9.016 ± 0.102 $\mu$M at the end of test, indicating no significant change during the deployment. The stated value of the standard from the company was 10.0 $\mu$M indicating a potential accuracy bias of 10-15 percent.

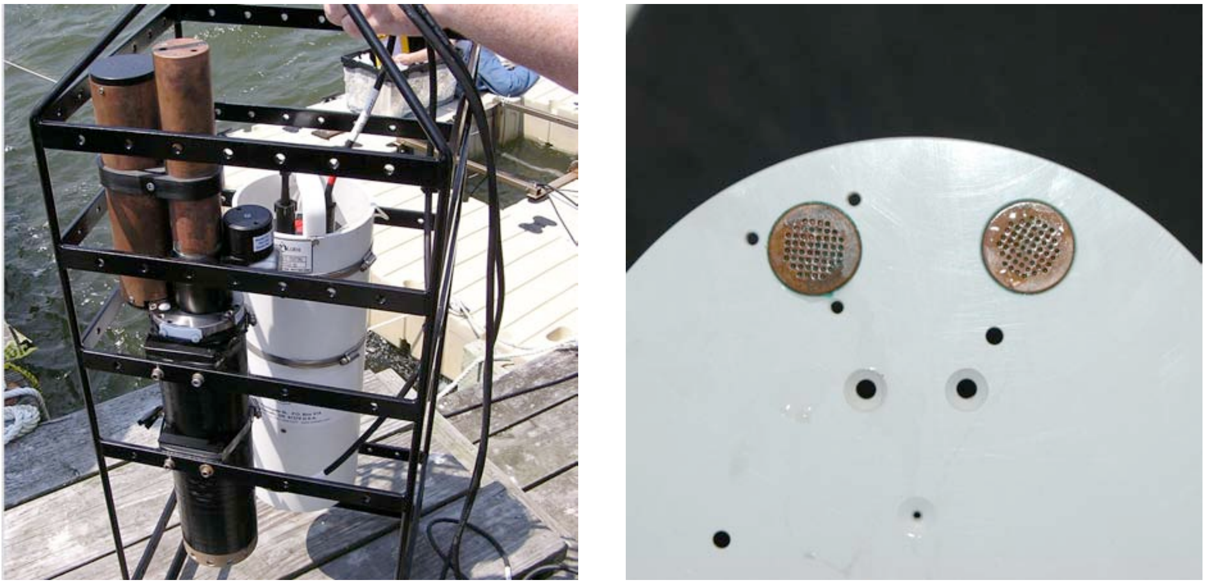
**Table 3.** Laboratory determined concentration of onboard phosphate standard at beginning and end of field test (mean, sd. for n=3 replicates). The stated value of the standard used to predict in situ concentrations by the Cycle-P was 10.0 $\mu$M.

| | **Pre-test Result ($\mu$M)** | **Post-test Result ($\mu$M)** |
|---|---|---|
| **On-board Standard** | 8.691 (0.119) | 9.016 (0.102) |

Weekly field sample spikes were conducted to help evaluate consistency in laboratory analysis and sampling handling. The reported percent recovery for the field sample spikes ranged from 114 – 167 percent, with a mean and standard deviation of 139 ± 23. This bias was not seen in any of the internal QA analysis performed by NASL (see Table 8).

## Instrument Photographs

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



WET Labs Cycle-P prior to deployment

Cycle-P sample inlet prior to deployment

WET Labs Cycle-P after deployment

Cycle-P sample inlet after deployment

**Figure 5.** Photographs of the WET Labs Cycle-P before and after deployment in Chesapeake Bay, MD.

## Moored Deployment Results in Resurrection Bay, Seward, AK

The mooring test in Resurrection Bay took place within the inlet of Humpy Cove on a floating dock attached to the end of a small fixed pier (Fig.6). The water depth of the test site was ca. 3 m. A programming error occurred for the CTD and salinity data are not available. Temperature data was taken from the test instrument itself and ranged from 11.5 - 16.4 ºC, with noticeable daily cycles related to tides (Fig. 7). The site water was very clear (mean % beam transmission = 86) with fairly low algal fluorescence (mean = 146 mV) (Table 1 and Fig. 7).

**Figure 6.** Site Photos from field deployment in Humpy Cove, Resurrection Bay, Alaska.

Reference sample phosphate concentrations in Humpy Cove ranged from 0.049 – 0.442 $\mu$M, with a mean of 0.130 ±0.071 $\mu$M during the deployment test (Fig. 8). Field trip blanks averaged 0.047 ±0.006 $\mu$M and represented approximately 36% of the average phosphate signal of the reference samples. The Cycle-P in situ measured concentrations generally tracked the overall pattern of reference sample concentrations throughout the deployment reporting a range in concentrations of 0.009 – 0.701 $\mu$M, with a mean of 0.182 ±0.108 $\mu$M (Fig.8). Sampling time interval for the Cycle-P averaged around 90 minutes instead of an expected 2h interval, so reference sampling timepoints did not always match up with instrument sampling. When times did not match the two Cycle-P measurements surrounding the reference sample were averaged for use in direct comparisons. A linear regression of directly compared instrument versus reference sample concentrations shows significant offset and variability (Fig. 9a). A time series plot of the ratio of corresponding instrument versus laboratory measurements indicates a positive bias in the Cycle-P measurements in the early part of the deployment that declined over time and became less variable after approximately 10 days (Fig. 9b).

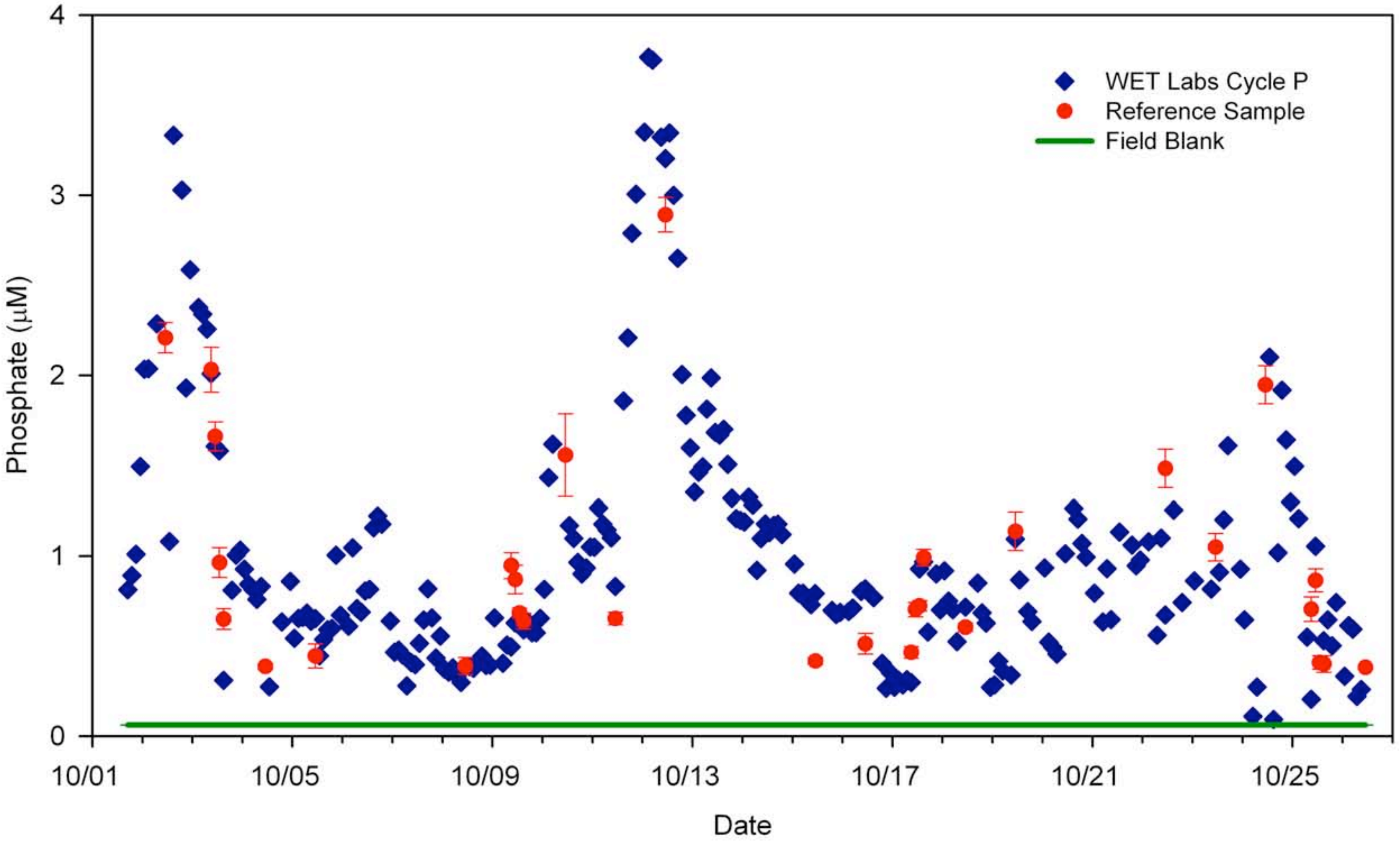


**Figure 13.** Time series comparison of WET Labs Cycle-P measured phosphate concentrations against laboratory measured reference samples and field trip blanks for the Clinton River, MI moored deployment test. (Laboratory measured concentrations represent mean ± sd., n=3).

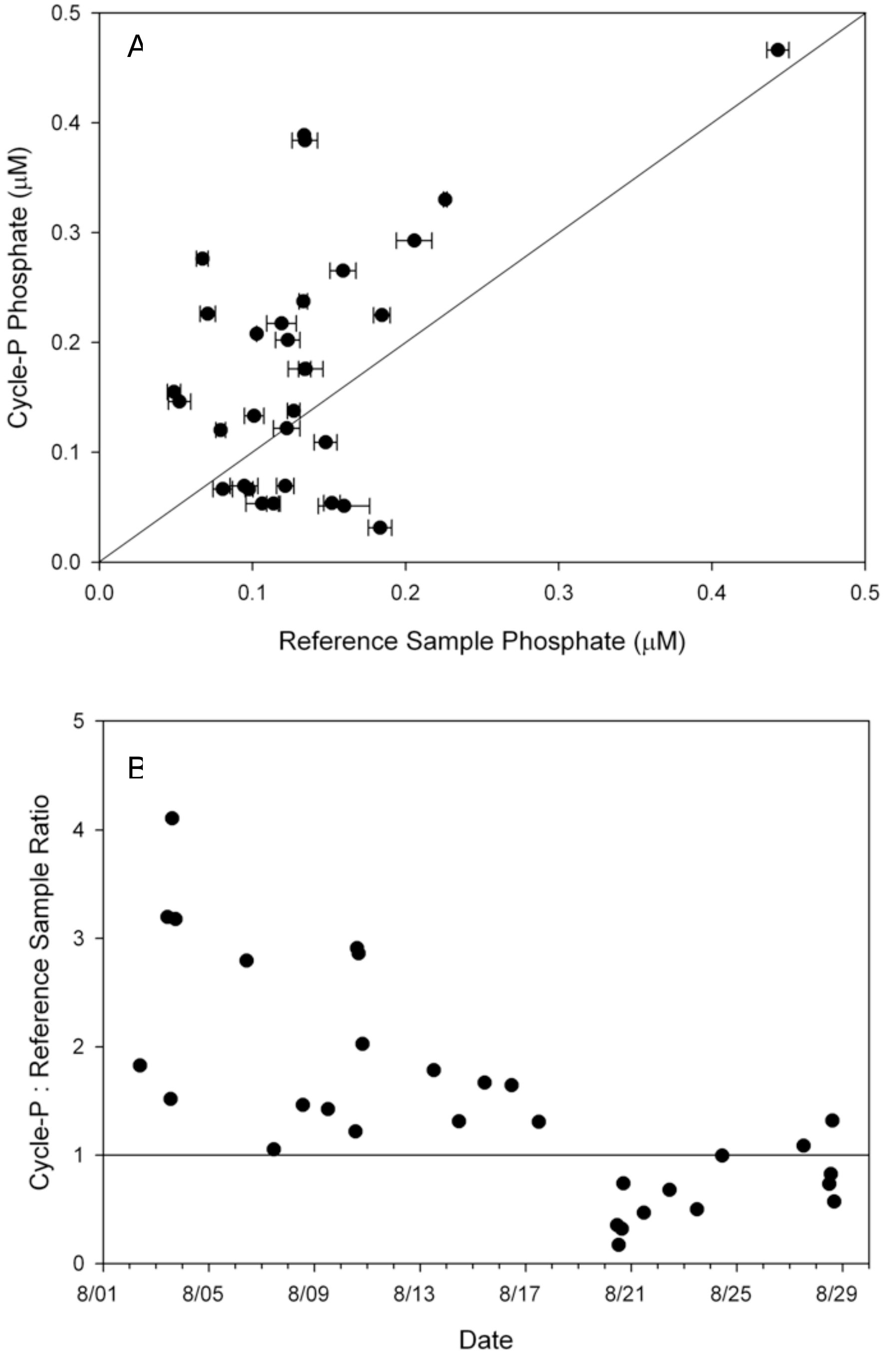


**Figure 9.** Analysis of test results from Resurrection Bay, AK test site. (A) One to one comparison of Cycle P in situ phosphate determinations versus laboratory determined concentrations for matching field reference samples. (B) Time series of the ratio of Cycle P versus laboratory determined phosphate concentrations for matching field reference samples.

**Moored Deployment Results in Resurrection Bay, AK (cont.)**

Only pre-test exposures to laboratory blanks (DIW) and reference standards where completed at this test site because the manufacturer was not able to return at the time of the retrieval. The Cycle-P measured a slightly negative value for the blank but was less than 1% different for the reference standard (Table 4).

**Table 4.** Comparison of predicted phosphate values for pre-test and post-test exposure to blanks and reference standards. (Lab results represent mean and (sd.) for n=3 replicates).

| | **Lab Result ($\mu$M)** | **Cycle P Result ($\mu$M)** |
|---|---|---|
| **Lab Blank-pre** | 0.036 (0.001) | -0.058 |
| **$PO_4$-P Standard - pre** | 3.504 (0.038) | 3.530 |

Laboratory analysis of the on-board nutrient standard conducted by NASL for a sample collected before and after deployment show both high accuracy and good stability (Table 5). The final concentration was only 2% different than the initial concentration, and the value was within 1-3% of the stated 10 $\mu$M concentration used by the Cycle-P to estimate in situ concentrations.

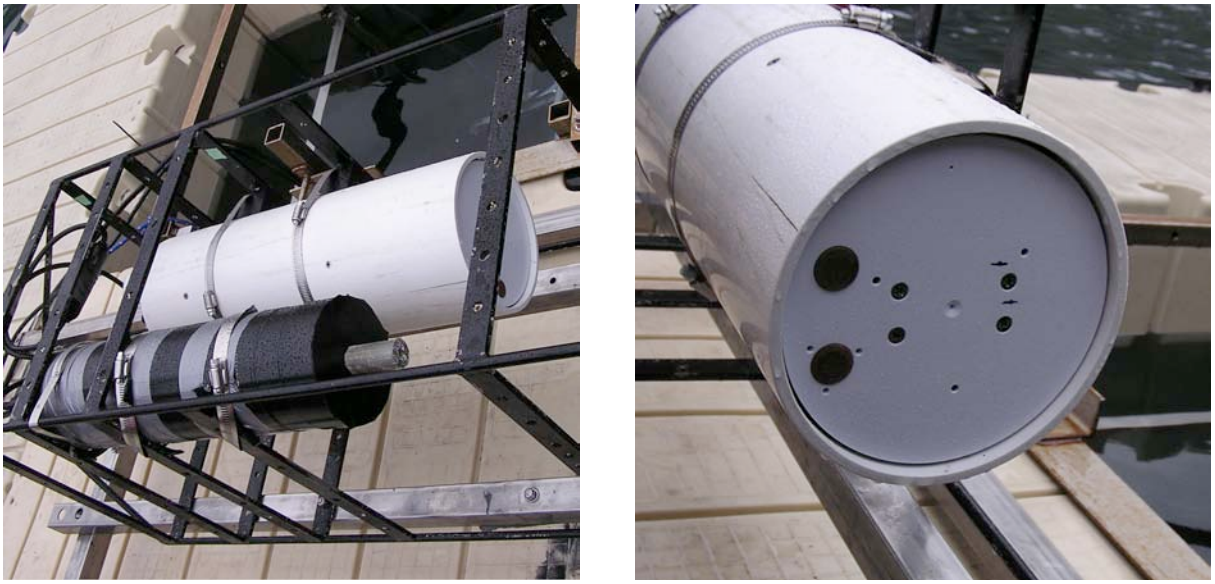
**Table 5**. Results of the laboratory measurements of the on-board phosphate standard at beginning and end of field test (mean and sd. for n=3 replicates). The stated value of the standard from manufacturer was 10.0 $\mu$M.

| | **Pre-test Result ($\mu$M)** | **Post-test Result ($\mu$M)** |
|---|---|---|
| **On-board Standard** | 9.9388 ( nd ) | 9.7338 (0.0202) |

Weekly field sample spikes were conducted to help evaluate consistency in laboratory analysis and sampling handling. The reported percent recovery for the field sample spikes at this test site ranged from 82 – 93 percent, with a mean and standard deviation of 87.4 ± 5.6. Again, this amount of deviation was not seen in any of the internal QA analysis performed by NASL (see Table 8). Results of other QAQC samples and repeated analysis of replicates, including those done in different batches, do not indicate any consistent problems in sample handling or analysis. There may be a slight deviation caused by changing the salinity of the spiked reference sample by 10 % due to the addition of the nutrient standards.

## Instrument Photographs

Before and after photos were taken of the instrument package and its sample intake to examine the extent and possible impacts of bio-fouling (Fig. 10). Almost no 'hard' bio-fouling occurred at this test site, and only minor amounts of periphytic algae adhered directly to the nutrient analyzer.



WET Labs Cycle-P prior to deployment

Cycle-P sample inlet prior to deployment

WET Labs Cycle-P after deployment

Cycle-P sample inlet after deployment

**Figure 10.** Photographs of the WET Labs Cycle-P before and after deployment in Resurrection Bay, AK.

## Moored Deployment Results in Clinton River, MI

The mooring test in Michigan took place at the end of a fixed pier located at the mouth of the Clinton River which drains into Lake St. Clair (Fig. 11). The water depth of the test site was 2.2 m. The site exhibited a fairly large fluctuation in conductivity, ranging from 300 - 900 uS/cm as shifting winds produce a varying mixture of river water and lake water and water temperature ranged from 11.5 - 24.7 (Table 1, Fig.12).

**Figure 11.** Site Photos from the field deployment in the Clinton River, Mt. Clemens, Michigan.

Reference sample phosphate concentrations in the Clinton River ranged from 0.382 – 2.892 $\mu$M, with a mean of 0.960 ± 0.629 $\mu$M during the deployment test (Fig. 13). Field trip blanks averaged 0.062 ±0.001 $\mu$M and represented approximately 6% of the average phosphate signal of the reference samples. The Cycle-P in situ measured concentrations tracked the overall pattern of reference sample concentrations quite closely throughout the deployment. The reported range in concentrations by the Cycle-P was 0.09 – 3.77 $\mu$M, with a mean of 1.02 ±0.71 $\mu$M (Fig.13). Sampling time intervals at this test site followed the expected 2h interval so reference sampling timepoints were closely matched with instrument sampling. A linear regression of directly compared instrument versus reference sample concentrations shows relatively close agreement ($R^2 = 0.77$) (Fig. 14a). A time series plot of the ratio of corresponding instrument versus laboratory measurements indicates an equal amount of positive and negative bias in the Cycle-P measurements with no apparent change in performance during in the deployment period (Fig. 14b).

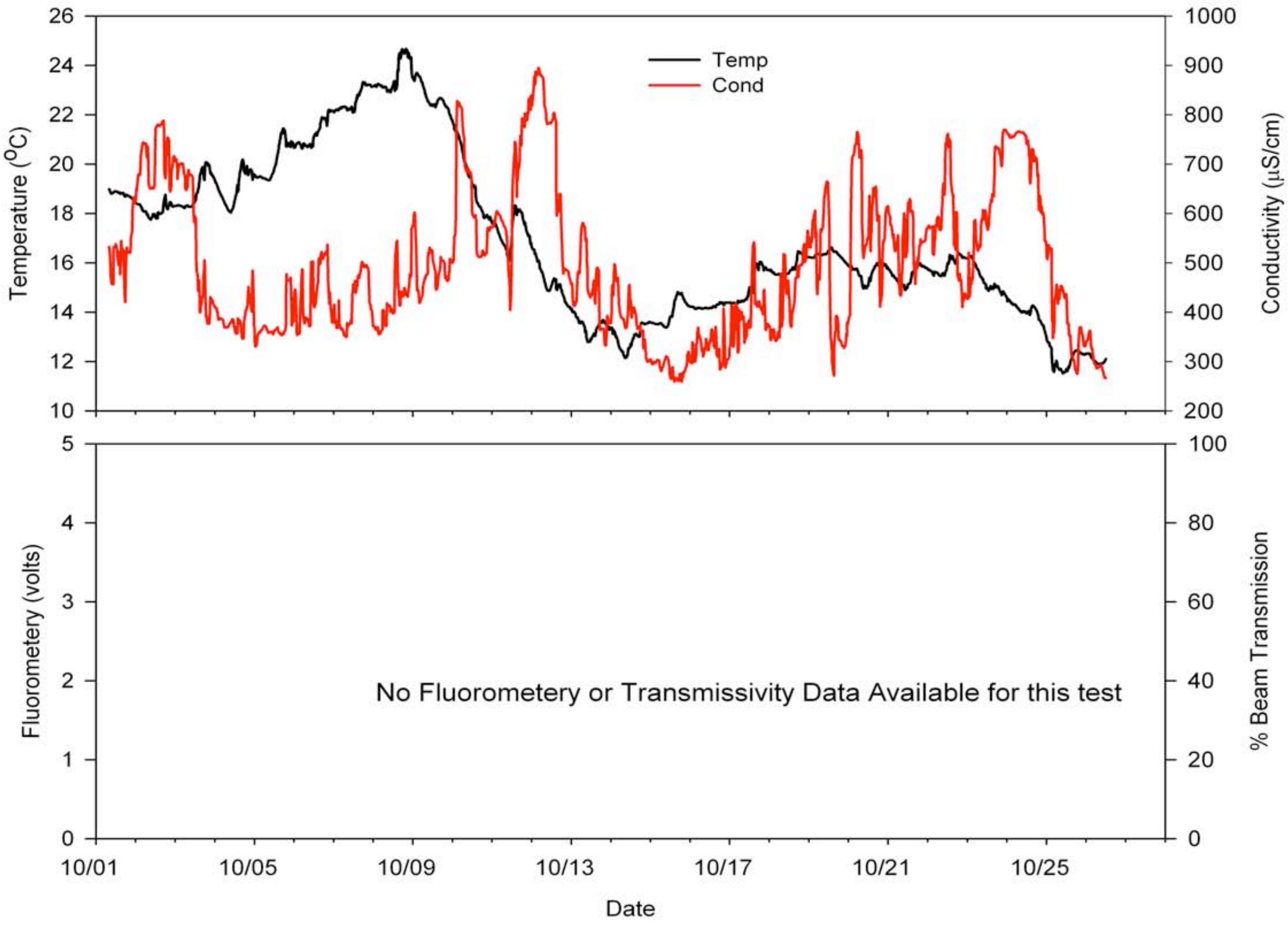


**Figure 12.** Ancillary data collected at the Clinton River, MI field test site describing conditions of temperature and conductivity. A data logging problem resulted in the loss of the time series data for fluorescence and water transparency.

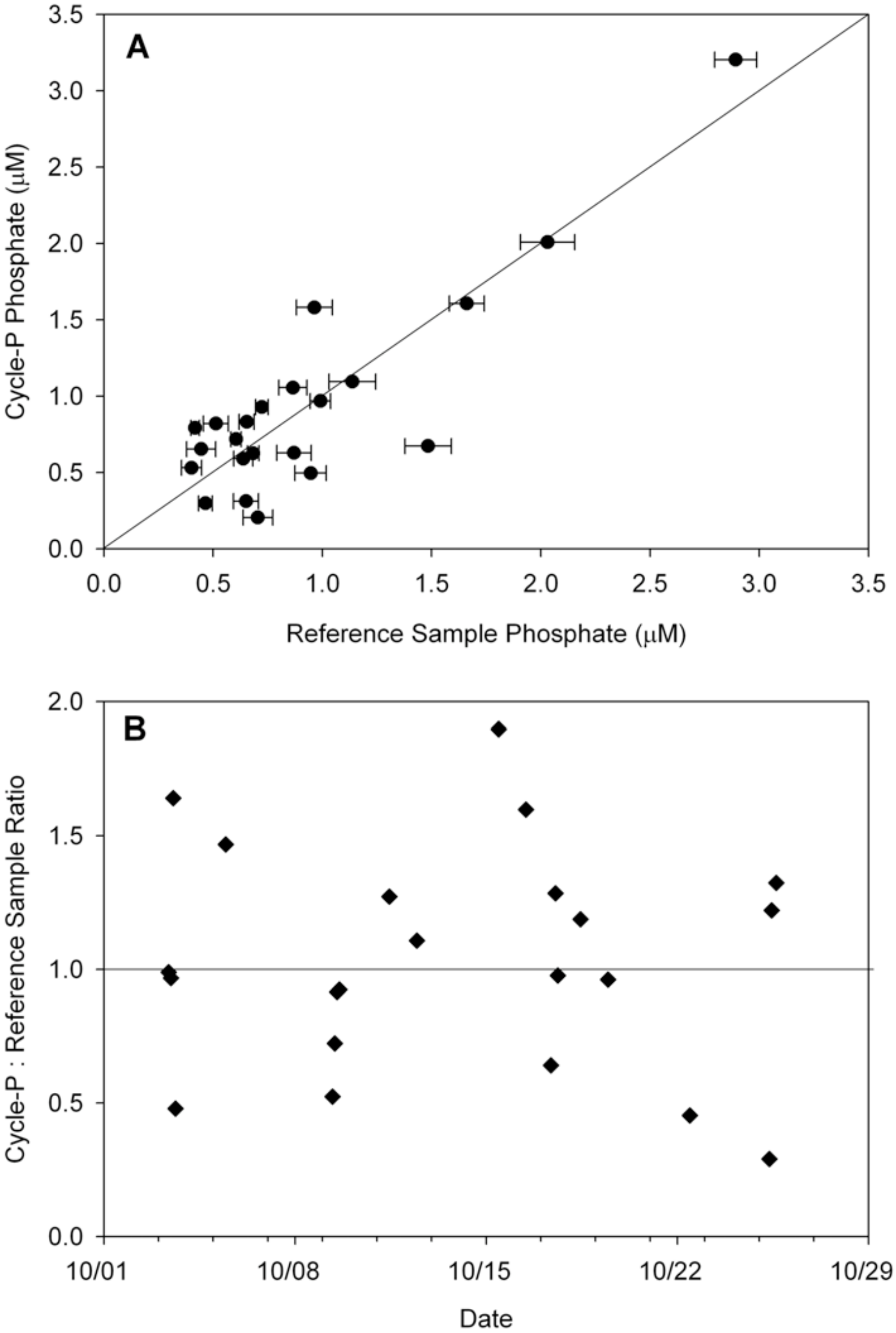


**Figure 14.** Analysis of test results from Clinton River, MI test site. (A) One to one comparison of Cycle P in situ phosphate determinations versus laboratory determined concentrations for matching field reference samples. (B) Time series of the ratio of Cycle P versus laboratory determined phosphate concentrations for matching field reference samples.

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

Results of pre- and post- exposures to laboratory blanks (DIW) and reference standards completed as part of the MI deployment test are presented in Table 6. The Cycle-P measured slightly below 0 for the blank solution both at the beginning and end of the test. The measured values of the reference standard were 15% and 22% higher than NASL determined concentrations at the beginning and end of the test, respectively. These results are consistent with the field reference comparisons and show a high level of consistency throughout the test, but in this case the potential bias appeared to be consistently positive.

**Table 6**. Comparison of predicted phosphate values for pre-test and post-test exposure to blanks and reference standards. (Lab results represent mean and sd. for n=3 replicates).

| | **Lab Result (μM)** | **Cycle-P Result (μM)** |
|---|---|---|
| **Lab Blank-pre** | 0.0543 (0.0004) | -0.041 |
| **$PO_4$-P Standard - pre** | 3.5040 (0.0375) | 4.018 |
| | | |
| **Lab Blank-post** | 0.0548 (0.0033) | -0.107 |
| **$PO_4$-P Standard - post** | 3.4332 (0.0126) | 4.18 |

Laboratory analysis of the on-board nutrient standard conducted by NASL for a sample collected before and after deployment show both high accuracy and good stability (Table 7). The final concentration was less than 2% different than the initial concentration, and the value was within 1-2% of the stated 10 μM concentration used by the Cycle-P to estimate in situ concentrations.

**Table 7**. Results of the laboratory measurements of the on-board phosphate standard at beginning and end of field test (mean and sd. for n=3 replicates). The stated value of the standard from manufacturer was 10.0 μM.

| | **Pre-test Result (μM)** | **Post-test Result (μM)** |
|---|---|---|
| **On-board Standard** | 9.7734 (0.0693) | 9.9291 (0.0202) |

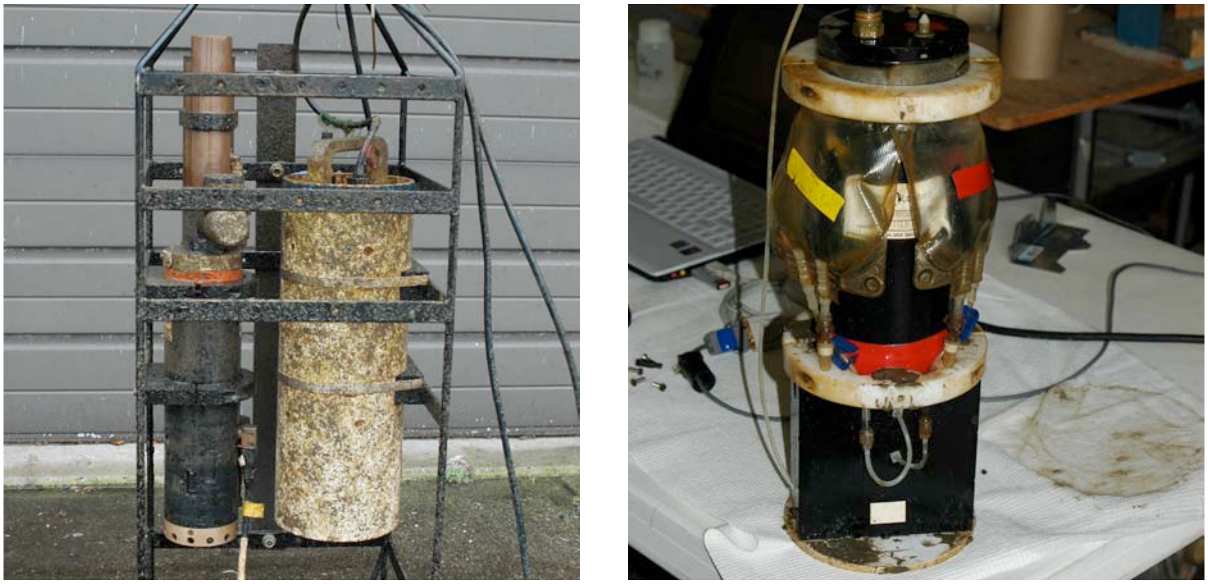
Weekly field sample spikes were conducted to help evaluate consistency in laboratory analysis and sampling handling. The reported percent recovery for the field sample spikes at this test site ranged from 100 – 107 percent, with a mean and standard deviation of 104.2 ± 3.5. These results, along with agreement among replicates and internal QAQC analysis done at NASL (see Table 8), indicate no problems in sample handling or analysis during the test.

### Instrument Photographs

Before and after photos were taken of the instrument package and its sample intake to examine the extent and possible impacts of bio-fouling (Fig. 15). No 'hard' bio-fouling occurred at this test site, and only minor amounts of periphytic algae adhered directly to the nutrient analyzer.

WET Labs Cycle P prior to Deployment

Cycle P sample inlet prior to deployment



WET Labs Cycle P after Deployment

Cycle P sample inlet after deployment

**Figure 15.** Photographs of the Cycle-P analyzer before and after deployment in the Clinton River, MI.

## QUALITY ASSURANCE / QUALITY CONTROL:

### Technical System Audits

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

### NASL nutrient analysis

NASL conducted internal laboratory checks on their accuracy and precision with every analytical batch of field samples. QA performance checks included duplicate analysis of field samples, analytical nutrient spikes of field samples, comparisons of expected absorption values of internal NASL standards based on long term averages, and measurements of external standards from certified solutions against internal calibration standards. A summary of the laboratory QA results, organized by test site, are presented in Table 8.

**Table 8.** Summary of the internal NASL laboratory QA results that were conducted during the analysis of phosphate on reference samples from each of the ACT test sites. Data represent the mean and standard deviation for the reported observations (denoted by 'N') submitted by NASL.

| | N | Lab Duplicates (% Diff) | N | Lab Spikes (% Rec) | N | Lab Stds (% Diff) | N | External Stds (% Diff) |
|---|---|---|---|---|---|---|---|---|
| **GL** | 36 | 1.84<br>(1.79) | 7 | 102.37<br>(4.44) | 3 | 1.97<br>(1.7) | 1 | 9.17<br>(-) |
| **AK** | 20 | 3.12<br>(3.48) | 13 | 99.71<br>(1.17) | 4 | 2.62<br>(1.95) | 2 | 9.75<br>(0.82) |
| **CBL** | 23 | 4.93<br>(5.00) | 11 | 97.44<br>(1.98) | 5 | 2.20<br>(1.26) | 3 | 2.51<br>(1.53) |
| **MLML** | 16 | 2.53<br>(3.03) | 7 | 99.98<br>(4.09) | 6 | 1.81<br>(1.53) | 3 | 5.94<br>(4.09) |

#### *Reference Sample Analysis*

All reference samples were analyzed in triplicate. Whenever results of triplicates yielded a coefficient of variation greater than 15%, the two reserved frozen samples were submitted to NASL for analysis. The two new values were added to the original database and then the three values which gave the minimum standard deviation were selected to calculate the final mean for that reference sample.

## RELIABILITY:

The WET Labs Cycle-P was successfully testing in a fixed surface mooring at three test sites including freshwater, brackish water, and saltwater coastal environments. The test instrument had to be exchanged on day 2 of the Chesapeake Bay test; otherwise the instrument functioned continuously during all three mooring tests. In terms of data recovery, there was a sub-section of data that was not reported back to us by the manufacturer because the in situ predicted concentrations were clear outliers. Specifically, for the Chesapeake Bat test 84% of the potential instrument readings were reported out and subsequently used in the presented analysis. Similarly, 79% of the potential data points were reported for the Michigan test and available for the analysis. For the Alaska test over 99% of the data were reported out and used in the analysis.

One concern that arose during the test was the sample timing interval used by the Cycle-P. The plan was for the test instrument to initiate sampling at fixed 2 hour interval starting on the top of the odd hours. In the Chesapeake Bay test the Cycle-P was initiating samples on the even hours instead. It is not known whether this offset occurred during the instrument exchange and was a simple over-sight in the programming. Due to the offset, we had to average two instrument readings that surrounded the reference sample timepoints. In the Alaska test the Cycle-P sampling interval was approximately 1.5 hours instead of 2 hours. Again, this produced an offset with the reference sampling intervals and we averaged instrument results for reported comparative concentrations. No timing problems occurred in the Michigan test, and both the sampling interval and timing of sampling was as expected throughout the test.

In general it appears that the fundamental technology has the capability to successfully measure in situ phosphate in situ under a variety of field conditions. Any difficulty in deploying instruments or collecting data during this ACT Demonstration where overcome and seemed to be related to packaging or processing issue that can likely be addressed with engineering refinements. The concerns over the sampling intervals are mainly an issue when one has to use reference data for comparisons or field calibration and are not related directly to instrument function as an internal timestamp is provided with all reported data. However, the ability to take meaningful reference samples in a highly variable environment is important and a consideration for optimal operations.

## ACKNOWLEDGMENTS:

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Mario Tam

**March 30, 2008**

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