PERFORMANCE DEMONSTRATION STATEMENT

American Ecotech NUT 1000

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

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

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

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EXECUTIVE SUMMARY:

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

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

In this Demonstration Statement, we present the performance results of the American Ecotech NUT 1000 during a surface mapping tests in Monterey Bay, CA. This field exercise was designed to demonstrate the capacity of test instruments for high frequency and resolution sampling of ambient phosphate concentrations provided in a flow through sampling stream drawn from 1m during an underway survey. Water sampling was conducted both in the highly turbid and eutrophic waters of the Moss Landing Harbor as well as more oceanic conditions of outer Monterey Bay during a 7 hour cruise. The reported measurements from the NUT 1000 were highly linear over an order or magnitude range between 10 – 120 µgP/L when regressed against laboratory analyzed reference samples. However, the initial calls from the instrument consistently over-estimated phosphate concentrations by around 50 µgP/L due to an internal calibration issue most likely associated with the higher refractive index of seawater relative to the calibration standard solutions. Simple post-correction of the instrument data provided excellent agreement with the laboratory reference data throughout the range of observed concentrations.

In addition to the surface mapping application, two moored application tests were attempted, but distinct instrument malfunctions resulted in aborted tests at both locations. It should be noted that this instrument as configured was not designed for mooring applications. In general, however, it appears that the fundamental technology of the NUT 1000 has the capability to successfully measure in situ phosphate concentrations over a wide range of water quality conditions and can be particularly effective for rapid, survey-based applications due to its high sampling rate capability and extreme portability. We encourage readers to review the entire document for a comprehensive understanding of instrument performance and to discuss results with the instrument manufacturer.
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 NUT 1000 nutrient monitor. 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). The manufacturer was offered an opportunity to submit a company response to this report but has declined this option and has stated that they accept the results and interpretations as presented.

TECHNOLOGY TYPE:

The concept for the Nutrient Monitor (NUT1000) was originally conceived by Monash University and was redesigned and subsequently commercialized by Ecotech Pty. Ltd. The Nutrient Monitor is designed to provide a low detection limit and a rapid response for filterable reactive phosphate. Conductivity and temperature are also measured and salinity is automatically calculated. The monitor can achieve a high sensitivity with detection limits for reactive phosphate of less than 3 \( \mu \text{g/L} \) and a typical response time per measurement of 30 seconds. This high sampling rate is due to the use of rapid sequenced reagent injection in combination with a multi-reflection flow cell. Due to the use of reagent injection, reagent use is minimized and over 1000 measurements can be performed using only 20mL of reagent. Automatic reference calibrations can be easily configured providing for a very high level of reliability. The NUT1000 is lightweight, portable and can be powered by 12VDC making it ideal for chemical mapping of surface waters. It can also be configured with an optional Global Positioning System input for these applications. Typical sample throughput is 100 measurements per hour thereby facilitating the construction of detailed plume profiles. The monitor can also be used permanently in one fixed-point where it will sample and calibrate itself independently. Used in this way the instrument can map changes in phosphate concentrations over time in catchments, streams, rivers or waste outfall sites.

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 verification 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. The amount of disagreement in measurements can be expressed as a percent of the signal or as an absolute difference. 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** – Reliability is 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 0.0007 mgP/L by prior laboratory studies. The typical working concentration range for the method and SOP is between 0.0035 – 1.48 mgP/L. A sample reagent blank was analyzed in conjunction with every sample and all internal standards were verified and calibrated using certified external nutrient standards. Additional internal QAQC samples including laboratory duplicates and nutrient recovery spikes of the reference samples were analyzed with each analytical batch.

**Moored Deployment**

Field demonstration tests of instrument performance in a moored application were conducted at two ACT Partner Institution sites. Prior to deployment, all instruments were set up and calibrated as required at the field sites by a manufacturer representative with assistance from ACT staff. As the NUT 1000 is non-submersible, its instrumentation box was placed in a weatherproof housing attached to the deck of the mooring platforms and the sample inlet held in place 1 m below the water surface. Instruments were programmed to record data based on a time interval that allowed for a 30 day deployment. Specific sampling intervals varied among test instruments with the sampling frequency ranging between 15 minutes and 2 hours. All instruments were delivered a low (0) and high (100 µgP/L) reference standard (made from certified reference standards) both before and after deployment as an estimate drift over time. A photograph of each individual instrument and the entire instrument rack was taken just prior to deployment and just after recovery to provide a qualitative estimate of biofouling during the field tests. Finally, a sub-sample of the on-board standard solutions were collected both immediately before and after the deployment period for independent analysis by CBL-NASL to help account for any possible accuracy offset and degradation of the standard over time.

A standard 2-L Van Dorn water sampler was used at each field test site to collect water samples for laboratory nutrient analysis. These samples were used as the reference samples for examining instrument performance and stability over time. The sampling frequency was structured to examine changes in nutrient concentrations over daily and whole-month time scales. Specifically once each week an intensive sampling event that consists of 4 consecutive samples spaced at two-hour intervals was conducted. For the remaining 4 days of the week water was sampled only once per day. Samples were taken while the instruments are initiating sample uptake.

**Surface Mapping Deployment**

The surface mapping field exercise was designed to demonstrate the capacity of the test instruments for high frequency and resolution sampling of ambient nutrient concentrations provided in a flow through sampling stream such as might be found in a underway WQ monitoring package such as a ‘ferry box’. This deployment was conducted by the ACT-Pacific Coast Partnership at Moss Landing Marine Laboratories making use of their coastal research vessel the R/V John H. Martin, a converted 56
foot Westport charter boat. While the JH Martin maintains a dedicated through hull WQ monitoring system, water sampling constraints required the use of MLML’s self-contained and portable Underway Data Acquisition System (PortUDAS) plumbed to draw water through hull from -1m near the portside stern section of the JH Martin. The PortUDAS is configured to draw water via a 12V DC pump through a 1 mm screen and de-bubbling chamber and the conditioned sampling stream passed through a SBE 38 Digital Thermometer, a SBE 45 Thermosalinograph, a SCUFA chlorophyll fluorometer and optical backscatter turbidimeter and a Wet Labs C-Star, 10cm transmissometer (archival data for all of MLML’s UDAS systems can be found at weathernew.mlml.calstate.edu/serveudas/udasmain.html). Sensor output (nominally 0.25Hz) is multiplexed with a GPS stream through a hardened on board computer and wirelessly transmitted to a logging computer for real-time display and geospatial mapping via a MatLab interface (L. Beatman, pers. comm.).

The outflow from the PortUDAS system delivered into a 10L acid washed plastic cooler which provided clean sampling access for field grab samples. The sampling frit of the NUT1000 was positioned at the inflow of the cooler and water samples taken from this region (Fig 1). Flow rate through the

Figure 1. Working configuration of instrumentation used in surface mapping deployment on Monterey Bay, 19 July 2007. (1) A portable WQ monitoring system was used to draw water from -1m through the stern hull of the R/V John H. Martin (left). The PortUDAS system recorded oceanographic WQ conditions as well as GPS location at the time of sampling. The outflow from the PortUDAS supplied the nutrient sensor packages maintained at ambient water temperature in the plastic tub (middle). Reference water samples were taken from the smaller cooler in the tub at fixed stations throughout the bay. (2) Close up of flow through configuration and the NUT1000 sample inlet frit positioned at the left side of the cooler.
PortUDAS system in this configuration was ca. 12 L/min. The NUT1000 at its highest rate, ca. every 20s (0.05 Hz) and internal baseline and reference standard sets run at least every 30 min during the deployment. Prior to deployment, all instruments were calibrated as suggested in individual manufacturer manuals and exposed to low and high reference standards similar to the moored test.

The sampling scheme for the deployment was designed to cover the broadest range in nutrient concentrations and WQ conditions accessible in the Monterey Bay region to provide a demonstration of the dynamic performance and stability of the instrumentation. Consequently water sampling was conducted both in the highly turbid and eutrophic waters of the Moss Landing Harbor as well as more oceanic conditions of outer Monterey Bay. The cruise track (Fig. 2), attempted a saw-tooth sampling pattern, on-shore off-shore southward along the coast of the bay followed by a NNW run towards the upwelling influence regions of the north bay. At each field grab sample site the vessel was asked to maintain position for 5 minutes while the sampling cooler was emptied an allowed to fill with new water best representing that locale. Acid washed sample bottles where then rinsed by three fillings with the new sample water and a 1 liter sample taken at the local time recorded. A sterile 0.2μ nylon filter with 500mL bottle was rinsed three times by filtering ca. 150mL of sample, then ca 500 mL collected and stored in the dark on ice until subdivided into analytical batches back at the lab.

Figure 2. Geospatial representation of the R/V John H. Martin cruise track (green) associated with the surface mapping field deployment 19 July 2007. Shaded topography provided to highlight position of sampling relative to major geomorphological features of the Monterey Bay region. Track spanned several watershed outfalls as well as coastal ocean conditions over the Monterey Canyon. Annotations (cyan) indicate positions of sequential field grab sampling stations. Local times at selected sampling stations are provided to help orient reader to locations associated with time-series plots of the WQ and nutrient datasets.
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. 32 µgP/L) 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 and comparing the observed versus expected amount of analyte recovery.

DEMONSTRATION RESULTS:

In general it appears that the fundamental technology has the capability to successfully measure in situ phosphate concentrations under a variety of field conditions. However, mechanical difficulties allowed for only one (surface mapping in Monterey Bay) of the three field deployments to be completed successfully. Results are presented only for this successful field deployment test. Difficulty in deploying instruments or collecting data during this ACT Demonstration seemed to be related to flow system components that can be addressed by engineering refinements as the instrument moves along the commercialization cycle.
Surface Mapping in Monterey Bay, CA

Highly variable water quality conditions were encountered at -1m during the 19 July 2007 surface mapping cruise aboard the R/V John H. Martin. Table 1 gives the overall ranges in physical/chemical water quality conditions encountered during the mapping test. Time series plots of physical/chemical data during the mapping test are presented in Figure 3. Strong gradients in Salinity (top panel), chlorophyll (middle panel) and water clarity (bottom) panel where associated with transition from the estuarine slough environments to the coastal ocean. The PortUDAS sampling system was able to detect sharp fronts in WQ associated with falling (morning) and incoming tides at the entrance to the Moss Landing Harbor. Waters over the axis of the Monterey Canyon (13:00 – 14:00) were colder, saltier and clearer than those near shore indicative of oceanic conditions and possible intrusion of recently upwelled waters.

Table 1. The range in physical/chemical water quality conditions at a depth of 1m observed during the surface mapping deployment of the NUT 1000 in Monterey Bay, CA.

<table>
<thead>
<tr>
<th>Monterey Bay, CA (Surface Mapping)</th>
<th>Temp. (°C)</th>
<th>Salinity</th>
<th>Fluorescence (mV)</th>
<th>% Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>12.1</td>
<td>21.8</td>
<td>10</td>
<td>6.0</td>
</tr>
<tr>
<td>Max</td>
<td>19.0</td>
<td>33.1</td>
<td>1860</td>
<td>96.0</td>
</tr>
<tr>
<td>Mean</td>
<td>15.8</td>
<td>33.4</td>
<td>286</td>
<td>86.2</td>
</tr>
</tbody>
</table>

The NUT1000 performed without interruption during this intensive sampling deployment. Raw data records clearly marked internal zero baseline and standard assay sample points. The instrument also flagged points as ‘-9999’ for samples producing erroneous readings. These data flags were used to subsample the raw data stream to limit analysis to ‘good’ measurements based on instrument criteria. At a quick look level of analysis, the NUT1000 provided a near continuous record of surface soluble reactive phosphorous although the raw instrument calls exhibited ca. 50 μg PO₄-P / L overestimate relative to the analytical grab samples (Fig. 4).

The constancy of this measurement offset (Fig. 5) while leading to overestimates of P loads by 1.5 to 4.5-fold in clear waters (Fig. 6), is generally indicative of a calibration issue. Analysis of internal standard measurements indicated a low and uniform reagent blank attenuation but a decline of ca. 0.002 in the attenuation of the 100 μg PO₄-P / L on board standard (Fig. 7). These changes do not account for the magnitude of the offset observed between NUT1000 and our analytical grab samples. This discrepancy indicated that a portion of the measurement offset could be attributable to optical effects due to differences in refractive index of natural seawater versus the reagent grade water used for preparation of internal standards and machine calibration. The NUT1000 detected analytical reference PO₄–P standards with reasonable accuracy and exhibited lower reagent blanks (Table 3). The analytical reference assay of the on-board analytical standard revealed a nominal 12 μg PO₄-P / L overestimate in the standard composition, again lower than the observed offset. The availability of consistent grab samples enabled post-cruise assessment of the optical response of the NUT1000. Comparison of the attenuation calibration response of the NUT1000 detector to field measurements of on-board standards with the attenuation response associated with seawater field grab samples revealed a consistent PO₄-P response factor (0.000077 AU/ [μg PO₄-P / L]), yet predicted a 4-fold higher baseline attenuation coefficient for natural seawater (Fig. 8). Simple post-cruise correction of the raw instrument calls by this baseline difference brings the NUT1000 measurements in line with the grab reference samples (Fig. 9) ranging between 0.5 and 1.5-fold offset. The consistent performance of the NUT1000 at the beta stage of product development demonstrates that this technology platform can provide robust in situ measurements of soluble reactive phosphorous provided suitable grab samples are provided.
Figure 3. Water Quality conditions in the sampling stream encountered by the NUT1000.
Figure 4. Time-series of surface soluble reactive phosphate (PO$_4$) concentrations determined in real-time by chemical assay within the NUT1000 during the surface mapping deployment. Instrument programmed to sample the flow through stream at ca. 0.05Hz. Data stream edited to remove internal standard and reagent blank assays. Equivalent standard method (STM) chemical assays of soluble phosphate on grab samples taken during along the cruise track after equilibrating the flow through stream on station for 5 min (<0.2 μ fraction green dots). A consistent overestimate (ca. 50 μg PO$_4$-P / L) is evident in the onboard NUT1000 calls compared to the reference sample assays. Field blanks (cyan) averaged 1.89 ± 0.470 μg PO$_4$-P / L.
Figure 5. Comparison of the soluble phosphate concentration calls by the NUT1000 in real-time against laboratory measured concentrations for reference samples taken from the flow-through stream. The NUT1000 data represents mean ± s.d. of initial concentration calls comprised of a 3 min window following the grab sampling time. Although a linear relationship is evident over a 1-order of magnitude span in natural phosphate concentrations ($[\text{PO}_4]_{\text{NUT}} = 0.927 [\text{PO}_4]_{\text{grab}} + 51.00, r^2 = 0.973$), the NUT1000 calls are clearly offset from the standard analysis, indicative of a calibration issue in the instrument setup.

Figure 6. Variation in the NUT1000’s calibration response during the course of the surface mapping trial, standardized against the associated reference sample value. No significant or consistent trend in the NUT1000’s relative calibration response was observed over the course of the 7 h deployment. However the instruments initial calls were significantly higher than the standard method assays (3.34 ± 1.00), but appeared to be more accurate in the lower salinity harbor waters.
Figure 7. Detection of on-board PO₄ standards (dark blue) and reagent blanks (cyan) by the NUT1000 analyzer during the surface mapping field test. Optical detection of the reagent blank solution was stable throughout the deployment (0.00083 ± 0.00030 AU), however, detector response to the on-board standard (set at 100 μg PO₄-P/L), exhibited a significant decay during the 7 h field deployment (-0.00034 AU h⁻¹). All reagents and standards were made up in the same batch of Type 1 water for the test.
Figure 8. Comparison of calibration response of the NUT1000 to on-board standards or post-hoc field grab sample. (a) In spite of the observed decay in on-board standard detection, the global calibration response to standards in a Milli-Q matrix was robust. (b) Employing a similar calibration approach against field grab samples exhibited a similar linear response slope (0.0000797 vs 0.0000745 AU/μg PO₄-P), but a significantly higher apparent reagent blank offset (0.004150 vs 0.000830 AU) indicating that the seawater matrix contributes significantly to baseline attenuation.
Figure 9. Variation in American Ecotech NUT1000 calibration response following post-cruise correction of dataset obtained during field mapping deployment (see Fig. 4). Application of simple linear correction based on offsets attributed to seawater matrix effects greatly improves accuracy of NUT1000 assays relative to the instrument’s real-time calls (1.02 ± 0.30 vs 3.34 ± 1.00 see Fig. 6). A decline in corrected calibration response is observed, consistent with the change in detector response to the on-board standard.
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 2.

Table 2. 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.
QA checks on Instrument Performance

Several additional instrument performance checks were conducted as part of the field deployment tests. Specifically, Table 3 describes the results of blank and mid-range phosphate standards that were presented to the NUT1000 at the beginning and end of the field test. In addition, we obtained a sample of the onboard standard immediately prior to the test and had it analyzed as a reference sample by our nutrient laboratory.

Table 3. Comparison of standard method chemical assay to NUT1000 calls of nutrient reference standards made as fresh dilutions from certified SPEX standard solutions. Instrument sample inlet immersed in fresh standard solution after MilliQ rinse and rinse with indicated pre- or post-reference solutions for at least 1 min. (ca. 3 samples). Exposures were conducted on-board the R\V John H. Martin immediately preceding or following the mapping trials. All solutions made in the same batch of freshly prepared Milli Q Type I water. Certified chemical analyses performed at the Nutrient Analytical Services Lab (NASL) at the Chesapeake Biological Laboratory. Values presented as μg PO₄-P / L representing mean (s.d.) of three assays of each standard solution.

<table>
<thead>
<tr>
<th>Standard Solution</th>
<th>[PO₄]NASL</th>
<th>[PO₄]NUT1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>MilliQ pre</td>
<td>1.88 (0.13)</td>
<td>0.28 (0.41)</td>
</tr>
<tr>
<td>PO4 pre</td>
<td>32.77 (0.32)</td>
<td>36.76 (5.90)</td>
</tr>
<tr>
<td>Milli Q post</td>
<td>2.00 (0.11)</td>
<td>-1.17 (2.90)</td>
</tr>
<tr>
<td>PO4 post</td>
<td>32.32 (1.18)</td>
<td>32.90 (1.21)</td>
</tr>
<tr>
<td>On board PO4 Std</td>
<td>88.80 (1.39)</td>
<td>106.16 (3.37)</td>
</tr>
</tbody>
</table>

Reference Sample analysis

Several additional levels of QA/QC were applied to the field test. First, field trip blanks were collected once a week during mooring tests and on two occasions during the surface mapping and vertical profiling tests. The mean and standard deviation of these results were plotted directly onto the time series plots (see figure 3) to provide a relative sense of the signal to noise ratio and to provide a measure of the cleanliness of our sampling processing. Secondly, nutrient spikes of reference samples were performed once a week during mooring tests and on two occasions during the surface mapping and vertical profiling tests. The results of the reference sample spikes are given in Table 4. Lastly we established a QA criteria that the results of triplicate analysis on each reference sample should not exceed 15%. If this value was exceeded than the two samples held in reserve were submitted for re-analysis. Subsequently, all five values were examined and the three values that produced the lowest coefficient of variation were selected to provide the final value of that reference sample.
**Table 4.** Percent recovery of phosphate added to field reference samples. Spikes were performed on two of the reference grab sample timepoints. All concentrations were determined on triplicates and expressed as \( \mu \text{g PO}_4\text{-P} / \text{L (s.d.)} \)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Field Sample</strong></td>
<td>26.1 (0.2)</td>
<td>22.5 (0.2)</td>
</tr>
<tr>
<td><strong>Field Sample + P-spike</strong></td>
<td>317.1 (17.5)</td>
<td>308.6 (16.9)</td>
</tr>
<tr>
<td><strong>Observed Spike</strong></td>
<td>291.1</td>
<td>286.2</td>
</tr>
<tr>
<td><strong>Expected Spike</strong></td>
<td>310.0</td>
<td>310.0</td>
</tr>
<tr>
<td><strong>Percent Recovery</strong></td>
<td>93.9</td>
<td>92.3</td>
</tr>
</tbody>
</table>

**RELIABILITY:**

A six-hour continuous underway surface concentration mapping application was successfully completed with 100 percent of the expected data reported and useable. In addition to the surface mapping application, two moored application tests were attempted, but distinct instrument malfunctions resulted in aborted tests at both locations. It should be noted that this instrument as configured was not designed for mooring applications. Any difficulty in deploying instruments or collecting data during this ACT Demonstration seemed to be related to packaging or processing issue that can likely be addressed with engineering refinements. In general, however, it appears that the fundamental technology of the NUT 1000 has the capability to successfully measure in situ phosphate concentrations over a wide range of water quality conditions and can be particularly effective for rapid, survey-based applications due to its high sampling rate capability and extreme portability. Finally, while real-time in situ measurements provide good proxies for relative differences in P concentration over sampling time and space scales, the utility of incorporating systematic grab samples to enable post sampling verification of the measurements cannot be overemphasized.
ACKNOWLEDGMENTS:

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