



## PERFORMANCE DEMONSTRATION STATEMENT

### *Satlantic ISUS V3 Nitrate Sensor*

---

<b>TECHNOLOGY TYPE:</b>	Field portable nutrient analyzer
<b>APPLICATION:</b>	In situ estimates of dissolved nitrate from moored, surface mapping and vertical profiling deployments
<b>GOALS:</b>	Demonstrate the capabilities and potential of this instrument
<b>TYPE OF EVALUATION:</b>	Field Performance Demonstration at four ACT Partner sites
<b>DATE OF EVALUATION:</b>	Testing conducted from May through October 2007
<b>EVALUATION PERSONNEL:</b>	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.

Questions and comments should be directed to: Dr. Thomas Johengen  
Alliance for Coastal Technologies  
c/o Chesapeake Biological Laboratory  
PO Box 38 / One Williams Street  
Solomons, Maryland 20688, USA  
Email: Johengen@umich.edu

**TABLE OF CONTENTS:**

Page No.

Executive Summary.....	3
Background.....	4
Type of Technology .....	4
Objectives of the Nutrient Analyzer Performance Demonstration .....	5
Parameters Evaluated .....	5
Summary of Demonstration Protocols .....	5
Demonstration Results, Surface Mapping Test.....	10
Demonstration Results, Vertical Profiling Test .....	15
Demonstration Results, Moored Tests .....	18
Patuxent River, Chesapeake Bay, MD (estuary) .....	19
Resurrection Bay, Gulf of Alaska .....	25
Quality Assurance/Quality Control.....	31
Reliability.....	32
Appendix 1. Manufacturer interpretation of results	

## 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 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 Satlantic ISUS V3 under diverse environmental conditions in surface mapping, depth profiling and moored deployment field tests. A total of three different field sites were used for testing, including estuary, open-ocean, and riverine environments. For the surface mapping deployment in Monterey Bay, the ISUS nitrate estimates closely tracked ambient nitrate concentrations (ISUS:Reference Sample =  $1.04 \pm 0.2$ ) over a two-order magnitude range and all expected data were reported. The ISUS successfully reported out nitrate concentrations at a 1 Hz sampling rate over two vertical profiling tests. Agreement with field reference sample concentrations was generally good, but a calibration offset was apparent from direct comparisons. The ISUS was successfully tested in a moored application at two of the three attempted test sites. A mechanical failure of the battery pack was encountered in the Michigan mooring test and no data were collected. All expected data were reported out from the other two mooring test sites in Chesapeake Bay, MD and Resurrection Bay, Alaska. In the Chesapeake Bay test the ISUS in situ measurements showed good overall agreement with reference samples ( $R^2 = 0.8$ ) but reported concentrations were positively biased at a ratio of around 2.1 times laboratory measured concentrations. In Resurrection Bay, AK the response of the ISUS was more variable, but within its specified accuracy of  $2 \mu\text{M}$ , and had a similar overall positive bias of 2.3 times laboratory measured concentrations. During the last 10 days of the AK deployment the ratio of instrument:reference samples improved to a mean ratio of 1.4. Exposure to both nitrate and nitrite samples singularly and in mixtures shows that while the ISUS responds to the presence of nitrite in either pure solution or mixtures that the calibrated optical algorithms provided with the instrument produce accurate estimates of nitrate in mixed standard solutions at similar molar ratios, as well as, in ambient waters where nitrite is typically less than 10% of nitrate concentrations.

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-test corrections to the data and the manufacturer's interpretation of the test results are presented in Appendix 1. In general, the ISUS V3 demonstrated the capability to successfully measure in situ nitrate concentrations under a variety of field conditions and in multiple sampling applications.

## 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](http://www.act-us.info)).

This document summarizes the procedures used and results of an ACT Demonstration to examine the performance of the ISUS V3 nitrate 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](http://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](http://www.act-us.info/evaluation_reports.php).

## TECHNOLOGY TYPE:

Satlantic's ISUS V3 nitrate sensor is the latest version of the original MBARI-ISUS nitrate sensor developed by Dr. Ken Johnson and Luke Coletti of the Monterey Bay Aquarium Research Institute (MBARI). The ISUS V3 uses the proven ultraviolet absorption spectroscopy technology to measure in situ dissolved chemical species and now comes with full USB communications and a Windows based program (ISUSCom) for system configuration and data download. The instrument consists of four key components: a stable UV light source, a UV spectrometer, a fiber optic sampling probe and a processing computer. All components are housed within a single pressure case with separate connectors for analog, serial and USB output. The instrument can be set to output nitrate concentrations in real-time at a rate of 1 Hz for profiling applications, or it can be programmed to sample on a defined schedule and log data internally for mooring deployments. An antifouling guard consisting of a combination of a 100  $\mu\text{m}$  Nitex screen and perforated copper shield is used to cover the sampling probe during long-term deployments to provide protection from fouling organisms.

The ISUS computes nitrate concentrations from the absorption characteristics of inorganic compounds in the 200-400nm range of the UV-spectrum. By illuminating a sample of seawater with UV light onto an UV spectrometer, the absorption spectra are measured. Calibration parameters for the nitrate calculations are determined at Satlantic by measuring absorption spectra of standard samples in the range of 0-40  $\mu\text{M}$  nitrate, 0-35 psu (practical salinity units) and 0-20  $^{\circ}\text{C}$  temperature. The resulting calibration coefficients fully characterize the instrument for a range of field applications.

## **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 four 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](http://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](http://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 instruments 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 multiple applications of surface mapping, vertical profiling, and 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 reference 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). The nitrate method employed 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), but modified to use an enzymatic reduction of nitrate instead of the traditional cadmium reduction method (Campbell, W.H. E.R. Campbell, and L. Egan 2006. Green Chemistry Nitrate Determination: An Alternative Nitrate Analysis Method. American Laboratory, February 2006).

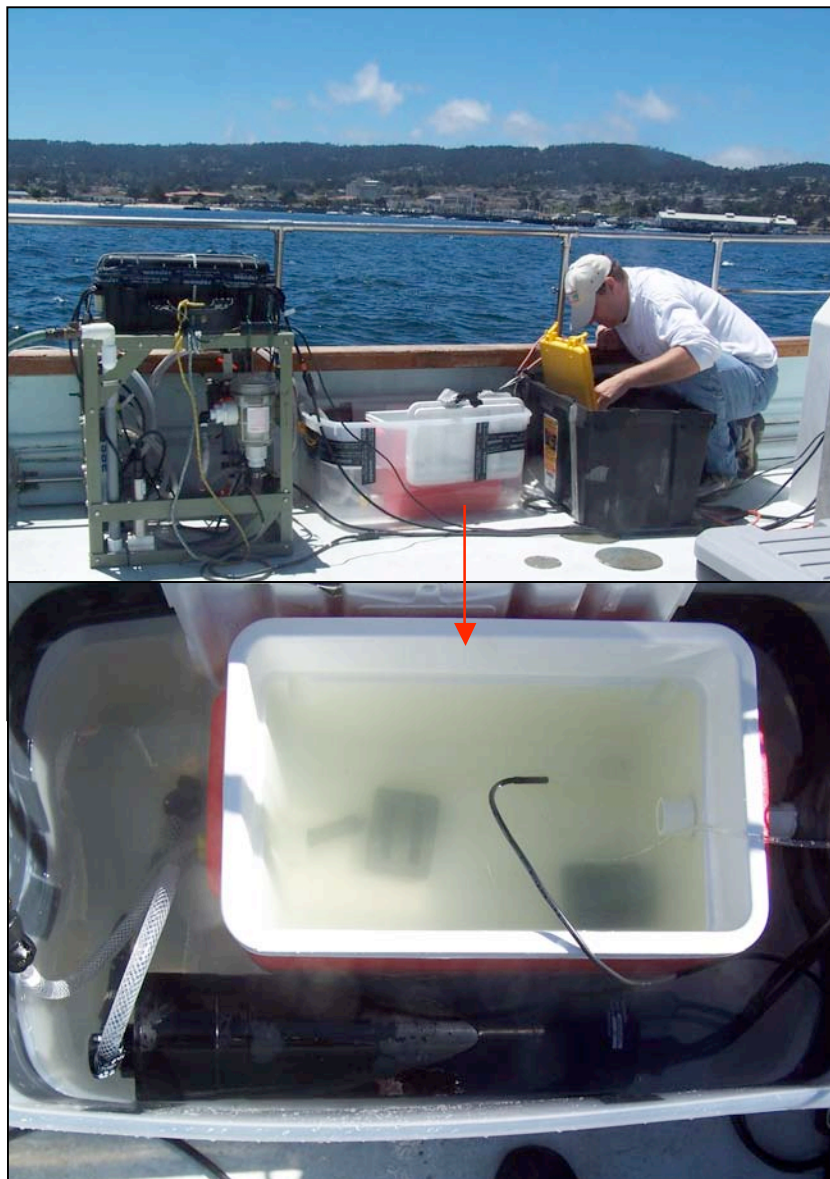
All laboratory nutrient analyses were conducted on an Aquakem 250. A statistically determined detection limit for this method has been established at  $0.05 \mu\text{M}$  and  $0.043 \mu\text{M}$  for nitrate and nitrite respectively, by prior laboratory studies. The typical working concentration range for the nitrate method and SOP was between  $0.0049 - 5.6 \text{ mgN/L}$  ( $0.35 - 20 \mu\text{M}$ ). The system contained an auto-dilutor to bring any higher concentrations down to the established linear calibration range. 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 duplicates and sample spikes were analyzed with each analytical batch.

### Surface Mapping Deployment

This 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 turbidometer and a Wet Labs C-Star, 10cm transmissometer (full descriptions and archival data for all of MLML’s UDAS systems can be found at <http://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 ISUS V3 flow-through cap was plumbed into the PortUDAS outflow stream and water delivered into a 10L acid washed plastic cooler which provided clean sampling access for field grab samples. Water samples were taken near the inflow of the sampling cooler. The sampling cooler and ISUS\_V3 were placed in a larger polypropylene tub which received overflow water, enabling maintenance of the sensor housing at ambient water temperatures while deployed on deck (Fig. 1). Flow

rate through the PortUDAS system in this configuration was ca. 12 L/min. The ISUS\_V3 was configured to sample at 1Hz and dark frames taken after every ten measurements. Data was monitored in real-time via the instrument's USB port and the ISUS V3 was powered via a regulated TPC-2000D Digital DC supply.

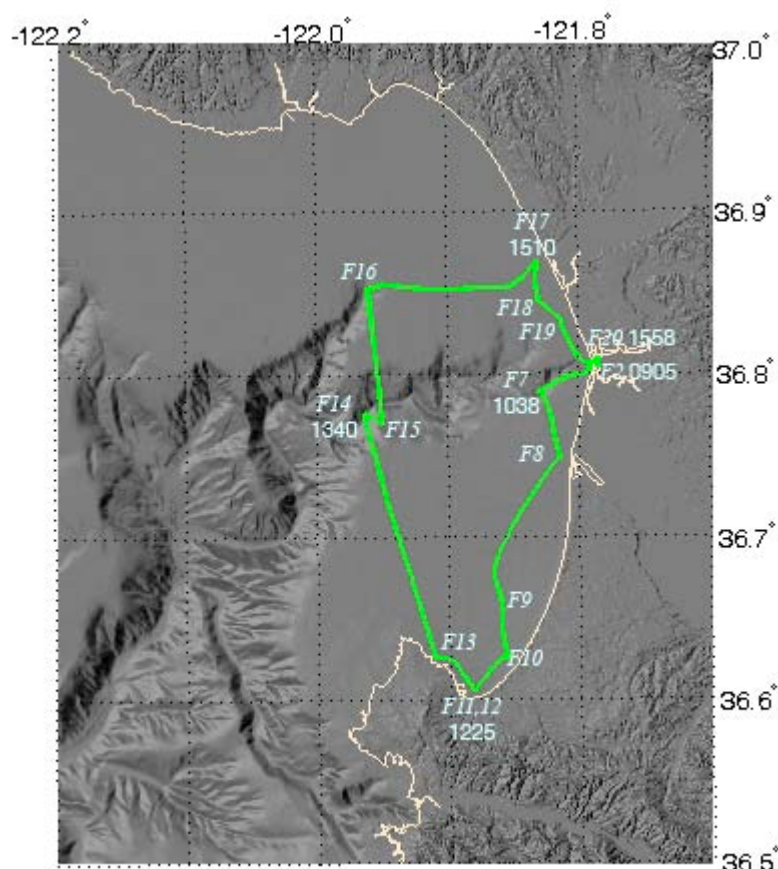


**Figure 1.** Working configuration of instrumentation used in surface mapping deployment on Monterey Bay, 19 July 2007. (1) A portable WQ monitoring system was used to draw water from -1m through the stern hull of the R/V John H. Martin (left). The PortUDAS system recorded oceanographic WQ conditions 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 at end of deployment for the ISUS V3.

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 and allowed to fill with new water best representing that locale. Acid washed sample bottles were then rinsed by three fillings with the new sample water and a 1 liter sample taken at the local time recorded. A sterile 0.2 $\mu$  nylon filter with 500mL



bottle was rinsed three times by filtering ca. 150mL of sample per rinse, finally ca 500 mL was collected and the sealed filtrate reservoir 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 is provided to highlight position of sampling relative to major geomorphologic features of the Monterey Bay region. The cruise 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.

### Vertical Profiling Deployment

A vertical profiling application was conducted in Resurrection Bay, Alaska. The vertical profiling test consisted of two independent profiles conducted at varying locations during a single cruise, where simultaneous instrument measurements and discrete samples are collected from the ship at six discrete depths throughout the water column. The rosette was maintained at sampling depths for 2 minutes prior to firing the sampling bottle to ensure that water has reached equilibrium with respect to any mixing influence from lowering the unit and that the bottle is well flushed. The ISUS V3 was mounted within a modified Niskin bottle rosette so that the sensor and bottles sampled near the same depth as physically possible. A standard and calibrated CTD package was attached to the rosette and programmed to provide an independent record of conductivity, temperature, depth and time during each instrument sampling event.

### 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 ISUS V3 was provided with a laboratory blank (type 1 deionized water, DIW) and reference standard (ca. 15-20  $\mu\text{M}$ ) 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.

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 instruments 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.

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 In Situ 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. Technical procedures included methods to assure proper handling and care of test instruments. All implementation activities were documented and are traceable to the test/QA plan and SOPs and to test personnel.

Various levels of QAQC were applied to the sampling and analytical protocols for each field test. First, ACT provided the companies with a laboratory blank (type 1 deionized water, DIW) and reference standard both before and after the field test deployment. All concentrations were confirmed by analysis at NASL. Secondly, field trip blanks were collected once a week during mooring tests and on two occasions during the surface mapping and vertical profiling 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.

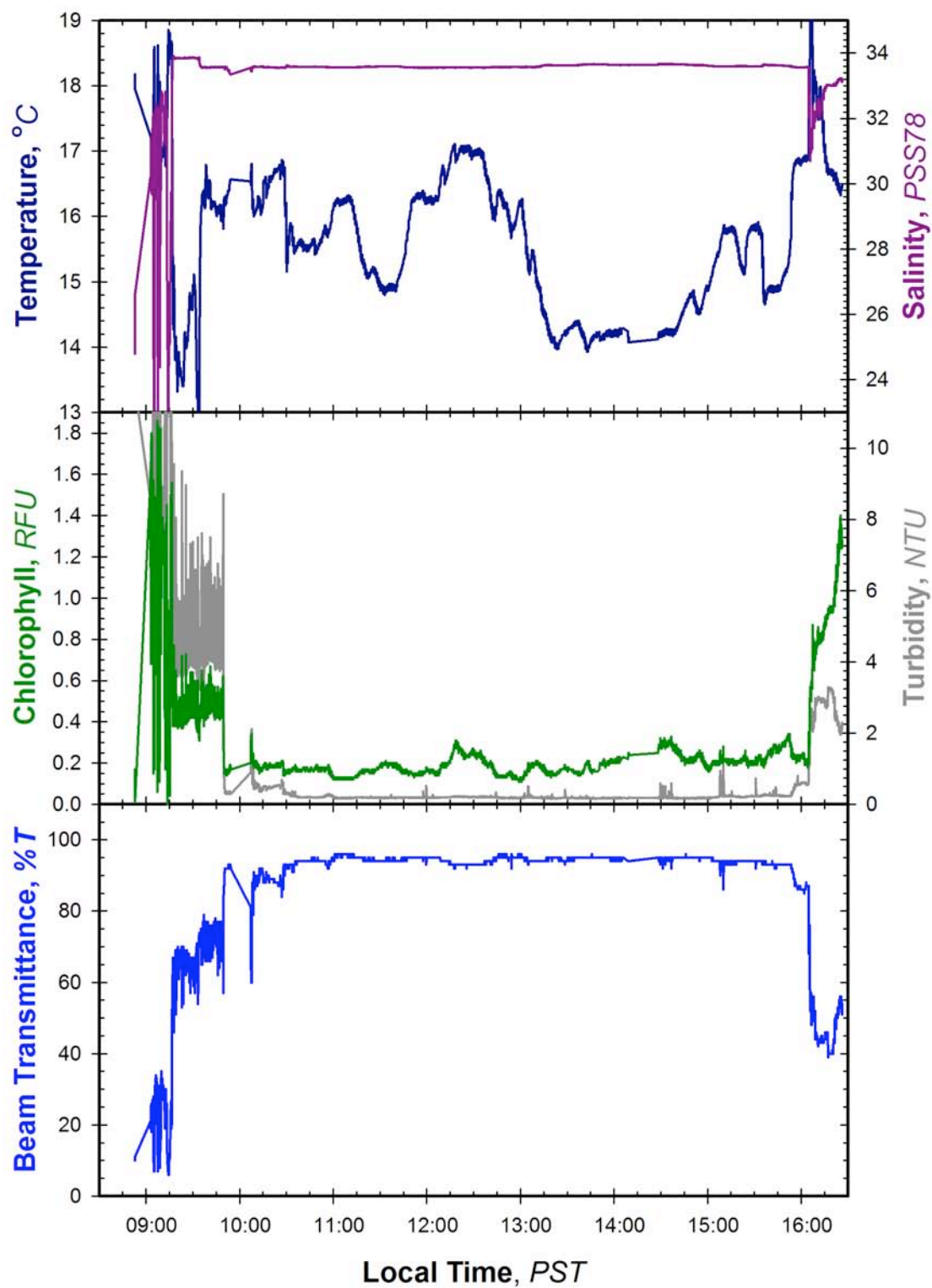
## DEMONSTRATION RESULTS:

The Satlantic ISUS V3 was successfully tested in a surface mapping application in Monterey Bay, CA, in a vertical profiling application in Resurrection Bay, AK and at two fixed mooring applications in Chesapeake Bay, MD and Resurrection Bay, AK. A third mooring test in the Clinton River, Mt. Clemens, MI was unsuccessful due to a mechanical power failure of the supplied battery pack. The instrument tried several times to initiate the sampling program but did not receive sufficient power from the battery. Therefore no data was collected from this test site.

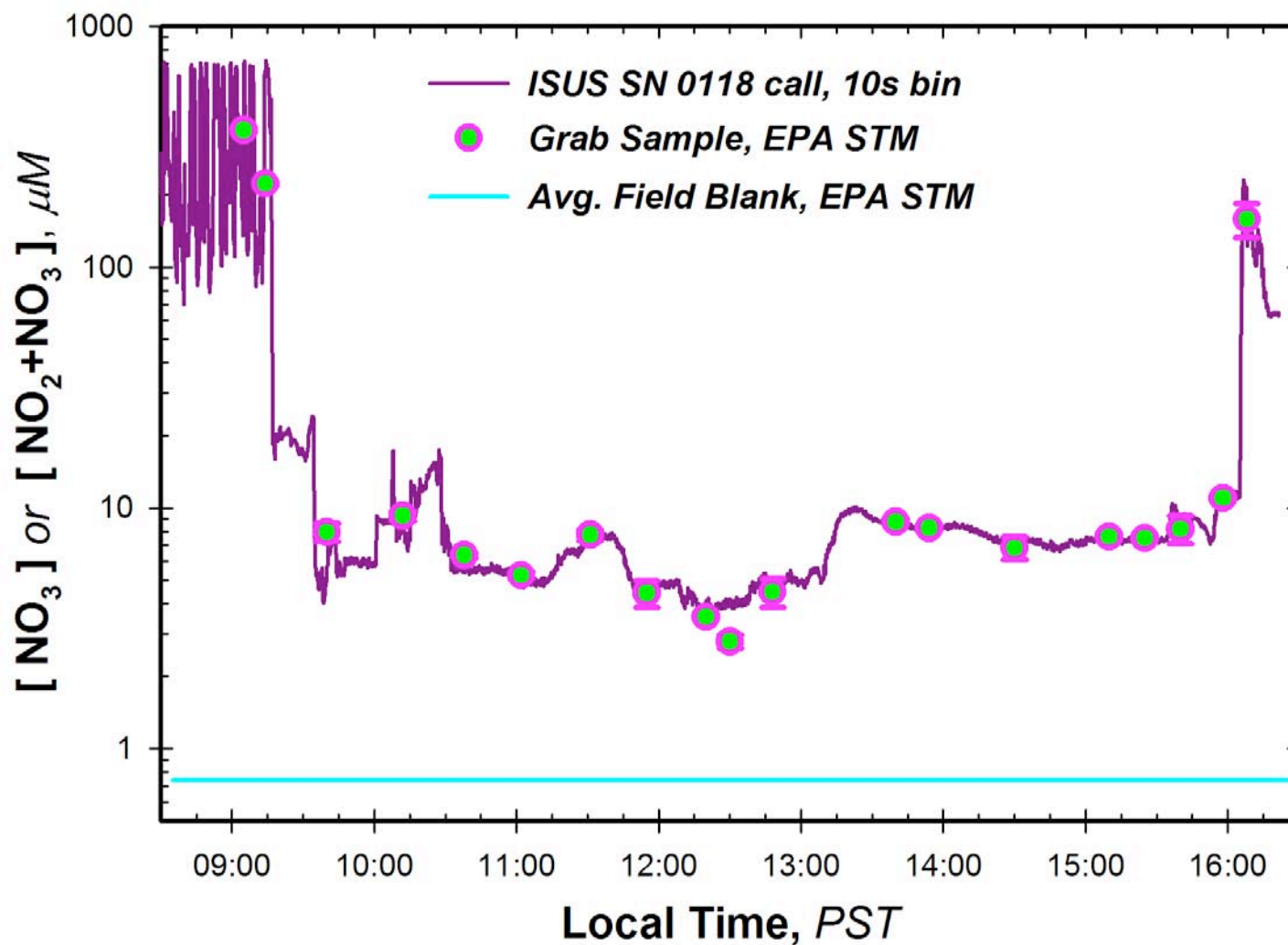
### Surface Mapping in Monterey Bay, CA

Highly variable water quality conditions were encountered at -1m during the 19 July 2007 surface mapping cruise aboard the R/V John H. Martin. 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 were 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.

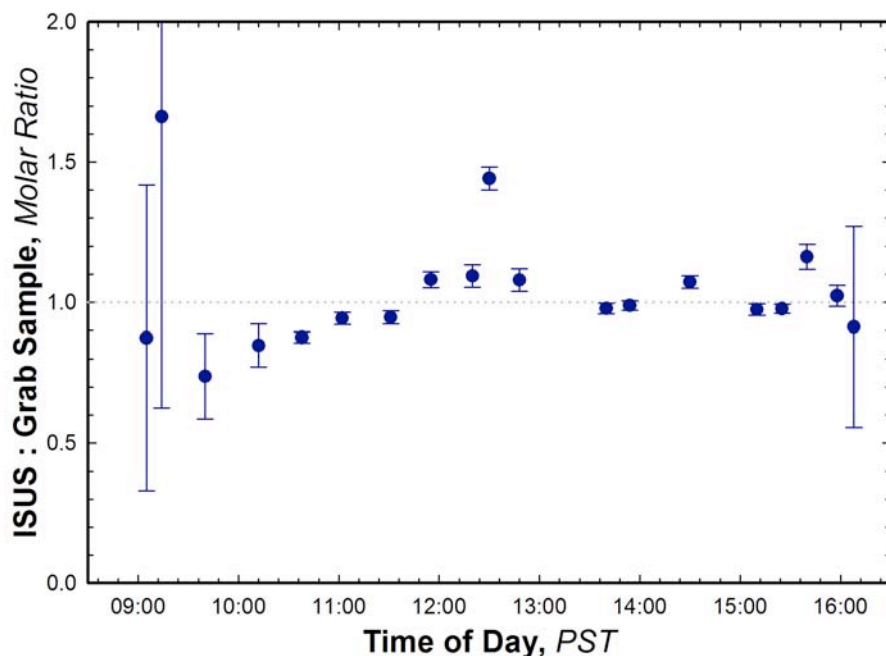
The Satlantic ISUS V3 collected data continuously during the deployment and no dropped frames were observed. Initial instrument calls of ambient nitrate concentrations ( $\mu\text{M}$ ) spanned two orders of magnitude along the cruise track (Fig. 4). ISUS V3 nitrate estimates were in excellent agreement with chemical measurements on field grab samples (ISUS:Grab  $1.04 \pm 0.21$ ) across this broad concentration range (Fig. 5). Instrument calls were more variable in the highly turbid harbor waters. Overall, the ISUS V3 response was linear with respect to traditional wet chemical analysis over encountered range of 3-400  $\mu\text{M NO}_2 + \text{NO}_3$  (Fig. 6). Although the ISUS V3 is capable of detecting nitrite ( $\text{NO}_2$ ) with high efficiency in pure solution, the calibrated optical algorithms provided with the instrument yield accurate measures of nitrate ( $\text{NO}_3$ ) in mixed standard solutions (4:1  $\text{NO}_3:\text{NO}_2$  molar ratio, Table 1) as well in natural waters encountered in the field survey where nitrite concentrations were below 1  $\mu\text{M}$  ( $0.15 \pm 0.21 \mu\text{M NO}_2$ ).



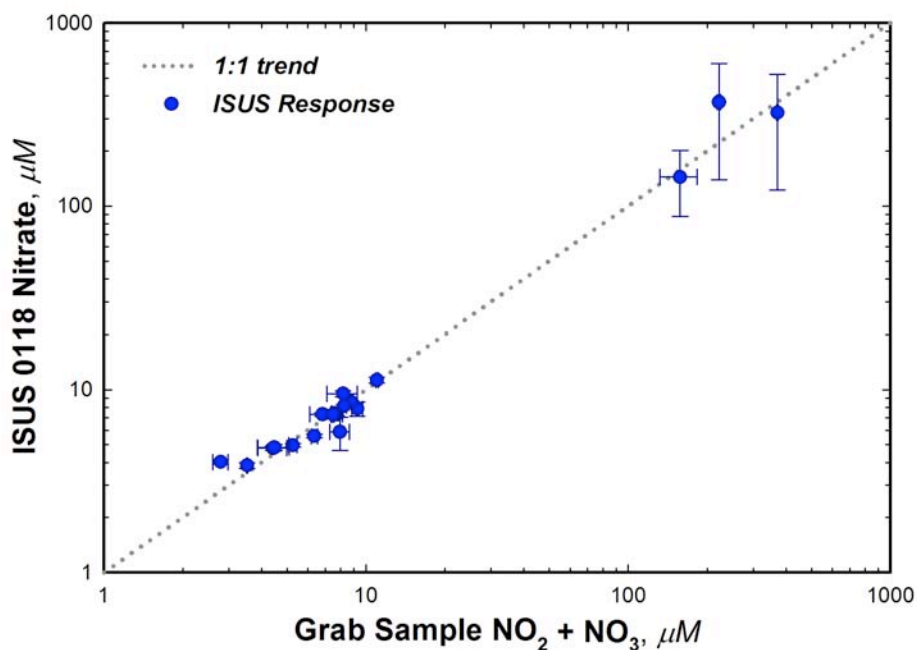
**Figure 3.** Water Quality conditions in the sampling stream encountered by the ISUS V3.



**Figure 4.** Time-series of surface nitrate concentrations determined optically by the Satlantic ISUS V3. Instrument programmed to sample the flow through stream at 1Hz. Data was binned to 10s average calls of nitrate concentration. Standard method (STM) chemical assays of total nitrates  $[\text{NO}_2 + \text{NO}_3]$  on grab samples taken during along the cruise track after equilibrating the flow through stream on station for 5 minutes. Logarithmic scale on nitrate concentration axis required to plot the 2-order of magnitude span in nitrate concentrations encountered during the field deployment. Field blanks averaged  $< 0.8 \mu\text{M}$ .



**Figure 5.** Comparison of the nitrate concentration calls derived optically by the Satlantic ISUS V3 and by standard method chemical analysis of total nitrates ( $\text{NO}_2 + \text{NO}_3$ ) taken from grab samples of the flow-through stream. ISUS data represents mean  $\pm$  s.d. of initial concentration calls comprised of a 3 min window around the grab sampling time. Over the 2-order of magnitude span in nitrate concentration encounter, the agreement between the two methods adheres to a 1:1 relationship.



**Figure 6.** Variation in the Satlantic ISUS V3 calibration response during the course of the surface mapping trail, standardized to the associated reference sample value. No significant or consistent trend in the ISUS's relative calibration response was observed over the course of the 7 h deployment at 1 Hz sampling and was in close agreement with traditional chemical assays of total nitrates ( $1.04 \pm 0.21$ ).

**Table 1.** Comparison of standard method chemical assay to ISUS\_V3 calls of select nutrient reference standards made as fresh dilutions from certified SPEX standard solutions of each nutrient. Instrument detector immersed in fresh standard solution after DIW rinse and rinse with indicated standard pre and post-reference solutions for at least 1 min. Exposures were conducted on board the R/V John H. Martin immediately preceding or following the surface mapping trials. All solutions made in the same batch of freshly prepared DIW Type I water. Certified chemical analyses performed at the Nutrient Analytical Services Lab (NASL) at the Chesapeake Biological Laboratory. Values presented as  $\mu\text{M}$  representing mean (s.d.) of three assays of each standard solution.  $[\text{NO}_3]$  determined as difference between  $[\text{NO}_3 + \text{NO}_2]$  and  $[\text{NO}_2]$  measured on aliquots of the same solutions.

Standard Solution	$[\text{NO}_3 + \text{NO}_2]_{\text{NASL}}$	$[\text{NO}_3]_{\text{NASL}}$	$[\text{NO}_3]_{\text{ISUS}}$
DIW- pre	0.0405 (0.0454)	0.0297	0.719 (0.267)
$\text{NO}_2$ -pre	19.9509 (0.1530)	0.0297	49.755 (0.394)
$\text{NO}_3$ -pre	16.3182 (0.1270)	16.3074	15.538 (0.304)
$\text{NO}_3 + \text{NO}_2$ -pre	41.3068 (0.2107)	31.6333	32.214 (0.318)
DIW -post	0.0653 (0.0663)	0.0522	1.334 (0.328)
$\text{NO}_2$ -post	19.9911 (0.1017)	0.0522	51.302 (0.471)
$\text{NO}_3$ -post	16.5383 (0.2262)	16.5253	16.295 (0.313)
$\text{NO}_3 + \text{NO}_2$ -post	41.5270 (0.7762)	31.9204	33.419 (0.412)

**Table 2.** Percent recovery of nitrate 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  $\mu\text{g} (\text{NO}_3 + \text{NO}_2)\text{-N} / \text{L}$  (s.d.).

	<i>1</i>	<i>2</i>
Field Sample	73.6 (2.7)	122.6 (2.9)
Field Sample + $\text{NO}_3$ -spike	744.5 (67.5)	862.8 (2.0)
Observed Spike	671.1	740.7
Expected Spike	700.0	700.0
Percent Recovery	95.9	105.8

### Vertical Profiling in Resurrection Bay, AK

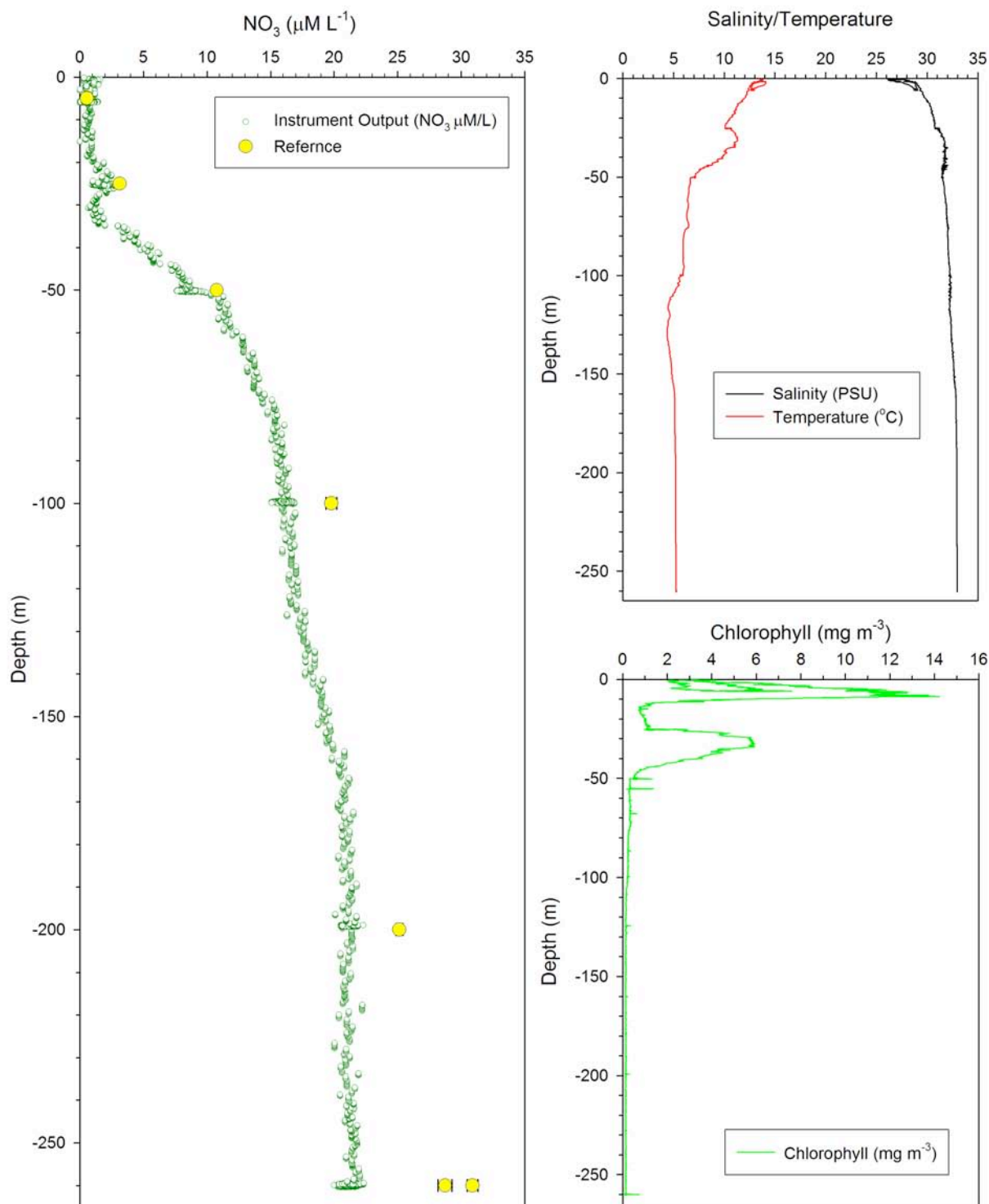
A vertical profiling test was conducting at two sites within Resurrection Bay, AK (Fig. 7). The first profile was taken at site GAK1, a relatively open ocean site, and consisted of a 260 m deep cast with the CTD/Rosette and ISUS V3. The temperature profile ranged from 4 -14 °C, salinity ranged from 27 – 33, and two distinct peaks in chlorophyll concentration occurred at 10 m and 40 m, with sub 1 mg m<sup>-3</sup> levels below 50m (Fig 8). Nitrate concentrations were severely depleted in the surface water (ca 0.6 μM) and increased to a maximum of 30 μM. The ISUS V3 called concentrations mapped the reference samples quite closely but with increasing offset at the higher concentrations at depth. The average of the ratio the directly compared ISUS V3 versus reference sample concentrations ranged from 0.55 – 1.07 with mean of  $0.78 \pm 0.16$  for the six comparisons. This amounts to a concentration offset of between 0 – 9 μM.



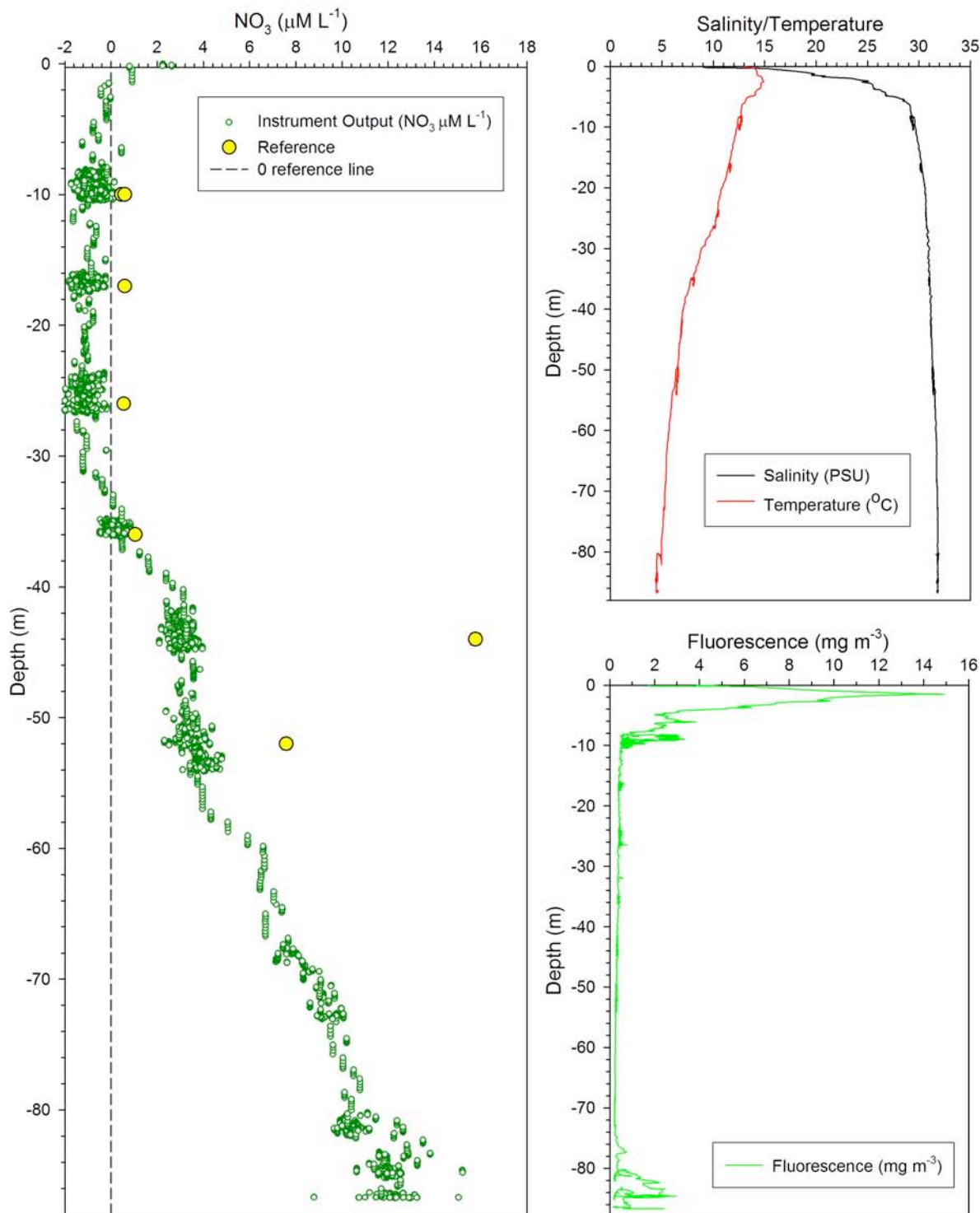
**Figure 7.** Profiling test site locations in Resurrection Bay, AK. The GAK1 site represents fairly open ocean conditions, while the SMC1 site was within influence of the plume of the Resurrection River

The second profile was taken at site SMC1, near Seward Harbor in a region influenced by the inflow of the Resurrection River. An 80 m deep cast was conducted, but reference samples were taken only to 52 m as the ship drifted into shallower water during the cast and the rosette had to be raised to a safe sampling depth. The temperature profile at this site also ranged from 4 -14 °C but salinity was ranged from 9 – 33 with a sharp pycnocline at 10 m. A single large peak in chlorophyll concentration occurred at 10 m with sub 1 mg m<sup>-3</sup> levels below 10m (Fig 9). Nitrate concentrations were severely depleted in the surface water (ca 0.5 μM) and increased with depths mostly below the reference sampling depths. There is no known reason for the unexpectedly high reference sample concentration at 42 m. The ISUS V3 called concentrations showed a low bias of approximately 1.3 μM for the first 4 depths increasing to 4.0 μM for the comparison at 52 m.





**Figure 8.** Vertical profile of nitrate concentrations determined optically by the Satlantic ISUS V3 versus laboratory measured reference sample concentration of total nitrates [ $\text{NO}_3 + \text{NO}_2$ ] collected by Niskin bottles at six discrete depths for site GAK1. Replicates bottles were collected at the deepest sampling depth. Ancillary data for temperature, salinity, and chlorophyll were recorded by a CTD profiler.



**Figure 9.** Vertical profile of nitrate concentrations determined optically by the Satlantic ISUS V3 versus laboratory measured reference sample concentration of total nitrates [ $\text{NO}_3 + \text{NO}_2$ ] collected by Niskin bottles at six discrete depths for site SMC1. Replicates bottles were collected at the deepest sampling depth. Ancillary data for temperature, salinity, and chlorophyll were recorded by a plus CTD profiler.

## Moored Deployments

The ISUS V3 was tested under a long-term (approximately 30d) mooring deployment at three different field sites that included Chesapeake Bay, MD, Resurrection Bay, AK, and Clinton River, Mt. Clemens, MI (see Table 3). A mechanical failure in the power source resulted in an unsuccessful test at the Clinton River site and no results are available for that deployment.

**Table 3.** The ACT Partners site, dates, and basic physical/chemical conditions observed during the moored deployment field tests for the ISUS V3 nitrate analyzer. Temperature and Salinity (Conductivity) were determined by a CTD, relative fluorescence was measured with a fluorometer and transmissivity was measured with a 25cm path length transmissometer.

SITES		Temp. (°C)	Salinity/ Conductivity ( $\mu$ S/cm)	Fluorescence (mV)	% Beam Transmission
<b>Chesapeake Bay, MD</b> (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
<b>Resurrection Bay, AK</b> (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

Results are reported out by individual site and summarize the complete time series of nutrient concentrations predicted by the ISUS V3 during the deployment as well as direct comparisons of the reference sample concentrations determined by NASL. In addition we report the results of exposure to blanks and standards performed immediately before and after the deployment.

### Moored Deployment Results in Chesapeake Bay, MD

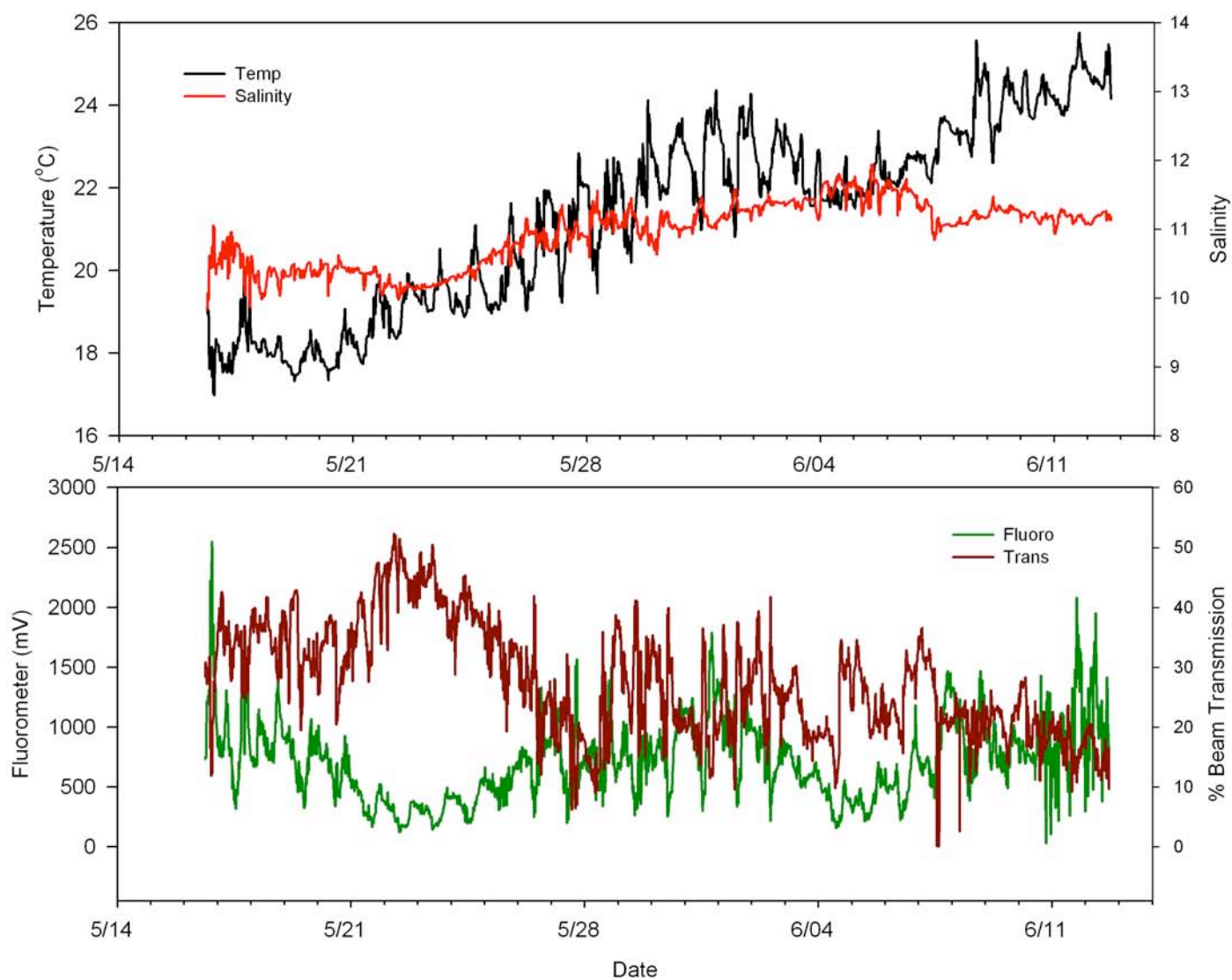
The mooring test in Chesapeake Bay took place at the end of a fixed pier located at the mouth of the Patuxent River on the campus of the Chesapeake Biological Laboratory (Fig. 10). The water depth of the test site was 2.2 m. The site water was generally brackish with salinity ranging from 9.8 - 11.9, and water temperature ranged from 17.0 - 25.8. (Fig.11). The water was quite turbid (mean % beam transmission = 27) with significant algal concentrations (mean fluorescence = 713 mV) (Table 3 and Fig. 11).



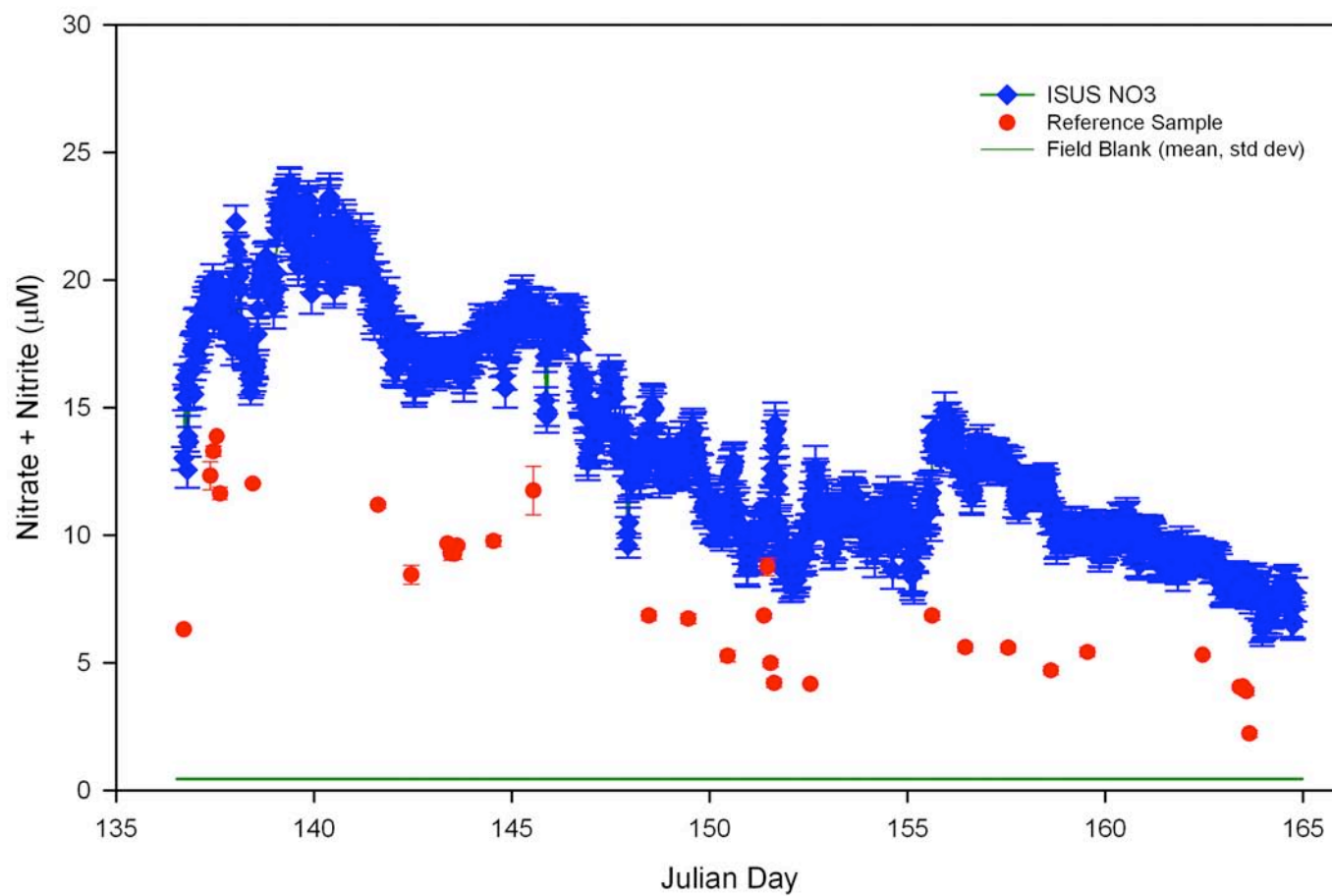
**Figure 10.** Site Photos from the field deployment in Chesapeake Bay, MD. Left - fixed pier at mouth of Patuxent River on the campus of the Chesapeake Biological Laboratory. Right - ACT staff collecting a reference sample next to the instrument sampling inlet through a well on the sampling platform.

Nitrate + nitrite concentrations generally declined over the course of the test, ranging from a maximum of  $13.9 \mu\text{M}$  to a minimum of  $2.22 \mu\text{M}$  based on reference sample measurements. Nitrite accounted for between 2 - 7 percent (mean = 4%) of the two species and nitrite results are not presented individually. Field trip blanks (N=4) averaged  $0.44 \pm 0.03 \mu\text{M}$ , accounting for, on average, less than 6% of the reference sample concentrations.

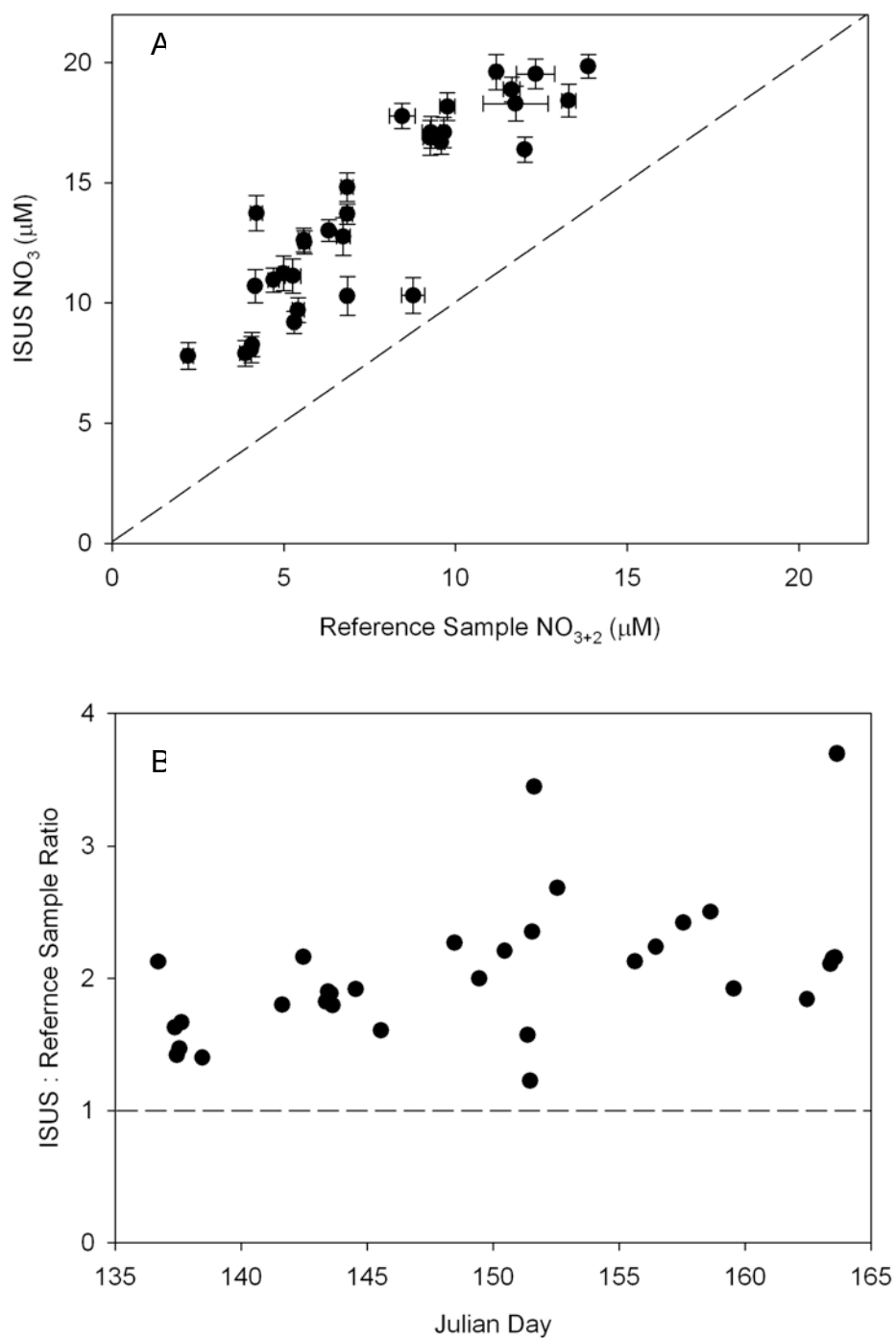
The ISUS V3 determined in situ concentrations closely followed the overall pattern of reference sample concentrations throughout the deployment but with a consistent positive bias (Fig.12). A linear regression of directly compared instrument versus reference sample concentrations confirms the strong correlation ( $R^2 = 0.80$ ) (Fig. 13a). A time series plot of the ratio of corresponding instrument versus laboratory measurements indicates the calibration offset was fairly consistent throughout the deployment, with a mean ratio of  $2.1 \pm 0.53 \mu\text{M}$  (Fig. 13b). This result suggests instrument response was not affected by time or concentration during the deployment.



**Figure 11.** Ancillary data collected at the Chesapeake Bay, MD field test site describing conditions of temperature, salinity, algal fluorescence and water transparency.



**Figure 12.** Time series comparison of the Satlantic ISUS V3 measured nitrate concentrations against laboratory measured reference samples and field trip blanks for the Chesapeake Bay, MD moored deployment test. (Laboratory measured concentrations represent mean  $\pm$  sd.,  $n=3$ ).



**Figure 13.** Analysis of test results from Chesapeake Bay, MD test site. (A) One to one comparison of ISUS V3 in situ nitrate determinations versus laboratory determined concentrations for matching field reference samples. (B) Time series of the ratio of ISUS V3 versus laboratory determined nitrate concentrations for matching field reference samples.



### Moored Deployment in Chesapeake Bay, MD cont.

A set of pre- and post-deployment exposures to blanks (DIW) and reference standards were to be completed in the laboratory as part of each field test. The ISUS V3 reported un-measurable (slightly negative) nitrate levels for the DIW blanks for both the pre- and post-test exposures. The ISUS V3 determined concentration for a pure nitrate standard was approximately 80 % of the laboratory determined value for measurement, with a low bias of between 2.7 to 3.3  $\mu\text{M}$  for the pre- and post-test exposures, respectively (Table 4). The ISUS V3 measured concentration for a pure nitrite standard was approximately 117% of the laboratory based measurement, or a positive bias of about 2.7  $\mu\text{M}$  for the pre-test exposure. We are unsure of the reason for the larger discrepancy in the post-test nitrate exposure but believe a sample handling error may have occurred as the lab results should have been similar to the pre-test value of 15.5  $\mu\text{M}$ . The ISUS response to this standard was very consistent between the pre- and post-test exposures.

**Table 4.** Comparison of reported blank, nitrate, and nitrite values for pre- and post-test exposures between for the ISUS V3 versus laboratory determined concentrations for the Chesapeake Bay, MD mooring test. (mean, s.d. for n=3 replicates).

	NASL Result ( $\mu\text{M}$ )	ISUS Results ( $\mu\text{M}$ )
<b>Lab Blank-pre</b>	0.062 (0.011)	-0.461 (0.525)
<b>NO<sub>3</sub> Standard-pre</b>	17.97 (0.38)	15.00 (0.56)
<b>NO<sub>2</sub> Standard-pre</b>	15.51 (0.56)	18.22 (0.57)
<b>Lab Blank-post</b>	0.048 (0.00)	-1.15 (0.48)
<b>NO<sub>3</sub> Standard-post</b>	16.93 (0.40)	13.00 (0.50)
<b>NO<sub>2</sub> Standard-post</b>	8.99 (0.46)	18.41 (0.64)

Weekly nutrient spikes of field reference samples were performed to examine the consistency of sample handling and NASL nutrient analysis (Table 5). The percent recovery for nitrate spikes ranged from 92 - 99 percent with a mean of  $96.5 \pm 3.4$ . The percent recovery for nitrite spikes ranged from 97 - 104 percent with a mean of  $100.6 \pm 2.7$ .

**Table 5.** Percent recovery of nitrate and nitrite added to field reference samples from the Chesapeake Bay, MD mooring test. Spikes were performed once each week at each of the test sites. All concentrations were determined on triplicates.

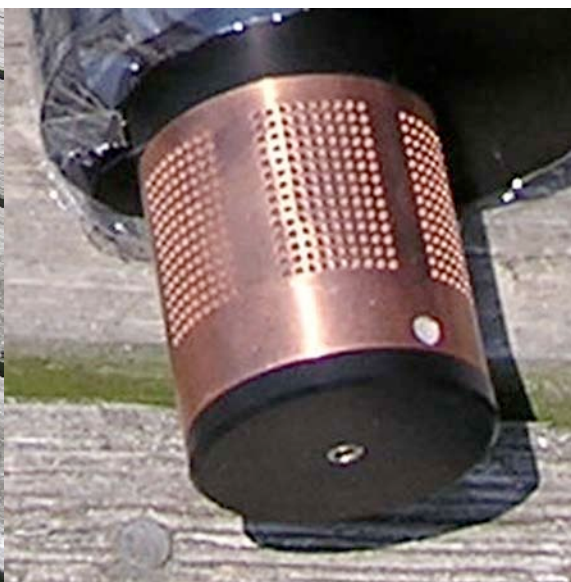
Week	Nitrate (% Recovery)	Nitrite (% Recovery)
<b>1</b>	97.6	103.8
<b>2</b>	91.5	101.3
<b>3</b>	98.6	100.1
<b>4</b>	98.2	97.3
<b>Mean (s.d.)</b>	96.5 (3.4)	100.6 (2.7)

### 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. 14). 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.



ISUS V3 prior to deployment



ISUS V3 sample inlet prior to deployment



ISUS V3 after moored deployment



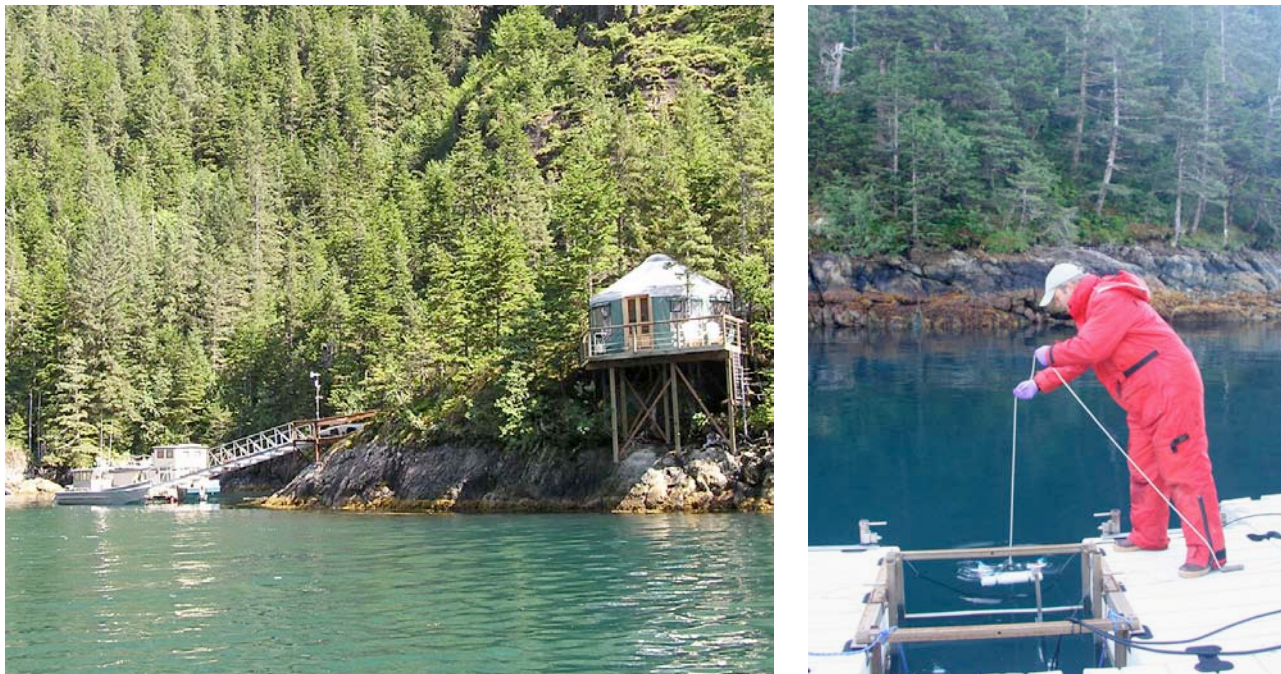
ISUS V3 sample inlet after moored deployment

**Figure 14.** Photographs of the Satlantic ISUS V3 before and after deployment in Chesapeake Bay, MD.



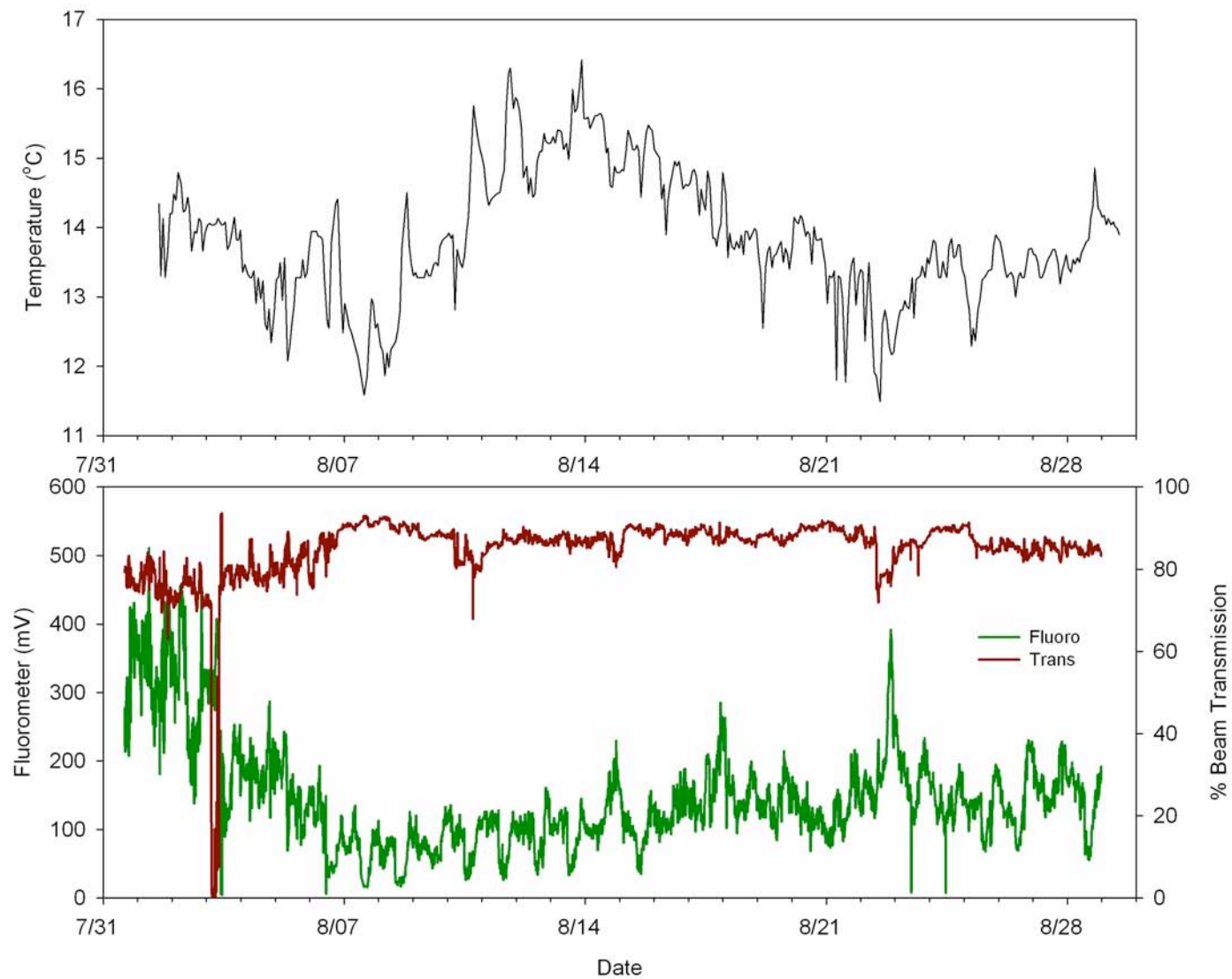
### Moored Deployment in Humpy Cove, Resurrection Bay, AK

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. 15). 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 °C, with noticeable daily cycles related to tides (Fig. 16). The site water was very clear (mean % beam transmission = 86) with fairly low algal fluorescence (mean = 146 mV) (Table 3 and Fig. 16).

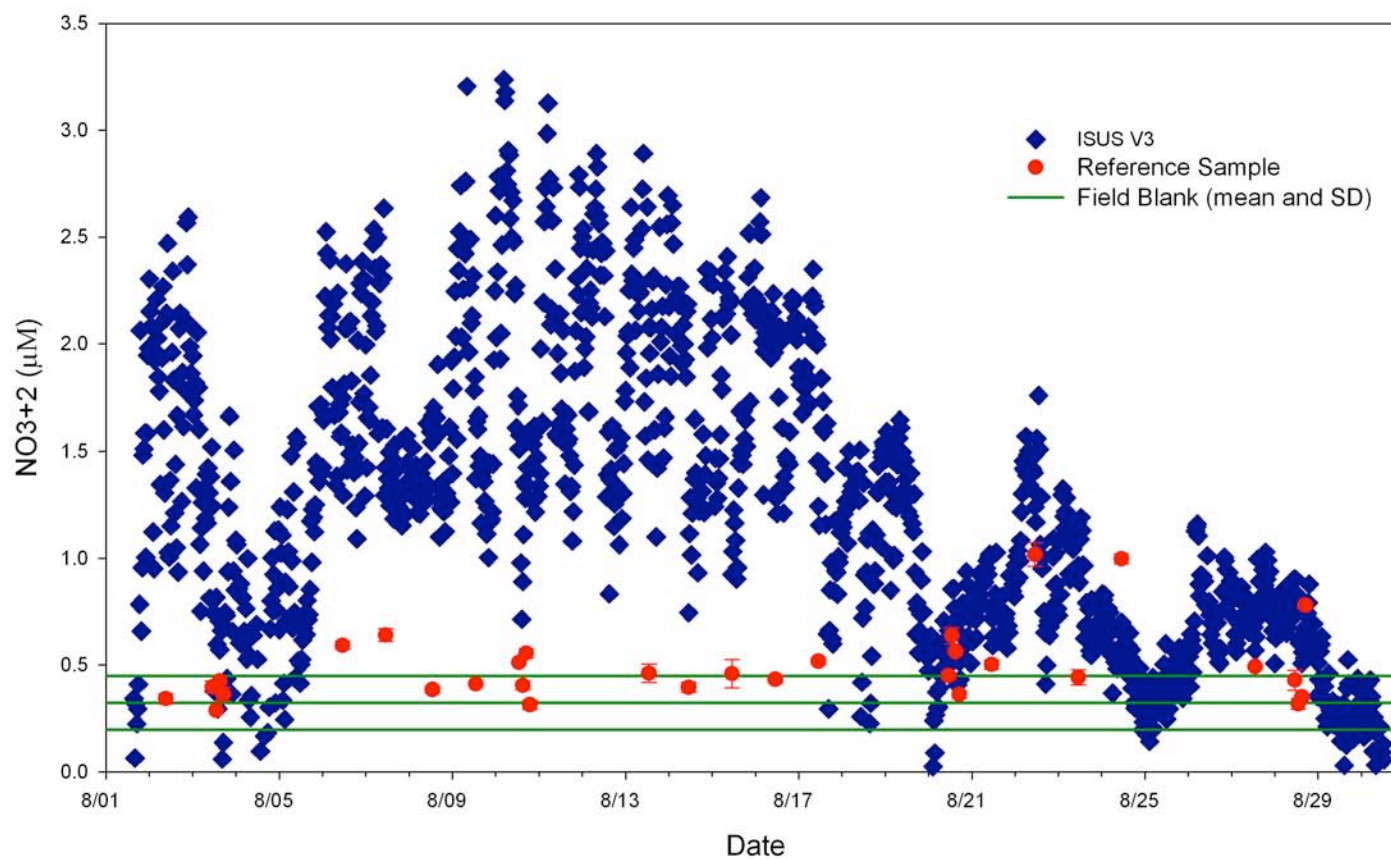


**Figure 15.** Site Photos from field deployment in Humpy Cove, Resurrection Bay, Alaska.

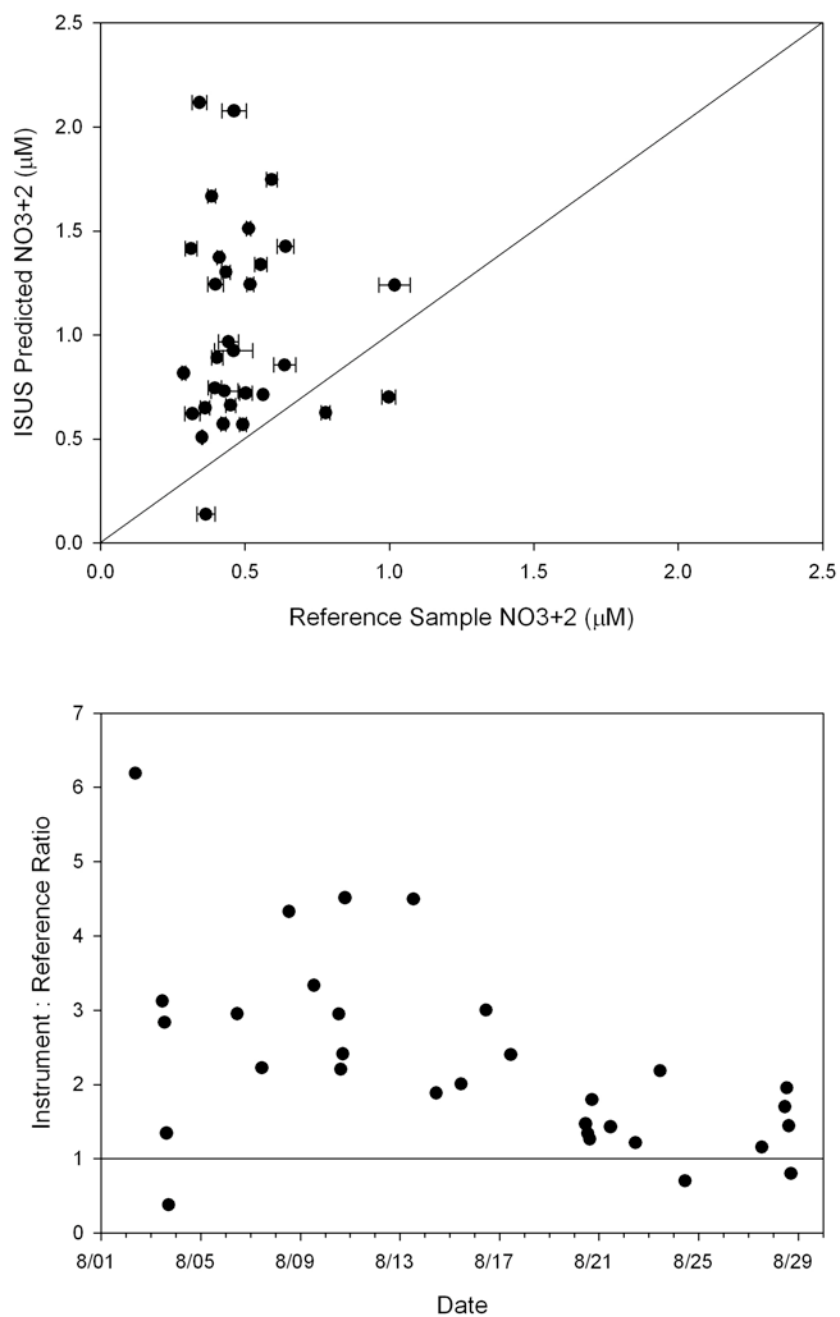
Reference sample nitrate+nitrite concentrations in Humpy Cove were quite low throughout the deployment, ranging from 0.28 – 1.02  $\mu\text{M}$ , with a mean of  $0.48 \pm 0.17 \mu\text{M}$  (Fig.17). The higher amount of visual scatter in the ISUS measurements is in part due to the much finer scale of resolution used to plot the data. Field trip blank averaged  $0.322 \pm 0.008 \mu\text{M}$  and represented, on average, 67% of the nitrate signal of the reference samples. The ISUS V3 in situ measured concentrations showed substantial variation on daily and weekly time-scales. Daily variations may have been due to tidal cycles and the transition of a sharp density layer across the sampling depth. Time series salinity data was not available to confirm this process but water layering was visibly noticeable near the sensor depth. A linear regression of directly compared instrument versus reference sample concentrations shows significant offset, however, the agreement improved significantly after about the 20<sup>th</sup> day of the deployment (Fig. 18).



**Figure 16.** Ancillary data collected at the Resurrection Bay, AK field test site describing conditions of temperature, algal fluorescence and water transparency.



**Figure 17.** Time series comparison of Satlantic ISUS V3 measured nitrate concentrations against laboratory measured field reference samples and field trip blanks for the Resurrection Bay, AK moored deployment test. (Laboratory measured concentrations represent mean  $\pm$  sd.,  $n=3$ ).



**Figure 18.** Analysis of test results from Resurrection Bay, AK test site. (Top) One to one comparison of ISUS V3 in situ nitrate determinations versus laboratory determined concentrations for matching field reference samples. (Bottom) Time series of the ratio of ISUS V3 versus laboratory determined nitrate concentrations for matching field reference samples.

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

A set of pre- and post-deployment exposures to blanks (DIW) and reference standards were to be completed in the laboratory as part of each field test (Table 6). Reference standards were made for a nitrate only and a nitrate plus nitrite mixture. The ISUS V3 measured concentration for blanks varied over a range of  $0.6 \mu\text{M}$  but were not significantly different than 0. The ISUS V3 calls on the pure nitrate standard were 99 % and 88 % of the laboratory measured concentrations for the pre- and post-deployment exposures, respectively. The ISUS V3 calls on the mixed nitrate + nitrite standard were 94 % and 77 % of the laboratory measured concentrations, respectively. All post-deployment measurements were lower than pre-deployment, indicating there may have been a slight drift in the response of the ISUS V3 over the deployment period.

**Table 6.** Comparison of reported blank, nitrate, and nitrite values for pre-test exposures between for the ISUS V3 versus laboratory determined concentrations for the Resurrection Bay, AK mooring test. (mean, s.d. for n=3 replicates).

	NASL Result ( $\mu\text{M}$ )	ISUS Results ( $\mu\text{M}$ )
<b>Lab Blank-pre</b>	0.049 (0.000)	0.461 (0.725)
<b>NO<sub>3</sub> Standard-pre</b>	16.36 (0.33)	16.25 (0.95)
<b>NO<sub>2</sub> Standard-pre</b>	27.27 (1.33)	23.97 (0.33)
<b>Lab Blank-post</b>	0.083 (0.030)	-0.121 (0.278)
<b>NO<sub>3</sub> Standard-post</b>	16.02 (0.16)	14.99 (0.31)
<b>NO<sub>2</sub> Standard-post</b>	27.99 (2.44)	21.67 (2.61)

Lastly, nutrient spikes for the reference samples were performed once per week to examine the consistency of the NASL nutrient determinations (Table 7). The percent recovery for nitrate spikes ranged from 95 - 107 percent with a mean of  $103 \pm 5.5$ . The percent recovery for nitrite spikes ranged from 104 - 108 percent with a mean of  $105.4 \pm 2.1$ .

**Table 7.** Percent recovery of nitrate and nitrite added to field reference samples for the Resurrection Bay, AK mooring test. Spikes were performed once each week at each of the test sites. All concentrations were determined on triplicates.

Week	Nitrate (% Recovery)	Nitrite (% Recovery)
<b>1</b>	103.4	108.4
<b>2</b>	107.4	104.5
<b>3</b>	95.1	103.9
<b>4</b>	106.1	104.6
<b>Mean (s.d.)</b>	103.0 (5.5)	105.4 (2.1)

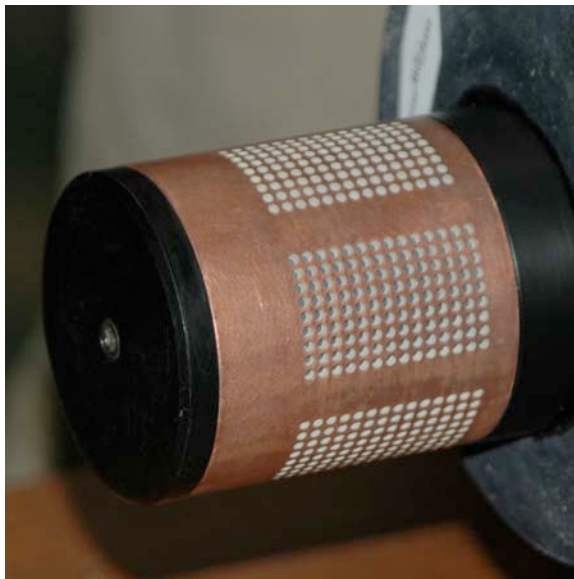


### Instrument Photographs

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. 19). Almost no 'hard' bio-fouling occurred at this test site, and only minor amounts of periphytic algae adhered directly to the nutrient analyzer.



Satlantic ISUS V3 prior to deployment



ISUS sampling inlet prior to deployment



Satlantic ISUS V3 after test deployment



ISUS sampling inlet after test deployment

**Figure 19.** Photographs of the Satlantic ISUS V3 before and after deployment in Resurrection Bay, AK.

**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.

Test Site	#	Lab Duplicates (% Diff)	#	Lab Spikes (% Rec)	#	Lab Stds (% Diff)	#	External Stds (% Diff)
MI	26	2.86 (2.38)	13	103.57 (3.84)	6	3.05 (1.37)	2	8.34 (1.94)
AK	24	4.64 (4.41)	17	104.24 (3.52)	10	2.83 (1.61)	2	7.95 (1.39)
MD	27	2.99 (3.84)	15	98.51 (3.67)	16	3.63 (3.14)	5	6.83 (5.66)
CA	21	1.68 (1.94)	7	98.01 (3.39)	10	1.84 (2.08)	3	6.14 (5.26)

**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 ISUS V3 nitrate sensor was tested in three different applications including continuous underway mapping of surface concentrations, vertical profiling on a standard CTD-rosette, and a fixed depth (1m) moored application. For the surface mapping deployment in Monterey Bay, the ISUS nitrate estimates closely tracked ambient nitrate concentrations over a two-order magnitude range and all expected data were reported. The ISUS also successfully reported out 100 percent of the expected nitrate concentrations at a 1 Hz sampling rate over two vertical profiling tests in Resurrection Bay, AK. The ISUS was successfully tested in a moored application at two of the three attempted test sites. A mechanical failure of the battery pack was encountered in the Michigan mooring test and no data were collected. All expected data were reported out from the other two mooring test sites in Chesapeake Bay, MD and Resurrection Bay, Alaska. In general, it appears that the fundamental ISUS V3 technology has the capability to successfully measure in situ nitrate concentrations under a variety of field conditions and in multiple applications including underway mapping, vertical profiling, and moored deployments.

**ACKNOWLEDGMENTS:**

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, J. Newton, C. D'Elia, L. Codispoti, P. Anderson, for their advice and direct participation in various aspects of this evaluation. E. 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, Coastal Services Center, Charleston, SC.

**March 30, 2008**

---

**Date**

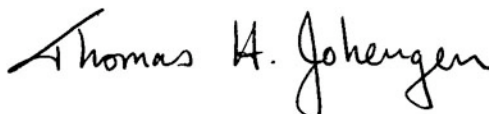


**Approved By: Dr. Mario Tamburri**  
**ACT Executive Director**

**March 30, 2008**

---

**Date**

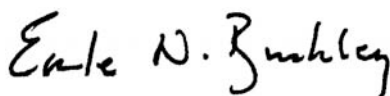


**Approved By: Dr. Tom Johengen**  
**ACT Chief Scientist**

**March 30, 2008**

---

**Date**



**Approved By: Dr. Earle Buckley**  
**Quality Assurance Supervisor**



YOUR PARTNER IN RESEARCH

## Satlantic Comments and Data Review

Satlantic was pleased to provide an ISUS V3 nitrate sensor for the ACT Performance Demonstration. The ACT program is a valuable proving ground for new technology and effective forum for analysis and feedback. All tests were well-executed and provided valuable quality assurance reference data for instrument evaluation. The collection of test sites provided a wide variety of environments and deployment conditions that effectively covers the typical experiences of traditional ISUS users.

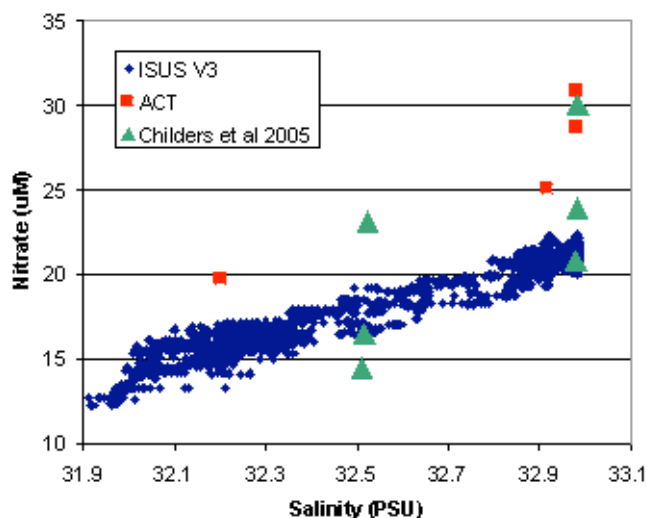
Overall we were very pleased with the performance of the ISUS V3 instrument at all four test sites. All planned data were recovered for the surface mapping, vertical profiling and two mooring tests. A power failure with the battery pack at the Michigan test site unfortunately resulted in no data being recorded during this test. However, standard samples run immediately before and after the deployment demonstrated that the ISUS V3 instrument was functioning properly and within specifications.

Bio-fouling protection was very good at all sites. The ISUS V3 anti-biofouling guard provided abundant protection over the month long deployments under significant fouling conditions. For longer deployments, the ISUS V3 has been deployed with a pumped flow cell that maintains the optical path free of contamination well beyond the 30 days used for the ACT mooring deployments.

Since the ACT deployments, Satlantic has released a new version of the ISUS technology called the Submersible Ultraviolet Nitrate Analyzer or SUNA. It uses the same nitrate measurement technology developed by MBARI for the ISUS sensor and is designed specifically for near-shore deployments with external data loggers.

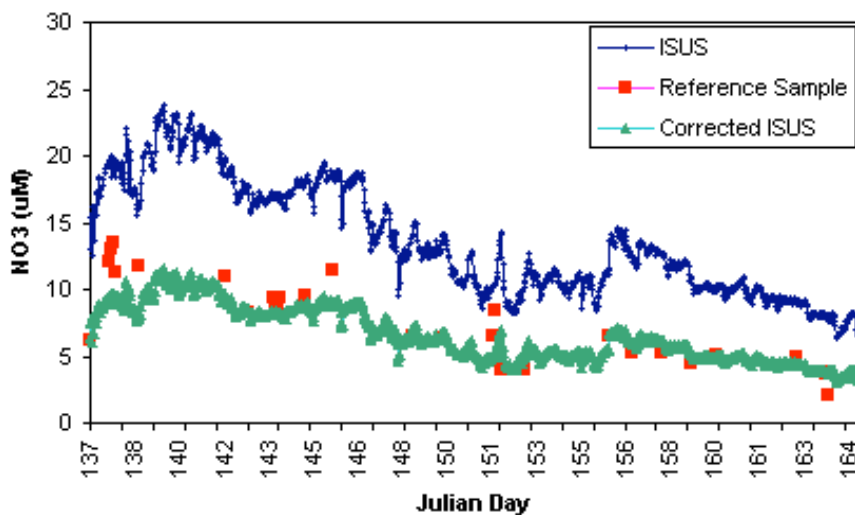
**Surface Mapping in Monterey Bay, Ca.** The ISUS V3 performed remarkably well over the highly variable water quality conditions during the surface mapping demonstration in Monterey Bay. This test showcased the instruments fast response time for accurate real-time nitrate calculations over two orders of magnitude. Previous surface mapping tests have been run independently by Satlantic in Chesapeake Bay and the Florida Everglades. To see results please visit: [www.Satlantic.com/ISUS](http://www.Satlantic.com/ISUS).

**Vertical Profiling in Resurrection Bay, AK.** The utility of the fast 1 Hz sample rate was also confirmed by the real-time vertical profiles of nitrate that correctly identified the depth and shape of the nitracline at both locations. The discrepancy between the ISUS calculations and the ACT reference samples taken below 100m prompted further investigation into possible explanations. A potential thermal influence was explored by applying post processing thermal correction algorithms developed by Carol Sakamoto, Ken Johnson and Luke Coletti (*An improved algorithm for the computation of nitrate concentrations in seawater using an in situ ultraviolet spectrophotometer*, In Press). Since the ISUS V3 internal calibration file contains a factor to compensate for temperature changes in the sampled medium, the post-processing correction did not significantly affect the results. Inspection of the raw data files verified very clean spectra (negligible interfering influences) that typically return accurate nitrate calculations. A look at the previously reported nitrate-salinity relationship from the Gulf of Alaska shelf including GAK1 (Childers et al. 2005) indicates that the 1.4 salinity to nitrate ratio reported by the ISUS V3 is in line with historical values (Fig 1).



**Figure 1.** Deep (>100m) nitrate:salinity relationship for the Gulf of Alaska.

**Mooring Deployment in Chesapeake Bay, MD.** The ISUS V3 time series recorded off the CBL Pier in the Patuxent River correctly resolved the episodic nitrate variations and overall hourly trend. However, the nitrate calculations produced by the ISUS V3 contain a consistent offset that is likely caused by the relatively high optical density of the water dominated by very high levels of CDOM. Absolute accuracy from the hourly times series can be obtained by applying a correction factor calculated from the reference samples (Fig. 2). Because bio-fouling was not an issue, the offset did not change over the course of the deployment and therefore a single correction factor (i.e. 2.06) can be used to correct the entire time series data set. This is the recommended procedure for optimizing accuracy from challenging environments with severe levels of interfering species.

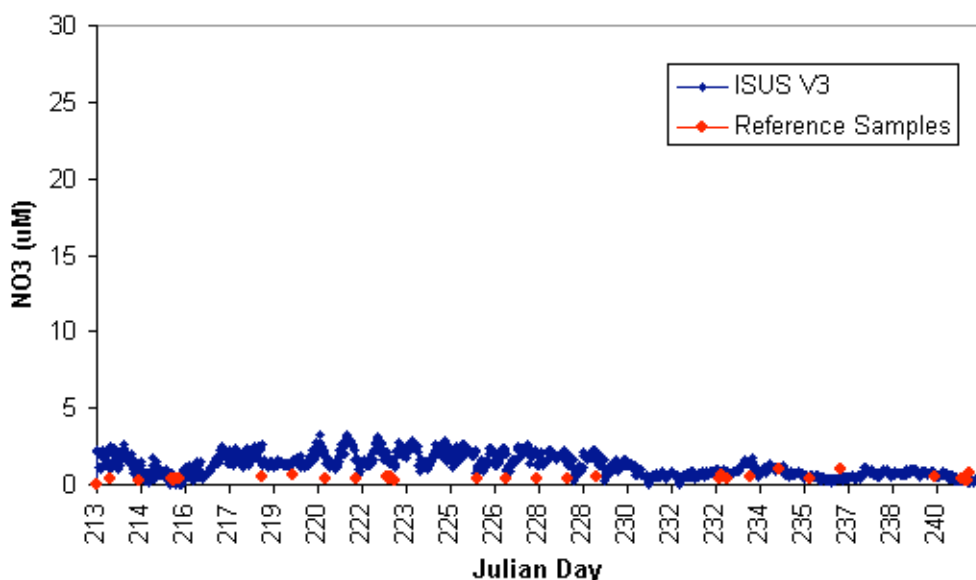


**Figure 2.** Corrected ISUS nitrate time series from Chesapeake Bay mooring deployment.



YOUR PARTNER IN RESEARCH

**Mooring Deployment in Resurrection Bay, AK.** The time series data from Humpy Cove, when plotted on a compressed scale implies a pattern of higher variability at the beginning of the deployment that gradually improves towards the end. This trend is typically reversed for long-term deployments as the calculated nitrate values can be affected by increased bio-fouling. As the data and pictures indicate, bio-fouling was not an issue for this 30 day deployment. When the data are plotted on the same scale as the CBL data (Fig. 3), it becomes evident that the ISUS data were relatively clean. It is also apparent that local nitrate levels were quite low for the duration of the deployment, with small variations successfully tracked by the ISUS. The low background nitrate level is expected for such a pristine environment far from anthropogenic influences.



**Figure 3.** ISUS V3 data from Resurrection Bay mooring test plotted with ACT field reference samples on same scale as Chesapeake Bay time series.

Satlantic would like to thank all ACT personnel and associated researchers for their commitment to performing this invaluable nutrient sensor demonstration with the highest standards. We feel that the technology evaluations and demonstrations they perform are an invaluable service to the greater scientific community.

Geoff MacIntyre  
Director of Product Development  
Satlantic, Inc.