Long-term in situ chemical sensors for monitoring nutrients: phosphate sensor commercialization and ammonium sensor development

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LONG-TERM GOALS

The long-term goals of this project are to 1) transition the CYCL-PO4 sensor from a prototype to a fully functional commercial product to enable sustained observations of phosphate concentration for detailed investigations of biogeochemical variability in open ocean and coastal environments, and 2) develop an in situ ammonium sensing capability along with the phosphate sensor, thereby allowing comprehensive research to be conducted on the impacts and controls of nutrient cycling in coastal and nearshore ecosystems.

OBJECTIVES

The overarching objective of our effort is to develop a reliable, accurate, long-term in situ nutrient sensing sensor system employing reagent-based colorimetric methods using fluidics (the so called "wet" chemistry method) that is easy to use and can be readily adapted for several nutrients. WET Labs has been developing a core set of technologies to utilize optical detection techniques for determination of nutrient concentrations using low volume, reagent-based reaction chemistry. Specifically in this work we focus on the commercialization of the CYCL-PO4 prototype in situ sensor. Evaluation of the *in situ* data obtained using this system has shown that the measurement technology is robust, and that the sensor provides stable and accurate readings. In Phase I of this project, we seek to transition this technology from prototype status to a commercial product. Our efforts broadly encompass design issues for simplifying manufacturing, maintenance, service, customer easy of use, and enhancements for integrating the sensor with other observing systems. Our focus has to build wet chemistry based commercial in situ sensors with easy to use cartridges, removing the need for users to deal with the chemistry. In doing so, we aim to develop a sensing platform which can be easily modified to measure other dissolved nutrients and analytes. In Phase II of this project, we aim to modify the reagent based detection methodology for the *in situ* determination of ammonium concentration.

APPROACH AND WORK PLAN

Our approach and work plan has two discernable phases; 1) commercialization and production of the CYCL-PO4 in situ dissolved phosphate sensor system, and 2) development of the CYCL-NH4 in situ dissolved ammonium prototype sensor. Each phase includes an extensive laboratory and field testing and validation effort conducted in concert with the research partners of this project. The work plan associated with these efforts concentrated on the commercialization of the phosphate sensor during year 1, with extensive field and laboratory testing of the sensor during the end of year 1 and through year 2 of the project. Phase II efforts focus on development of the reagent chemistry and fluidic modifications of the CYCL-PO4 platform for the measurement of dissolved ammonium in late year 1, followed by production of several CYCL-NH4 in situ prototype sensor systems for laboratory and field testing in years 2 and 3 of the project. A brief summary of the tasks associated with each project is given below.

Tasks for Phase I – Commercialization of the in situ CYCL-PO4 Phosphate sensor

- Utilize easy to replace reagent cartridges to allow user to keep “hands off” chemicals
- Improved reliability of internal calibration.
- On board calculation of phosphate concentration.
- Easy exchange of intake filters, covers and other expendable items.
- Seamless integration of sensor with other data acquisition/control systems (interoperability).
- Employ anti-biofouling measures to allow for unattended deployment for three months.
- Extensive field testing by WET Labs and the project partners.

Tasks for Phase II – Development of the in situ CYCL-NH4 Ammonium sensor

- Identification and testing of the most suitable method for the determination of dissolved ammonium.
- Development of ammonium chemistry for long-term storage.
- Design new manifold based on the above.
- Obtain precision and detection limit (0.1 and 1 μM, respectively).
- Extensive testing by the Project Team.

WET Labs project team consists of Dr. Andrew Barnard (lead PI), Casey Moore (corporate lead), Dr. Ron Zaneveld (Science liaison), Bruce Rhoades (senior engineer), John Koegler (senior application engineer), Alex Derr (senior mechanical engineer), Cale Wetzel (production engineer) and a post-doctoral research fellow (Dr. Corey Koch). As the goal of phase I of this project is to transition the existing prototype from engineering to production, several WET Labs production personnel are also involved in the fabrication of the commercial sensor. One of the major foci in the early stages of our work has been to stage the release of the commercial sensor to production (personnel, training, manufacturing, machining, etc) in tandem with the necessary engineering. As such, we have involved from the onset, our applications and production engineering teams to help facilitate the design of the system and to ensure that the system is inherently manufacturable by our staff.

Our project partners include SubChem Inc (Dr. Al Hanson, Peter Egli, and Scott Veitch) who assist with operational performance evaluation, sensor stability, and water sample verification, as well as provide assistance with the ammonium sensing methodology development, and Dr. James Ingle (Professor Emeritus, Oregon State University) who is acting as a consultant to the project on the ammonium sensing wet chemistry methodology and sensing design. Several other groups are involved with this project including Dr. Burt Jones (University of Southern California), Dr. Ru Morrison and Tom Gregory (University of New Hampshire), Robert Campbell (Prince William Sound Science

Center), Dr. Dan Doolittle (Kachemak Bay Research Reserve), and Doug Wilson (NOAA Chesapeake Bay Office). These groups will be acting as CYCL-PO4 sensor evaluators for the project, and as such will be provided with CYCL-PO4 sensors to deploy on there established monitoring platforms. Their primary role is to provide the project with nutrient validation data, assist with issues related to integration with data systems, and to act as independent evaluators of the CYCL-PO4 sensing capabilities.

Over the past year, our project team has added a post-doctoral research fellow, Dr. Corey Koch, who recently completed his Ph.D. thesis at Oregon State University in Chemistry. Corey accepted a post-doctoral research fellow position at WET Labs to assist with the phosphate sensor validation program and with the ammonium sensor development efforts. WET Labs has also added a production technician to our staff to lead the commercial CYCL-PO4 production build efforts.

We have also encountered changes to our partner team over the last year. Dr. Ru Morrison left the University of New Hampshire this past summer, and is now the executive director for NERACOOS. Dr. Jonathan Pennock, Director of University of New Hampshire Marine Program and New Hampshire Sea Grant College Program, agreed to serve as the partner PI in Ru's place. Tom Gregory continues to facilitate and manage the CYCL-PO4 testing and validation program associated with the University of New Hampshire project efforts. Dr. Dan Doolittle, Kachemak Bay Research Reserve, left the reserve in 2008. In subsequent discussions with the new Science Director for the Reserve, it was not clear if the Reserve would have efficient resources to support the partner goals for the CYCL-PO4 validation efforts, and thus a decision was made to delay their involvement until a later date. Finally, the project has significantly benefited from the involvement of Dr. Joseph Needoba, research scientist at Oregon Health and Sciences University. He has an extensive research background in nutrient cycling dynamics and experience in the development of in situ nutrient sensors. Dr. Needoba's involvement with the project has been facilitated through our collaborations with the Coastal Margin Ocean Prediction (CMOP) National Science Foundation Science and Technology Center.

WORK COMPLETED

The majority of our work efforts to date have focused completing engineering modifications requited to transition the CYCL-PO4 in situ phosphate sensor from prototype to a commercial product. Work completed associated with these efforts include:

- Mechanical design improvements to incorporate many easy-of-use features for the user serviceable components of the CYCL-PO4 sensor including reducing the number of plumbing connections, easy to replace of intake filters, snap-in style reagent cartridges, and easy to remove/replace protective shell
- Incorporation and production of easy to replace reagent cartridges to allow user to keep "hands off" chemicals simplifying the process of replacing the reagents.
- Flow cell modifications to reduce the impact of bubbles within the system and to aid in efficient flow of sample through the fluidic components
- Redesigned electronics to enhance the capabilities and functionality of the sensor, and to reduce the cost of the components, thereby increasing the manufacturability for commercial production
- Firmware modifications to increase the functionality and reliability, including on board phosphate concentration calculation, interruptible, two way communications during any period of the measurement cycle, programmable and adjustable standard measurement cycles, reagent

use monitoring and reporting, and command and control communications to facilitate interoperability with other data control systems

- Completed user host software development for the CYCL-PO4. Features include sensor configuration, deployment/mission planning, data offload and metadata recording, raw data and PO4 concentration plotting, reagent and standard usage tracking, as well as integrated QA/QC data analyses functions.
- Completion of engineering and manufacturing documentation including completed bill of materials, machine drawings, assembly documentation, users guides, shipping documentation, characterization procedures, specification sheets
- Held a 2 day project partner kick-off meeting at WET Lab to demonstrate and train the project partners on the use and operation of the CYCL-PO4 system, discuss deployment and testing activities (including issues of sensor integration and communication), as well as discussed the long-term testing and evaluation plans associated with the CYCL ammonium sensor development.
- Completed construction of several CYCL-PO4 sensors. Builds were conducted in two series of batches, involving both engineering and production staff. Gage studies were conducted to determine performance metrics and repeatability of the manufacturing process.
- Extensive field testing by WET Labs and the project partners.

Work completed associated with our Phase II, development of an in situ ammonium sensor, efforts include:

- Hiring of a postdoctoral research fellow, Dr. Corey Koch, to aid in the development of the chemical analytical techniques, specifically the ammonium sensing methodologies.
- Evaluated absorption and fluorescence based analytical techniques for the measurement of dissolved ammonium concentrations
- Evaluated and tested several reagent combinations for use with the OPA detection methodology
- Conducted a series of laboratory experiments to determine the reaction kinetics and fluorescence characteristics
- Conducted a series of experiments to evaluate the potential matrix interference effects of the OPA detection methodology
- Constructed two alpha prototypes of the ammonium sensor based on the CYCL-PO4 sensor design

RESULTS

The work performed in the initial year of this project has focused primarily on the engineering aspects of transitioning the CYCL-PO4 sensor to a commercial product. The design modifications incorporated into the pre-commercial product version have been completed, resulting in a completed bill of materials, machine drawings, and assembly documentation. The method for the determination of soluble reactive phosphate in natural waters is based on the reaction of phosphate ions with an acidified molybdate reagent to yield a phosphomolybdate complex, which is then reduced with ascorbic acid to a highly colored blue compound, molybdenum blue.

The primary components of the CYCL-PO4 sensor include intake filters, pumps for the sample water, reagent and calibration standard cartridges, a mixing manifold, an optical flow cell, the electronics that control the system and perform post-processing, and a protective outer sleeve (Figure 1). Sample water is drawn through dual 10µm stainless steel intake filters (covered with copper screens to prevent biofouling) to remove particulate matter that might interfere with the measurement or clog the flow path. The bottom center section of the meter, the mixing manifold, is a solid block with four fluid ports for the ambient water sample, two reagents and calibration standard. Small lengths of tubing are used to connect the pump manifold to the intake filter and the reagent and standard cartridges. The ports are labeled and color coded to match the reagent cartridges and ambient water. The micropumps mount on to the mixing manifold, and utilize flexible diaphragms to remain at ambient pressure when the instrument is submerged and are necessary to maintain accurate metered pump flow. The pressure housing, containing the electronics and optical detection cell, is mounted on the end of the pump manifold. The CYCL-PO4 also includes an external thermistor located at the top of the instrument near the electrical bulkhead connector.

The reagents and phosphate calibration standard are housed in hard plastic shells for protection and to allow for easy replacement. Each of the cartridges is color coded and indexed to ensure proper installation. Installation and removal of each cartridge is made easy by using quick disconnect fittings and guide pins to insure proper alignment. The outer sleeve with carry handle is meant to provide the instrument protection from minor impacts and biofouling and is removable to provide access to the reagent cartridges and intake filters.

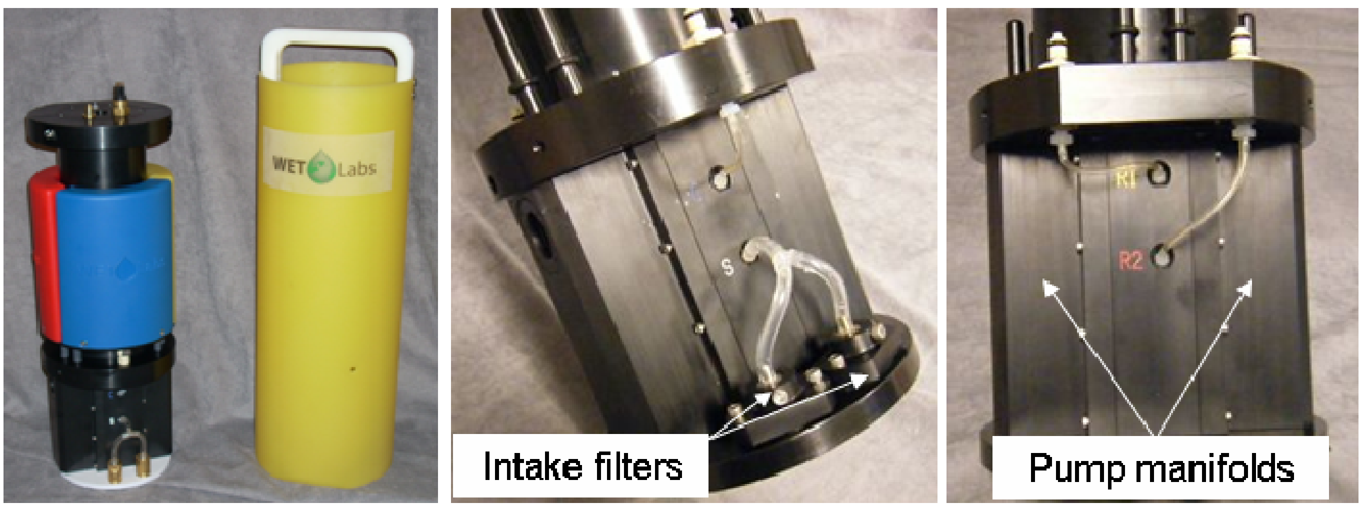

Figure 1 – Left panel: The CYCL-PO4-PO4 sensor with outer protective sleeve removed. Middle panel: The mixing and pump manifold assembly located on the lower portion of the sensor. Right panel: Opposite side view of the manifold showing the fluid connections for the two reagents.

The CYCL-PO4 sensor system determines phosphate concentrations by measuring the optical transmittance of the resulting molybdenum blue and comparing it to a baseline transmittance measurement of the ambient water alone. The optical cell's reflective walls minimize light loss due to scattering and hence the transmittance approximates the absorptance of the sample, or the light loss due to absorption by molybdenum blue. Ambient water is filtered through a 10µm filter and brought into the optics cell via a mixing chamber by a micropump. The transmittance of this sample is then measured to provide the baseline value (ambient measurement). Ambient water and the two reagents are then pumped into the mixing chamber in precisely controlled amounts. The resulting mixture is then pumped into the optics cell and its transmittance is measured (sample measurement). Additionally, a known concentration of phosphate (standard) may be added to the same amounts of ambient water and reagents as used in the previous sample, and the transmission of the resulting mixture (sample + calibration “spike”) is measured. From these three measurements, the concentration of phosphate in the ambient water sample may be determined and referenced to the calibration concentration.

The CYCL-PO4 instrument is factory calibrated to output reactive phosphate concentration in micro-Molar (µM) units. The measurement precision and accuracy of the CYCL-PO4 sensor are 50 nM and 150 nM phosphate respectively, with a dynamic range from ~0 to 10 µM. Incorporation of an on-board phosphate standard in the CYCL-PO4 allows the user to monitor and verify the calibration stability of the sensor to due to changes in system performance, fouling, or changes in instrument behavior. The cartridges contain enough reagents to perform more than1000 phosphate measurements before needing replenishment. With proper care and maintenance, the CYCL-PO4 sensor is expected to support six field deployments before needing to be returned to WET Labs for service and re-calibration.

The CYCL-PO4 Host software program is written in the Java™ language developed by Sun Microsystems Inc. The host software interface includes a wide range of tools to program and control the sensor, monitor reagent usage, interact, view, and download phosphate and raw transmission data. A step-by-step graphical user interface is also included to aid in configuring the CYCL-PO4 for deployment in the field. The control electronics and associated firmware and software allow the user to program the CYCL-PO4's sampling scheme, and output. There are four major operating modes for integrating the CYCL-PO4 instrument into a system. Together, these modes of operation provide maximum flexibility in configuring, controlling, and integrating the CYCL-PO4 system with most deployment platforms. Regardless of which mode the instrument is operated in, the raw data and engineering units for each sample sequence are stored on the CYCL-PO4's internal flash disk for post-deployment retrieval.

Field evaluations of the long-term performance and measurement validation of the CYCL-PO4 sensor by our project partners are ongoing. As part of process, WET Labs hosted a project partner team kick-off meeting on 25-26 March 2009 at our Philomath, OR offices. The purpose of this meeting was to provide an overview of the CYCL-PO4 sensing technology, review the project goals, and plan for partner participation in support of nutrient sensor development. The workshop introduced the CYCL-PO4 phosphate sensor system, and demonstrated its operation and use through a hands-on training session to assist partners with planning and field use of the system in deployments at various sites. All of the participants presented an overview of current and planned nutrient monitoring activities and discussed the phosphate validation (i.e. water sample) capabilities for each group. The second day of the workshop also included a planning session related to the development of an in situ ammonium sensor based on the CYCL-PO4 technology. One of the outcomes of this workshop was the development of CYCL-PO4 training and operation materials.

Following this meeting, CYCL-PO4 units were delivered to the project partners in the summer of 2009. Subsequent field deployments have been conducted by the project partners in a variety of locations including Lake Eyak, AK (PWSSC), Narragansett Bay, RI (SubChem), Santa Monica Bay, CA (USC), and the Great Bay Estuary, NH (UNH). Field deployments by are project partners were conducted in September through October 2009. While several of the partners encountered some problems with the integration and setup of the CYCL-PO4 sensor, most of the initial partner deployments of the CYCL-PO4 were successful. Only in the case of the Lake Eyak deployment, did the CYCL-PO4 fail to collect any data, which was determined to be due to a configuration error at the time of deployment. We have subsequently made modifications to the setup procedure to address this issue. Data analyses and validation measurements are currently ongoing by our partners and the WET Labs project team. Several more deployments are either currently ongoing by our project partners or are planned for the next six months. These include deployments in Prince William Sound (currently deployed; PWSSC), a pier near Redondo Beach, CA and in Santa Monica Bay, CA (USC), Narragansett Bay, RI (SubChem), Great Bay Estuary, NH (UNH), and in Chesapeake Bay, MD (NOAA Chesapeake Bay Office). In addition, we recently delivered a CYCL-PO4 sensor to Dr. Joe Needoba, OHSU, who has deployed the sensor in the lower Columbia River Estuary, OR.

As part the field evaluation effort, we have installed a CYCL-PO4 sensor in Yaquina Bay, Newport, OR. WET Labs and Satlantic Inc maintain a permanent field station in Yaquina Bay, which includes an environmental sampling system called the Land/Ocean Biogeochemical Observatory (LOBO). The LOBO system provides hourly measurements of surface ocean temperature, conductivity, chlorophyll fluorescence, turbidity, dissolved oxygen, nitrate and colored dissolved organic material. A CYCL-PO4 unit was installed at this site in March of 2009 to evaluate the long-term operational performance. The unit is periodically field serviced (~ every 6 weeks) to replace reagent cartridges and filters, as well as general cleaning. The CYCL-PO4 sensor deployed in Yaquina Bay, OR has collected over 4500 phosphate measurements and continues to operate as of the time of reporting.

The dissolved phosphate concentrations measured using the CYCL-PO4 sensor have ranged from 0 to 4 μM (Figure 2) and were within the range of other reported values for Yaquina Bay [1, 2]. Phosphate fluctuations within the bay were generally well correlated with changes in nitrate concentration, particularly during periods of high salinity and colder temperatures. Yaquina Bay, a small river estuary located in the central Oregon coast, is subject to strong tidal forcing as well as nutrient and particulate inputs from the Yaquina River. Previous researcher's results have shown that high levels of dissolved nutrients within the bay are associated with periods of coastal upwelling during the late spring through early fall periods [1, 2]. These periods of strong coastal upwelling are typically also associated with increased chlorophyll concentrations, which lag upwelling favorable winds by ~6 days [1]. Our continuous monitoring shows similar trends, with increases in nutrients associated with high salinity flushing events (presumably periods of coastal upwelling) and corresponding increases in chlorophyll and percent dissolved oxygen saturation approximately 7-10 days after the increased nutrient appearances in the bay. During these upwelling periods, the ratio of dissolved inorganic nitrate to phosphate was consistently close to the Redfield ratio (16:1). A corresponding increase in chlorophyll and dissolved oxygen approximately 7-10 days after the flushing event indicates that the upwelled nutrients fuel a phytoplankton bloom in the bay.

IMPACT AND APPLICATIONS

Economic Development

The CYCL-PO4 in situ chemical sensor development will result in a new product line for WET Labs. The expectation is that initial sales of the phosphate will result in $500,000 in new revenue over the first year of sales, with sales doubling in the ensuing 1-2 years. As the customer acceptance of the CYCL-PO4 product builds, we expect that sales of the product will double within 2 years of release of the product fulfilling the in situ phosphate chemical sensing need in the oceanographic, freshwater, and water quality. As we develop the ammonium sensor, we expect that the chemical analytical skills and manufacturing capabilities will experience further growth, thus resulting in an expansion of our production base. Finally, this result of this project will establish new technology basis from which to expand and develop new products from.

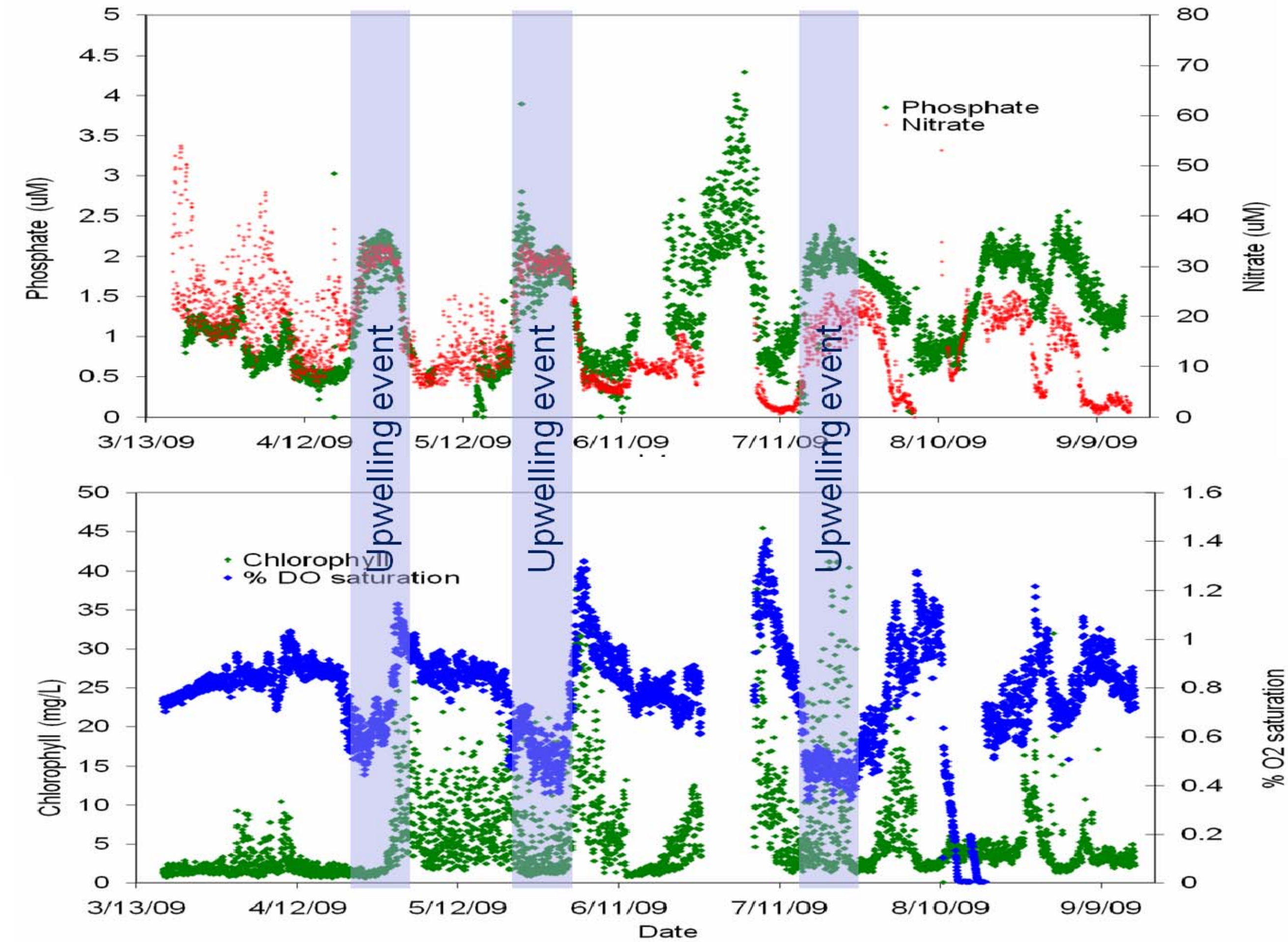

Figure 2 – Time series of environmental data collected in Yaquina Bay, OR. Top panel: Time series of phosphate concentration as measured by the CYCL-PO4 sensor and nitrate concentration (using Satlantic ISUS in situ sensor; note 16:1 ratio in N:P scales). Bottom panel: Corresponding time serial of chlorophyll and percent dissolved oxygen saturation (using WET Labs WQM sensor).

Quality of Life

Dissolved nutrient dynamics broadly affect issues related to public health, ecosystem status, and resource sustainability, including impacts of climate variability, eutrophication, harmful algal blooms, carbon cycling, and species composition among others. The need for *in situ*, autonomous, real-time nutrient monitoring capabilities has been clearly documented in several national reports on ocean observing. Providing a commercial in situ nutrient sensor to the broader ocean observing, resource management, and freshwater water quality communities that is easy to use, reliable, accurate and long-term deployable, will allow for improved monitoring of public and ecosystem health, coastal resource management, and science research on anthropogenic impacts to be conducted on a routine basis.

Science Education and Communication

Combined with other real time data systems such as the LOBO, the CYCL-PO4 and ammonium sensors will offer the opportunity to learn and teach about nutrient cycles. WET Labs has been involved in an education and outreach project over the past year, through financial and technical support of a marine resource management masters student from Oregon State University, to improve the public's understanding of water quality monitoring in coastal estuaries. As part of this project, the student is building in a module to describe the impact of riverine nutrients on coastal/estuarine ecosystems utilizing data from our water quality monitoring station (http://yaquina.satlantic.com). The addition of a CYCL-PO4 to this system will expand the discussion and presentation on nutrients by including real time observations of phosphate concentration.

TRANSITIONS

Economic Development

As mentioned above, the commercialization of the CYCL-PO4 phosphate sensor will set WET Labs on a definitive new product pathway focused on in situ nutrient measurement capabilities. This will not only expand our core suite of technologies, but will also facilitate economic growth of the company through market expansion. This in turn will increase our production capacity (through increased staff and production personnel), thus contributing to the economic health and viability of our region.

Quality of Life

As described in this report, several of the partners involved in this project are currently maintaining, or are a part of active resource management, coastal ecosystem health, and/or water quality monitoring programs. By involving these partners as active participants in the development and commercialization process, we will ensure effective transition of the CYCL-PO4 sensor into key local, state, federal monitoring programs as well as ocean observing and science research projects.

RELATED PROJECTS

This project will significantly overlap with the Coastal Margin Ocean Prediction (CMOP) National Science Foundation Science and Technology Center, lead by Dr. Antonio Baptista, Oregon Health and Science University. As part of this project, WET Labs has built and maintained a water quality monitoring station (termed Saturn04) within the lower Columbia River estuary. The monitoring station is providing real time hourly observations of surface temperature, salinity, pressure, chlorophyll concentration, turbidity, dissolved nitrate and dissolved oxygen (http://columbia.loboviz.com). In the next 8 months, a CYCL-PO4 sensor will be integrated with this system, and thus will be providing real time phosphate observations. Through this project, will also be addressing issues of sensor integration/interoperability with the WET Labs WQM (www.wetlabs.com) and Satlantic Inc LOBO (www.satlantic.com) instrumentation systems.