

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 CYCLE-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. Our focus has been 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. As part of supplemental funding provided to this project, we are also investigating and developing a methodology for measuring HAB/toxins using the CYCLE platform. This work is being conducted by our post-doctoral fellow, Dr. Bruno Soffientino.

APPROACH AND WORK PLAN

Our approach and work plan has two discernable phases; 1) commercialization and production of the CYCLE-PO₄ *in situ* dissolved phosphate sensor system, and 2) development of the CYCLE-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 CYCLE-PO₄ platform for the measurement of dissolved ammonium in late year 1, followed by production of several CYCLE-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 CYCLE-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 CYCLE-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.

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 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), Dr. James Ingle, Dr. Burt Jones (University of Southern California), Dr. Jonathan Pennock and Tom Gregory (University of New Hampshire), Robert Campbell (Prince William Sound Science Center), Dr. Joseph Needoba (Oregon Health and Sciences University), and Doug Wilson (NOAA Chesapeake Bay Office). These groups have been acting as CYCLE-PO4 sensor evaluators for the project, and as such have been provided with CYCLE-PO4 sensors, to deploy on 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 CYCLE-PO4 sensing capabilities. Dr. Corey Koch (WET Labs), has been serving as a post-doctoral fellow on the project and manages the phosphate/ammonium sensor development and validation program.

WORK COMPLETED

The majority of our work efforts since our last report have focused on completing the transition of the CYCLE-PO4 from R&D to a released product, continuing a comprehensive validation program, and further refining user ease (primarily software) based on NOPP partner feedback:

- Evaluated typical instrument-to-instrument variability (small differences in PO4 response) during field deployments to be from 25-150 nM PO4.
- Implementation of a production level, more comprehensive calibration procedure.
- Demonstrated instrument performance to high levels of phosphate (up to 40 μM).
- Evaluated effectiveness of on-board standard addition and found differences in factory and field calibrated samples to be statistically insignificant and within understood factory precision.
- Firmware and software modifications to increase file offload speed and robustness.
- Enhanced field service through development of a simple field optics cleaning procedure to reduce the time interval for factory service.
- Complete integration with the Land Ocean Biogeochemical Observatory system (LOBO).

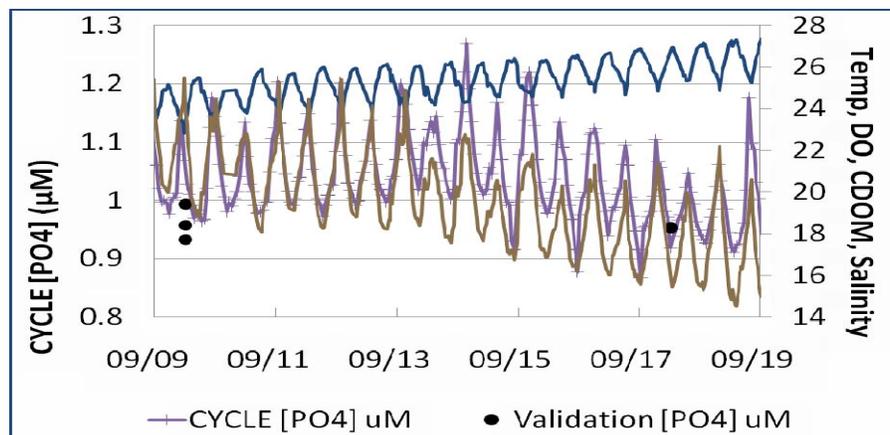
- Enhancement of the field validation program. Blind standards in saline and fresh water matrices have been prepared and sent to partners to be analyzed with each partners grab samples to evaluate method accuracy.

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

- Optical channels significantly different from those published in the literature were identified, tested, and developed. These channels demonstrated faster reactivity at ambient temperature and thus facilitate a higher-temporal resolution sampling capability with lower power.
- Development of a low power (0.5 W), heated/thermostated, optical cell to provide for analysis times fast enough to achieve 2 ammonium analysis per hour.
- For the novel optical channel we have demonstrated equivalent (if not enhanced) selectivity compared to a primary amine, the amino acid alanine, which has imparted the highest error in published studies.
- Reagent stability studies have indicated that reagents can be combined and stored for up to 3 months and still provide for accurate ammonium analysis. This and the aforementioned developments make the CYCLE-NH₄ comparable to the CYCLE-PO₄ in terms of size, deployment length/service interval, operation, sampling frequency, and reagent lifetime.
- Development of MatLab code for analytical signal extraction, for Beta level firmware.
- Transferred alpha prototype design into mechanical drawings for production of Beta level units. Currently the build phase for CYCLE-NH₄ has been initiated and Beta units are on schedule for spring delivery to the NOPP partners.

RESULTS

The work performed in this year has focused on: completing transition of the CYCLE-PO₄ sensor to a product, field validation of the CYCLE-PO₄ performance by the NOPP partners, and development of the CYCLE-NH₄. The NOPP partners have been very successful in collecting novel field data using the CYCLE-PO₄. In the 2010 summer season we have 6 nearly continuous data sets from the partners' unique deployment sites. Figure 1 displays data collected by Tom Gregory and Jonathan Pennock, of the University of New Hampshire, in the Great Bay, NH. CYCLE-PO₄ ortho-phosphate data is plotted with Colored Dissolved Organic Matter (CDOM), and salinity. A tidal relationship is evident, with PO₄ correlated well with CDOM, and inversely correlated with salinity, indicating a terrestrial source of PO₄ with dynamics controlled by tidal forcing. The black dots



represent grab samples analyzed in the laboratory via accepted autoanalyzer methodologies. In the Columbia River estuary, transient PO₄ spikes to extreme estuarine values were observed that were not captured by the grab sampling program and correlate with other variables (ammonium, low oxygen, etc). These novel results will be presented at Aquatic Sciences in February 2011 by researchers from CMOP.

Figure 1. Great Bay, NH time series of CYCLE-PO₄ phosphate (purple cross, μM), CDOM (brown line), salinity (blue line), and PO₄ grab sample data (black dots).

In addition to these partner data sets, the integration of the CYCLE-PO₄ with the Land Ocean

Biogeochemical Observatory (LOBO) in Newport, OR provides the longest high-resolution phosphate time series ever collected (to our knowledge). A nearly continuous two year data set is represented with many other

biogeochemical parameters (nitrate, chlorophyll, CDOM, salinity, oxygen, temperature, etc.). Nearly a year of this data is publicly accessible online (<http://yaquina.loboviz.com/>).

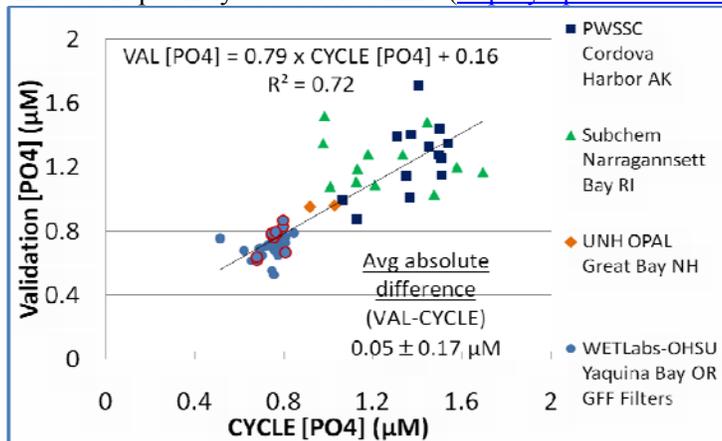


Figure 2. Comparison of grab sample data from NOPP partners to in-situ CYCLE-PO4 phosphate values.

Figure 2 presents data summarizing the validation effort carried out by the partners. The data is presented as a scatter plot of PO4 values obtained from the partners various lab analyses compared to the CYCLE-PO4 phosphate values. A regression line demonstrates good agreement between the values and a data analysis indicates on average a 50 nM (single standard deviation: 170 nM) difference between in-situ and validation values. A fundamental challenge in validating in-situ instrumentation is that accuracy can only be proven within the precision and accuracy of the accepted laboratory methods, which can often encompass more error due to sampling and chain of custody concerns.

In inter-laboratory studies using the same sample, there is often a lack of precision due simply to different laboratories. If we evaluate accuracy just from the WETLabs-OHSU data set (blue dots) we find the average difference between CYCLE-PO4 and the lab methods to be 32 nM with a single standard deviation of 76 nM. We therefore estimate the accuracy to be better than 150 nM and likely the CYCLE-PO4 is more accurate than 100 nM. Over the past year we have endeavored to improve our estimation of the CYCLE-PO4 accuracy by implementing interlaboratory study practices in the form of a series of blind standards prepared in both a seawater and freshwater matrix. NOPP partner Subchem Systems Inc. has led this effort and we are currently gathering and analyzing recent results.

Progress has been made on the CYCLE-NH4 in the laboratory. Reagent combinations have been identified. Reagent preparation and stability studies have been performed. A significant technical challenge that we faced with implementing NH4 analysis chemistry in-situ was that the typical reaction times were on the order of hours. This time frame was not amenable to making an in-situ standard addition for quality control nor was it

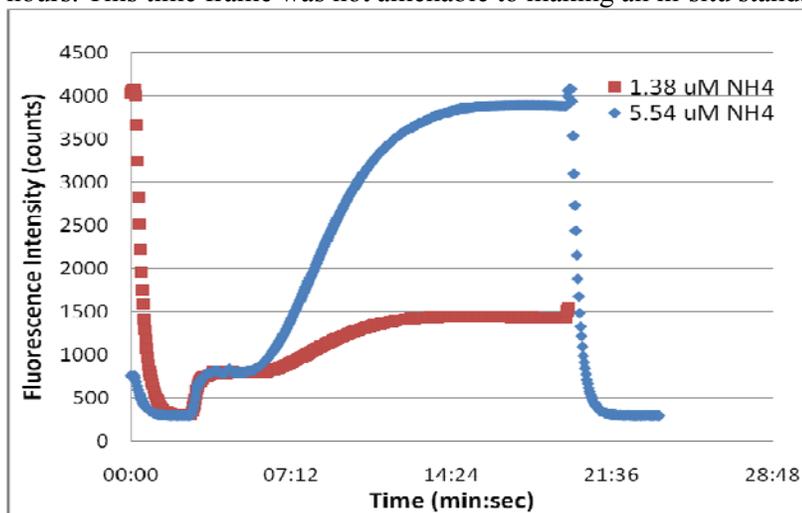


Figure 3. Example NH4 alpha prototype data illustrating the ability to make high-temporal resolution measurements using recent technological developments.

appropriate for high temporal resolution monitoring. Research was invested to overcome this challenge and novel optical solution combined with slight heating (0.55 W) of the reaction cell was found to provide for an analysis time comparable to that for the CYCLE-PO4. Figure 3 presents a reaction curve from the CYCLE-NH4 prototype illustrating 1.38 and 5.54 µM NH4 solutions reacting completely (thus achieving maximum analytical sensitivity) in a period of ~15 min. This ability to make measurements every 30-60 min is critical for biogeochemical applications where measurements of many parameters on a similar time scale are used in conjunction to understand the environmental system. Currently we are producing beta prototypes for disbursement to the NOPP

partners for field testing.

As part the field evaluation effort, we installed a CYCLE-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 CYCLE-PO4 unit was installed at this site in March of 2009 to evaluate the long-term operational performance. In April of 2010, the CYCLE-PO4 was fully integrated with the LOBO system, and the data is now available online to the public.

Our continuous monitoring shows 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.

Development of a methodology to detect the presence of HAB/toxins using the CYCLE architecture has been a focused activity over the last year. These efforts have led to the construction of a benchtop prototype which has been used to develop the fluidics, detection cells, and the reagent/labeling system based on an immune-assay

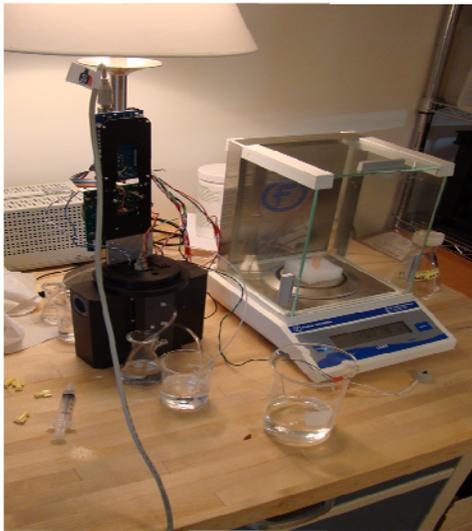


Figure 4. Picture of the bench top prototype of the immune-based assay in the CYCLE.

approach (Figure 4). The approach is based on the ELISA (enzyme linked immunosorbent assay) analysis platform and utilizes a fluorescently labeled antibody to bind to the target body (e.g. whole cell or toxin). The advantage of the ELISA approach is that there are already commercially available antibodies for several HAB toxins and species, and new antibodies can be produced for unique target analytes, thus providing a high degree of analyte flexibility for the CYCLE-HAB platform. Having demonstrated the feasibility of this approach in a laboratory setting, we are currently working to fully automate the analysis and completely adapt it the CYCLE architecture. The ELISA method requires a separation, incubation, and washing step and we have developed a unique optical cell design to facilitate separation of labeled magnetic beads from the sample using the CYCLE platform as seen in Figure 4. The novel optical cell for magnetic bead separation is being adapted and a novel, automated, mixing methodology is being developed for the optical cell to create a combined detection/reaction chamber. Prototype electronics and source/detector optics are being generated to control and probe the system. The temperature controlled optical cell developed for the CYCLE-NH4 is also being leveraged into this work to control incubation and ensure a reproducible analysis under

variable environmental temperatures. It is expected that a proof-of-concept, benchtop, CYCLE-HAB will result from this work and we will demonstrate toxin detection at environmentally relevant levels. This will provide a strong foundation for the rapid development of an *in situ* deployable sensor with significant potential impact.

IMPACT AND APPLICATIONS

Economic Development

The CYCLE-PO4 *in situ* chemical sensor development has resulted in a new product line for WET Labs. The product was successfully transitioned to our manufacturing operations, and is now supported by retail sales of the product. Currently, over 10 sensors have been sold or loaned to customers. The expectation is that initial sales of the phosphate will result in \$500,000 in new revenue over the 2 years. As the customer acceptance of

the CYCLE-PO4 product builds, we expect that sales of the product will double within 4 years of release of the product fulfilling the *in situ* phosphate chemical sensing need in the oceanographic, freshwater, and water quality monitoring fields. WET Labs has invested in setting up manufacturing facilities out of our internal funds in order to meet the expected growing demand for the sensors. As we develop the ammonium sensor, we expect that the chemical expertise, 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.

Quality of Life

Dissolved nutrient dynamics broadly affect issues related to public health, ecosystem status, and resource sustainability. These include 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 CYCLE-PO4 and ammonium sensors will offer the opportunity to learn and teach about nutrient cycles. The data from the Yaquina Bay, Oregon website, including the CYCLE-PO4 data, has been used by oceanography professors at Oregon State University as part of their teaching curriculum on nutrient cycling and real-time data analyses. WET Labs was previously involved in an education and outreach project, through which an educational display on the water quality monitoring in coastal estuaries was developed. This display is on exhibit at the Hatfield Marine Science Center in Newport, Oregon.

TRANSITIONS

Economic Development

As mentioned above, the commercialization of the CYCLE-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 CYCLE-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 within the lower Columbia River estuary which is providing real time hourly observations of temperature, salinity, pressure, chlorophyll concentration, turbidity, nitrate and dissolved oxygen (<http://columbia.loboviz.com>). The CYCLE-PO4 sensor has been integrated with this system, and is providing real time phosphate observations.