Development and Commercialization of Autonomous Chemical and Biological Instrumentation for Water Quality Monitoring

Third Quarterly Project Report
6/1/2016

Christopher Palmer, Michael DeGrandpre, Orion Berryman Steve Amish and Gordon Luikart, PIs, University of Montana, Missoula

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Objective 1: A small organic sensor for arsenate: Orion Berryman

Hirings:
Two new hires were made during this period. Currently five people are supported by the grant including Christopher Grubb (undergraduate student), Evan McManigal (undergraduate student), Ben Palmer (research assistant), Asia Riel (graduate student) and Casey Massena (graduate student).

Equipment Purchased:
No additional equipment has been purchased during this report period.

Travel:
Current results from this project have been presented at the ISXB2 conference (International Symposium on Halogen Bonding).

Progress towards Milestones:

A. Publications: Since the last report one publication related to this work has been submitted. In total, two publications citing this funding source have been published and one is under review.

- "Advantages of organic halogen bonding for halide recognition" N. B. Wageling; G. F. Neuhaus; A. M. Rose; D. A. Decato; O. B. Berryman, **2015**, *DOI: 10.1080/10610278.2015.1118101*
- "Protonation and alkylation induced multidentate C-H---Anion binding to Perrhenate" A. M. S. Riel; D. A. Decato; O. B. Berryman, **2015**, *Crystal Growth and Design*, *DOI: 10.1021/acs.cgd.5b01524*
- "A Halogen Bond Induced Triple Helicate Encapsulates Iodide." C. J. Massena; N. B. Wageling; D. A. Decato; E. M. Rodriguez; O. B. Berryman* **2016**, *submitted*.

B. Calculations: The first round of calculations with an external collaborator has been completed. The second round of calculations is anticipated once initial synthesis and experimental studies are complete.

C. Synthesis, Crystallography and Quantification: Initial hydrogen bonding ligands have been synthesized. Crystallizations have been initiated to structurally characterize hydrogen bonding ligands. Initial quantification of arsenate binding is in progress. Additionally, new fluorescent halogen bonding ligands have been prepared and initial characterization studies are underway. Crystallizations have been set up to structurally characterize halogen bonding complexes.

D. Separations: Initial steps to prepare new halogen bonding stationary phases are underway to improve anion separations in collaboration with in collaboration with the Palmer group.

The following activities have been undertaken under this objective:

A provisional patent has been filed with Joe Fanguy, director of technology transfer at UM, based on new technology discovered through this funding. A disclosure form was filled out on January 26th, 2016.

Total amount of expenditures as of 6/8/16:

Total Budget:	$319,054	
All Expenditures:	$76,317	Salary, benefits, tuition, equipment and supplies
All Encumbrances:	$23,900	Salary, benefits and tuition

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Objective 2: Field capable capillary electrophoresis methods and instrument: Christopher Palmer

Hirings:

Three additional hires have been made during this period. Professor Palmer received summer support during the first quarter reporting period. The project continues to support a graduate student (William Penny) and an undergraduate student (Tristan McGettrick). Three additional UM undergraduate students are being supported by the project: Xi Qing, Hung Ngai Chuk and Morly Jessop. A Blackfeet Community College student, Stephanie Bullchild, is working on the project with support from an NSF-funded Research Experiences for Undergraduates program.

Equipment Purchased:

No additional major equipment was purchased during this period.

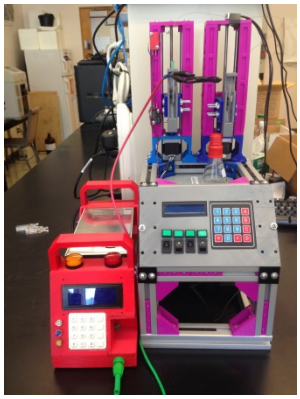

Figure 1: Working laboratory prototype portable CE instrument.

Progress towards Milestones:

A. Robust laboratory CE method for analysis of anions (nutrients, fracking return) and cations (fracking return): Substantial progress has been made on a laboratory CE method for the separation and analysis of anions bromide, chloride, nitrate, nitrite, sulfate, fluoride, bicarbonate and phosphate. An internal standard has been identified and is well separated from the analyte ions. Thiourea has been introduced as a unique additive to improve separation selectivity between bromide and chloride. Calibration and analysis indicates that anions can be detected at the low PPM level without sample preconcentration. Background research into preconcentration methods to allow detection at lower levels in natural waters has been completed and laboratory work on the development and optimization of these methods has commenced.

B. Working field-able CE instrument technology addressing power source, detection, sample introduction, and data collection and analysis. Adaptation of methods from bench-top CE to field-able technology. A fully-functional prototype instrument has been developed and constructed from commercial and 3D-printed parts and components (Figure 1). This design has been tested in laboratory experiments and is a workable instrument for ion analysis by CE. Electronics and control modules have been developed and tested. Autocad designs for instrument components have been produced and parts are being machined to those design specifications at Vintage Lab Tech, LLC in Missoula, MT. Components to be incorporated to make the instrument fully portable and independent of laboratory power have been selected and ordered. Background research into the theory and principles of operation of conductivity detectors has been conducted with the intention to develop and optimized a more sensitive detector for surface water analysis.

C. Analysis of field samples, demonstrating accurate (in comparison to accepted laboratory method. Christopher Palmer attended the Bitterroot Water Symposium in Hamilton, MT on April 29, 2016. There was significant interest in the level of nutrients in groundwater and surface water in the Bitterroot Valley among presenters and attendees. Levels of interest for nitrate are in the 0.2-5 ppm range. Chris Palmer made contacts with Michael Howell, director of the Bitterroot River Protection Association and Douglas Nation, a volunteer with Bitterroot Trout Unlimited, who are both interested in re-establishing a surface water nutrient monitoring program in the valley. These groups can provide access to river sampling sites as well as volunteer help to implement, operate and retrieve instruments from the field.

Total amount of expenditures as of 6/8/16:

Budget:	$286,350	
All Expenditures:	$ 61,760	Sal.& ben., tuition, instrumentation and supplies
All Encumbrances:	$ 3,834	Salary & Benefits, Supplies

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Objective 3: Testing and optimization of large-volume water sampling and filtration techniques for the autonomous collection of eDNA samples using DNA tests for multiple invasive and rare/threatened species along with related environmental data (water temperature, flow, and turbidity): Steve Amish and Gordon Luikart

Hirings:
Two students were added to the project for the summer, a volunteer from Whitman University and a UM undergrad who will be working part-time. In addition, three people are supported by the grant including Seth Smith, Jenna Schabacker and Steve Amish.

Supplies & Equipment purchased:

Purchased supplies for the construction of additional eDNA stream samplers. Purchased additional qPCR assays needed for testing samples. Purchased additional reagents needed for extracting DNA from samples.

Progress towards Milestones:

1. Collect preliminary data on sensitivity of existing eDNA sampling methods. Develop qPCR assays for detection of species of interest.
    a. Redesigned the manual stream sampler to reduce the variation among replicate samples, and added the ability to filter multiple particle sizes during a single sampling event.
    b. Modified the sampling protocol to make the process quicker and easier.
    c. Fabricated eighteen nets and dolphin buckets using the new modified design.
    d. Collected, extracted DNA, amplified target DNA, and quantified the amount of target DNA present using qPCR for preliminary samples using the new stream sampler.
    e. Paired sample comparison between the two extraction protocols (column and bead) on 30 samples to determine the quantity and quality of DNA being extracted using the two methods showed that PCR inhibitors were being passed at a higher rate for the bead extractions.
    f. Attended the 100th Meridian Initiative Columbia River Basin Team meeting in Spokane on aquatic invasive species.

    g. Attended Aquatic Invasive Species meeting in Helena hosted by Montana Fish, Wildlife, and Parks to coordinate research and sampling efforts within the state.
    h. Finalized a sequence database of existing native and invasive species to be used in the design of qPCR assays.
2. Analyze preliminary data. Design autonomous eDNA sampling prototype.
    a. Using samples collected during experiments at the U.S. Fish & Wildlife Service's Creston Hatchery, found a correlation between biomass of rainbow trout and the number of copies of target DNA detected using current eDNA sampling methods.
    b. Using samples collected during experiments at the U.S. Fish & Wildlife Service's Creston Hatchery, found a correlation between sampling volume and the number of copies of target DNA detected using our stream sampler prototype and traditional filter methods.
    c. Using 2015 samples from NW Montana, found a correlation between field samples collected from low-density and high-density bull trout populations using high volume stream samples.
    d. Based on the revised version of the manual stream sampler, we developed a preliminary design using high volume sampling for development of an autonomous eDNA sampler prototype.
3. August 1, 2016 – February 1, 2017: Field-test autonomous eDNA sampling prototype.
    a. Not yet started
4. February 1 – July 31, 2017: Analyze data, prepare intellectual property documents, and prepare research publications.
    a. No new progress to report.

Total Amount of Expenditures as of June 1, 2016:

Total Budget	$396,023	
All Expenditures	$ 71,317	Salary, benefits, & supplies
All Encumbrances	$ 5,622	Minor equipment

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Objective 4: Lab testing of a combined pH and alkalinity system for in situ freshwater measurements: Mike DeGrandpre

Hirings:

Taymee Brandon, an undergraduate previously supported on this grant, has graduated and has been hired as a research assistant for the summer.

Equipment Purchased:

No equipment has yet been purchased.

Progress towards Milestones:

A. December 2015: Begin building prototypes working with Sunburst Sensors (the only company that can readily build these instruments). This will be supported by Sunburst Sensors and UM's research office.

We have now built three new autonomous alkalinity systems. DeGrandpre's research lab manager, Cory Beatty, oversaw this effort. Post-doctoral researcher Chun-Ze Lai and graduate student Adam Prody assembled the instruments. They are currently conducting basic tests to ensure that the electronics are assembled correctly. The next phase will be to analyze alkalinity standards in the lab to determine if the instruments' performance is consistent with the existing instrument.

B. February 1, 2016: Build two combined alkalinity-pH instruments for field testing

We are waiting to see how the new alkalinity systems perform and will then implement and test the addition of a pH measurement. Sunburst Sensors is also supporting this effort by providing software and hardware design (supported by the UM Research Office).

C. July 2016: Complete laboratory testing of the prototype instruments, begin field studies

We are on track to complete the laboratory testing of the alkalinity systems within this time frame. We plan to take the instruments to Scripps Institution of Oceanography in mid-August to conduct extensive tests in a large seawater tank. Adam Prody will lead this effort. At the same time, Chun-Ze Lai will lead local deployments of the instrument in the Clark Fork River.

D. August 2016 – February 2017: Continue field-testing. Work on design improvements.

Not yet started.

E. February 1 – July 31, 2017: Analyze data, prepare intellectual property documents, prepare research publications

With the help of Lisa Mueller from the Michael Best Law Firm in Chicago, we have verbal confirmation from the U.S. Patent Office that our patent application for the autonomous alkalinity system will be allowed. This is very exciting news after our protracted effort to protect the intellectual property.

This grant has also supported completion of a manuscript closely related to the grant objectives, cited here.

Lai, C-Z., DeGrandpre, M. D., Wasser, B., Brandon, T., Clucas, D., Jaqueth, E. J., Benson, Z., Beatty, C. M. and R. S. Spaulding **(2016)**. Spectrophotometric measurement of freshwater pH with purified meta-Cresol purple and phenol red, submitted to *Limnol. Oceanogr. Methods.*

Total amount of expenditures as of 6/8/16:

Total Budget:	$290,971	
All Expenditures:	$96,844	Salary, benefits and supplies
All Encumbrances:	$13,171	Salary, benefits and tuition