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

Second Quarterly Project Report
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Objective 1: Small organic sensor for arsenate: Orion B. Berryman

Hirings:

No new hires were made during this period. Currently three people are supported by the grant including Christopher Grubb (undergraduate student), Asia Riel (graduate student) and Casey Massena (graduate student).

Equipment Purchased:

We have purchased, installed and begun to use a Teledyne High Pressure Liquid Chromatography-UV-Vis instrument to purify synthesized compounds.

Progress towards Milestones:

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

- "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**, *in preparation*.

B. Calculations: The first round of calculations with external collaborator have been completed. Target molecules for selective binding to arsenate have been identified.

C. Synthesis and Crystallography: Initial progress toward synthesizing selective ligands has been made. Additionally, crystallizations have been set up to structurally characterize halogen bonding complexes.

D. Separations: Additive compound has been identified and tested to improve separation of halides in capillary electrophoresis (in collaboration with Palmer group).

The following activities have been undertaken under this objective:

We are working with Joe Fanguy, director of technology transfer at UM, to assess feasibility for a patent application based on new technology discovered through this funding. A disclosure form was filled out on January 26th, 2016.

Total amount of expenditures as of 2/23/16:

Total Budget:	$319,054	
All Expenditures:	$52,420	Salary, benefits, tuition, equipment and supplies
All Encumbrances:	$13,669	Salary, benefits and tuition

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

Hirings:

No additional hires have been made during this period. Professor Palmer received summer support during the previous reporting period. The project continues to support a graduate student (William Penny) and an undergraduate student (Tristan McGettrick)..

Equipment Purchased:

No additional major equipment was purchased during this period.

Progress towards Milestones:

A. Robust laboratory CE method for analysis of anions (nutrients, fracking return) and cations (fracking return):

Substantial progress continues to be 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. Work has begun on the design of preconcentration methods to allow detection at lower levels of necessary.

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

An initial mechanical design for the field-able instrument has been developed and a prototype instrument has been constructed from commercial and 3D-printed parts and components. This design will be tested in laboratory experiments.

C. Analysis of field samples, demonstrating accurate (in comparison to accepted laboratory methods) and reproducible results at relevant concentration levels

Not yet started.

Total amount of expenditures as of 11/23/15:

Budget:	$286,350	
All Expenditures:	$42,310	Sal.& ben., tuition, instrumentation and supplies
All Encumbrances:	$10,228	Salary & Benefits, Supplies
Total:	$52,538	

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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)

Hirings:
Dulaney Miller was hired as a lab technician to help extract additional eDNA samples from previous field tests. Currently three people are supported by the grant including Seth Smith, Jenna Schabacker and Shabnam Qureshi.

Supplies & Equipment purchased:
Had qPCR thermocycler necessary for analysis of eDNA samples repaired. 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. Purchased additional materials for the construction of improved stream samplers.

Progress towards Milestones:

1. August 1, 2015 – February 1, 2016: Collect preliminary data on sensitivity of existing eDNA sampling methods. Develop qPCR assays for detection of species of interest.
    a. Began implementing design modifications to the eDNA sampler ('stream sampler') to simplify the collection protocol, and improve the sensitivity of the method.
    b. Extracted DNA, amplified target DNA, and quantified the amount of target DNA present using qPCR for additional samples.
    c. Began development of a modified DNA extraction protocol. Current protocols are extremely inefficient at removing DNA from high volume (25mL) samples. The new protocol will be scalable to even higher volumes without a significant increase in cost.
    d. Conducted paired sample comparison between the two extraction protocols on 30 samples to determine the quantity and quality of DNA being extracted using the two methods.
    e. Attended Montana American Fisheries Society (AFS) meeting in Helena and presented two posters summarizing our work to date comparing sensitivity of existing eDNA field methods.
    f. Began development of a sequence database of existing native and invasive species to be used in the design of additional qPCR assays.
2. February 1 – August 1, 2016: Analyze preliminary data. Design autonomous eDNA sampling prototype.

    a. Summarized preliminary experimental data comparing biomass of rainbow trout and the number of copies of target DNA detected using current eDNA sampling methods.
    b. Summarized preliminary experimental data comparing sampling volume and the number of copies of target DNA detected using our stream sampler prototype and traditional filter methods.
    c. Began running qPCR tests on remaining field samples comparing existing eDNA sampling methods. Samples were extracted using 2 different approaches, including a newly developed protocol, which promises to increase DNA yield from difficult samples.
    d. Researched existing commercially available filters for evaluation of their potential use in 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 February 29, 2016:

Total Budget	$396,023.00	
All Expenditures	$28,767.45	Salary, benefits, & supplies
All Encumbrances	$7,428.32	Salary & benefits

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

Professor Mike DeGrandpre

Hirings:

No new hires were made during this period. Four people are currently supported by the grant including Joe Clinch (undergraduate student), Reba Van Beusekom (graduate student), Cory Beatty (Research Associate) and Dr. Chunze Lai (post-doctoral scientist). We have been using a local electronics engineer for electronic repair and design (Doug McIntosh).

Equipment Purchased:

We are purchasing parts for the alkalinity systems but the instruments are being built at UM.

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 made significant progress towards this goal. Chunze Lai and Cory Beatty have been working together to begin building three new alkalinity systems. These activities include:

- design and assembly of prototype electronic boards by electronics engineer Doug McIntosh
- review of mechanical drawings of the alkalinity system
- submitting the drawings to Sunburst Sensors. Sunburst is overseeing the fabrication of the housings by Big Sky Machining (Superior, MT).
- ordering all other necessary parts
- most of the parts have now arrived and Cory and Chunze are beginning to assemble the instruments.

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

We are going to wait and see how the new alkalinity systems perform before adding pH to the system (which further complicates the design). The combined system will first be tested by Reba, using her benchtop prototype.

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

Not yet started.

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*

The following activities have been undertaken under this objective:

- We are working with Joe Fanguy, director of technology transfer at UM, and a Chicago law firm, Michael Best and Friedrich, to push a patent application through based on the proposed technology. A response to the latest patent office decision was submitted on December 21, 2015.
- Chunze Lai is also working on a research paper focused on freshwater pH measurements using indicators. The proof-of-concept publication is essential for advertising the technology to the research community and thereby facilitating commercialization of the method.

Total amount of expenditures as of 2/23/16:

Total Budget:	$290,971	
All Expenditures:	$46,916	Salary, benefits, tuition and supplies
All Encumbrances:	$61,138	Salary, benefits and tuition