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

First Quarterly Project Report
12/1/2015

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

Objective 1: Small organic sensor for arsenate: Orion B. Berryman

- Hirings – Casey Massena (graduate student) and Asia Riel (graduate student)
- Equipment Purchased – Preparation scale HPLC (pending)
- Progress towards milestones:
    - Publications (2)
        - "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**, *Submitted*.
    - Calculations with external collaborator have been initiated
    - Initial synthesis and crystallographic studies have been initiated
- Expenditures by report date: Actual amount ($18,344.37), Encumbrances ($16,183.33)

****

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

Hirings:

Graduate student William Penny has been hired as a research assistant with a full assistantship including tuition. Undergraduate student Tristan McGettrick has also been hired as a part time laboratory assistant. An undergraduate exchange student from Austria has been volunteering on the project for laboratory research experience.

Equipment Purchased:

A high quality water purifier was purchased ($4781) to generate water with low conductivity and very low or no contamination with analyte ions. Water of this type is necessary to develop and evaluate CE methods.

A new conductivity detector head was purchased ($600) to allow laboratory-based CE separation and conductivity detection of ions.

A computer system was purchased ($414.90) to use for data acquisition and control in the laboratory.

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. Analytes are easily detected at low part per million or high part per billion levels.

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

Schematics have been developed and commercial parts are being identified to construct a portable and field-able CE instrument.

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:	17,813.45	Salary and benefits exp. $5,903 enc. $15,645
All Encumbrances:	16508.44	Tuition exp. $1,462
Total:	$34,322	Instruments and supplies exp. $10,395 enc. $863

****

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 & Gordon Luikart

1. August 1, 2015 – February 1, 2016: Collect preliminary data on sensitivity of exiting eDNA sampling methods. Develop qPCR assays for detection of species of interest.
    - Hiring
        - Shabnam Qureshi hired in October as full time technician to help extract and analyze eDNA samples.
        - Jenna Schabacker hired in December as part-time technician to oversee extraction and analysis of samples, and development of additional qPCR assays.
        - Seth Smith hired in November as part-time technician to help collect eDNA samples, improve eDNA stream sampler, and to optimize extraction protocols.
        - Advertised for and interviewed an applicant for post-doc position. No acceptable candidates were found. We will be re-advertising position in

December, perhaps as a collaborative position (employee) shared with USFS Rocky Mountain Research Lab.

- Supplies & Equipment purchased
    - Ordered portable water pump and necessary supplies for collecting eDNA water filter samples. Received quotes for automated water sampling pumps and turbidity monitoring equipment. Purchased supplies for the construction of additional eDNA stream samplers.
- Progress toward milestone
    - Designed and produced prototypes for a new eDNA sampler ('stream sampler') designed to collect eDNA samples from high volumes of water.
    - Collected preliminary data to test the sensitivity of existing sampling methods. Existing sampling methods involve collecting eDNA via low volumes of water (2-5L) passing through a filter ('filter sample'), or using high volumes of water (1000-4000L) through the stream sampler. Experimental designs for testing the sensitivity involved manipulating rainbow trout biomass in hatchery raceways to control the amount of eDNA present, and collecting field samples from streams with low and high density bull trout populations. In total, approximately 220 samples were collected comparing two sampling methods.
    - Extracted DNA, amplified target DNA, and quantified the amount of target DNA present using qPCR for all samples.
    - Attended Montana Aquatic Invasive Species (AIS) meeting in Helena to strengthen collaborations with Montana Fish, Wildlife & Parks (MTFWP) and to advocate for the increased use of eDNA for monitoring AIS.
    - Researched publically available sequences and qPCR assays for pondweed.
    - Outlined design modifications to the stream sampler to improve sampling ease, efficiency, and repeatability.

2. February 1 – August 1, 2016: Analyze preliminary data. Design autonomous eDNA sampling prototype.
    - Progress toward milestone
        - Summarized preliminary experimental data comparing correlation between biomass and copies of target DNA present in different samples.
3. August 1, 2016 – February 1, 2017: Field-test autonomous eDNA sampling prototype.
    - Not yet started
4. February 1 – July 31, 2017: Analyze data, prepare intellectual property documents, and prepare research publications.
    - Progress toward milestone
        - Met with Joe Fanguy (Director of Technology Transfer, UM) to discuss patent potential of stream sampler and qPCR assays designed to detect species present in an eDNA sample.

Total Amount of Expenditures as of Dec. 1:

- Total Budget $396,023.00
- All Expenditures $5,787.96 Salary, benefits, & supplies
- All Encumbrances $5,135.23 Salary & benefits

****

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

Hirings:

Graduate student Reba Van Beusekom has been working on the project as a graduate research assistant since August. Undergraduate students Taymee Brandon and Joe Clinch are also working on the grant (Taymee is obtaining credits and is not paid). On November 23rd, Chunze Lai began working on the grant as a post-doctoral scientist. We are fortunate to recruit Chunze Lai. She is here in Missoula because her husband is a new faculty member on campus. However, it still took almost two months to get the paperwork through the system.

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.

With the hiring of Chunze Lai in late November, we can begin to make progress on this milestone. She will interface with Sunburst Sensors to facilitate the design and construction of two additional alkalinity systems. She is currently looking into different data logger designs. Graduate student Reba Van Beusekom is conducting the benchtop testing and Chunze will use Reba's results to construct the benchtop prototype.

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

We are on track to complete this milestone. Reba and another graduate student (Adam Prody) supported on an NSF grant are both making good progress on the automated/autonomous alkalinity system. Sunburst Sensors is also supporting this effort by providing electronic troubleshooting and design.

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

We are working with Joe Fanguy, director of technology transfer at UM, to push a patent application through based on the proposed technology.

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

Total Budget:	$290,971	
All Expenditures:	$8340	Salary, benefits and supplies
All Encumbrances:	$54,133	Salary, benefits and tuition