

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

Final Report. July, 2017

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

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

Summary:

The objective of this sub-aim was to develop sensors for spectroscopic detection of arsenate and iodide. In collaboration with the Palmer lab, this project was also developing new stationary phases for analytical device separation of anions using halogen bonding interactions. This sub-project met the objectives by developing new anion receptors and sensors for iodide and arsenate. Initial results from the MREDI award enabled an additional \$675,000 in grant funding to be leveraged from the National Science Foundation. The results have been published as four journal articles and one book chapter which is in press. Preliminary results developed on new iodide binding and self-assembly were used to apply for a provisional patent on this technology. Private partnership has been established with theoretical chemist at the Supramolecular Research Institute.

Results from this MREDI award will be leveraged for additional grant money. Additionally, the research that this lab and the Palmer lab have been performing on halogen bonding anion separation has strong patent prospects.

Grants received:

NSF CAREER award CHE 1555324 "CAREER: Fundamental Studies of Multidentate Halogen Bond Donors for Supramolecular Catalysis." Total: \$675,000.

Grants in development:

NIH R35 MIRA award "Halogen Bonding Self-Assembly of Triple Helices and their Development as Synthetic Anion Channels." To be submitted October 2017

Partnership formed:

Private partnership with the Supramolecular Research Institute to run theoretical calculations on developed systems.

Publications:

In total, four publications related to this work have been published and one is in press.

- "Advantages of organic halogen bonding for halide recognition" N. B. Wageling; G. F. Neuhaus; A. M. Rose; D. A. Decato; O. B. Berryman, *Supramolecular Chemistry*, **2016**, *28*, 665-672. 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*, *16*, 974-980. DOI: [10.1021/acs.cgd.5b01524](https://doi.org/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**, *Angewandte Chemie International Edition*, *55*, 12398-12402. - Front cover artwork, featured by NSF EPSCoR/IDeA Foundation, SciShow, UM press release, Montana Associated Technology Roundtables, Montanan Magazine, UM President’s Report, and UM We Are Montana tour.
- “Experimental Evidence of Halogen Bond Hard-Soft Acid-Base Complementarity.” A. M. S. Riel; M. J. Jessop; D. A. Decato; C. J. Massena; V. R. Nascimento; O. B. Berryman, **2017**, *Acta Crystallographica Section B*, *B73*, 203-209. – Invited contribution
- “Aspects of Multi-component Crystals: Synthesis, Concepts and Function.” D. A. Decato; O. B. Berryman, **2017**, Tiekink, E. R. T., Eds; De Gruyter Publishers. *In press*.

Equipment:

An Agilent Cary 60 UV-Vis and Cary Eclipse fluorimeter was purchased to quantify analyte binding to synthesized compounds and to evaluate their capability as sensors.

Patents:

Working with Joe Fanguy, director of technology transfer at UM, a provisional patent application was filed (application number 62/362,226) based on new technology discovered through this funding.

Jobs Created:

Funding from this award enabled six different individuals to be hired. Two of the hires worked as technicians, one undergrad was hired and three graduate students contributed to this work.

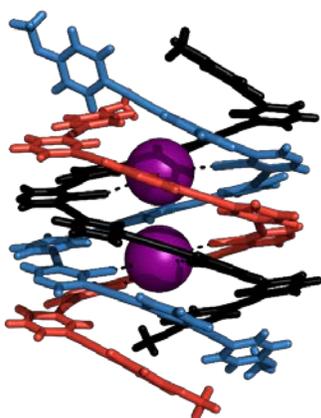


Figure 1: Strong and selective iodide binding

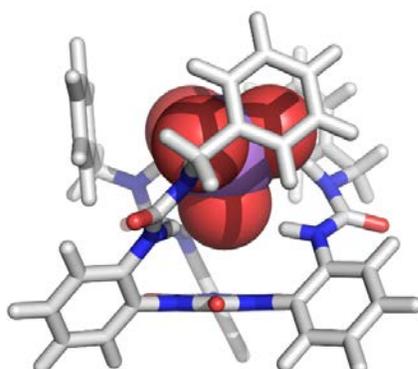


Figure 2: New anion receptor binds arsenate



Figure 3: Inherently fluorescent compounds as sensors

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

Summary:

The objectives of this sub-aim were to develop a small, battery-powered capillary electrophoresis (CE) system capable of field analysis of surface or ground water for anionic nutrients and for cations associated with fracking return water and to demonstrate applicability to natural waters.

Several prototype instruments (Figures 1, 2) were built and tested, resulting in a final prototype that can be transported in a small suitcase sized case. The instrument is fully functional and runs on 12V battery power. Detection of separated ions is currently achieved using a commercial conductivity detector running on laboratory power, but this can be easily configured to run on the DC power systems associated with the instrument.

Robust methods for the analysis of anions or cations in natural waters have been developed. Anion separations are able to detect ions at less than 200 ppb (Figure 3). Transfer of these laboratory-based methods to the portable CE system is nearly complete, although some further optimization is necessary to achieve complete separation of chloride and nitrite on this system. The method has been applied on both laboratory based and portable instruments for the analysis of Clark Fork River water (Figure 4), and anions are detectable at ordinary background concentrations in this water.

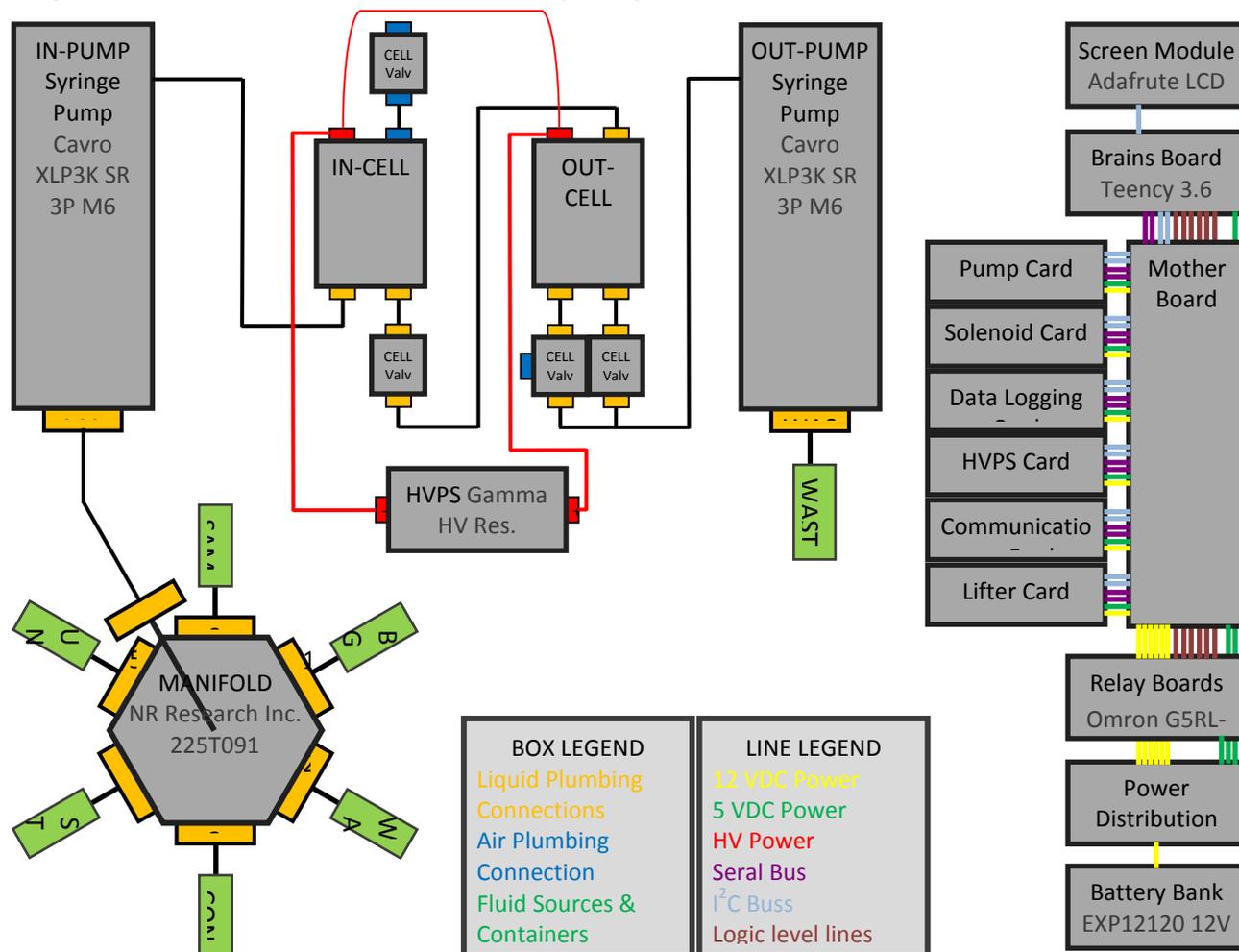


Figure 1: Schematic diagram of portable CE system

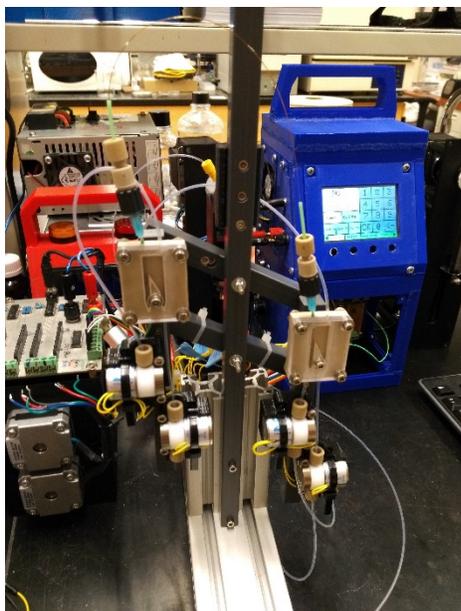


Figure 2: Prototype portable CE instrument showing electronic and plumbing components as well as central control system.

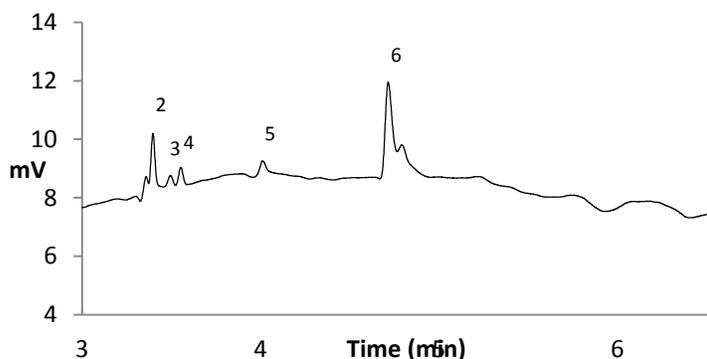


Figure 3: Separation of anions at 200 ppb. 1. Bromide, 2. Chloride, 3. Nitrite, 4. Nitrate, 5. Sulfate, 6. Bicarbonate

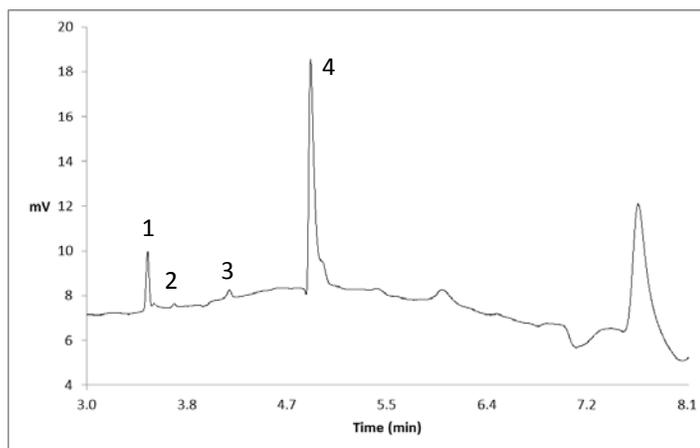


Figure 4: Analysis of Clark Fork River water. 1. Chloride, 2. Nitrate, 3. Sulfate, 4. Bicarbonate.

Partnership formed:

A private partnership with Vintage Lab Tech, LLC in Missoula MT facilitated much of the electronics and software development for the portable CE system.

Publications:

One publication related to this work or facilitated by this funding has been published and two have been submitted.

“Phospholipid Bilayer Affinities and Solvation Characteristics by Electrokinetic Chromatography with a Nanodisc Pseudostationary Phase,” William M. Penny, Harmen B. Steele, J.B. Alexander Ross, Christopher P. Palmer, (2017) *Electrophoresis*, 38, 738-746.

“Evaluation of Poly([2-(Acryloyloxy)ethyl]trimethylammonium Chloride) Cationic Polymer Capillary Coating for CE and Electrokinetic Chromatography Separations,” Julie R. McGettrick and Christopher P. Palmer, (2017) *Journal of Separation Science*, submitted.

“Effects of phospholipid nanodisc composition on solvation characteristics as determined by electrokinetic Chromatography,” William M. Penny, Christopher P. Palmer, (2017) *Electrophoresis*, submitted

Equipment:

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 was purchased (\$1600) 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.

Personnel Supported:

Personnel supported by this sub-project were one postdoctoral fellow (10 months), one graduate student full time for 2 years, three graduate students for partial summer support, and four undergraduate students.

Objective 3: Testing and optimizing large-volume water sampling, for the autonomous collection of environmental DNA (eDNA) samples and environmental data (water temperature, flow, and turbidity), for early detection of invasive, rare, or threatened species.

PI: Steve Amish, University of Montana, Flathead Lake Biological Station and Department of Biological Sciences

Co-PI: Gordon Luikart, University of Montana, Flathead Lake Biological Station and Department of Biological Sciences

Our novel water sampling approaches have the potential to transform the field of “environmental DNA” in two ways. First, by collecting larger quantities of environmental DNA (eDNA) they will improve biologists’ ability to detect aquatic invasive species (AIS) like zebra and quagga mussels, pathogens (e.g. fish-killing parasites in the Yellowstone River), and endangered native species like bull trout (Fig. 1). Early detection of aquatic invasive species threatening Montana’s public waters is critical for preventing their spread and devastating economic and ecological impacts.¹ Second, by collecting larger volumes of water with each sample we increase our efficiency and probability of detecting DNA from species present at low densities in lakes or rivers (Fig. 2). Automating the collection of eDNA samples at regular intervals will further increase sampling efficiency and detection rates. The MREDI grant has allowed us to: (1) design and produce a new manual high volume stream sampler for eDNA collection, (2) design, test, and apply several new eDNA qPCR assays (diagnostic tests) for the detection of aquatic invasive species, pathogens, and native species of Montana, and (3) to design and produce an autonomous eDNA sampler prototype.

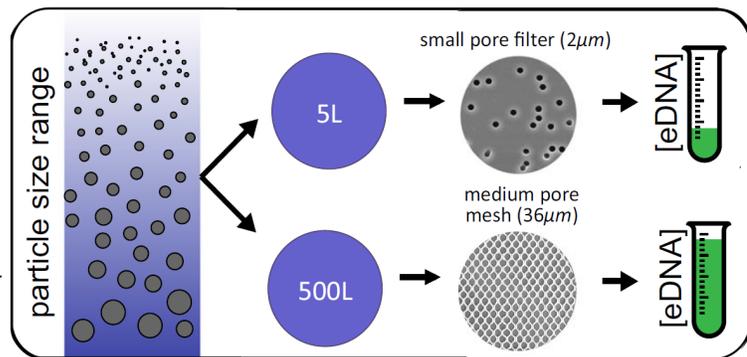


Figure 1. Medium pore size mesh collects less eDNA per liter of water, but can filter larger volumes of water (e.g. 500L) without clogging thereby collecting more eDNA.

¹ “Without increased and immediate action, quagga and zebra mussels will cause irreparable damage to western waters and long-term costs will be in the billions. In Idaho, the conservative estimate of state-wide direct and indirect costs from establishment of Dreissenid mussels is \$94,474,000” (WRP-ANS 2010).

- **Collect preliminary data on sensitivity of existing and new manual eDNA sampling methods**

We compared three different manual eDNA sampling methods to determine the relative detection sensitivity, quantity of eDNA collected, and limitations of standard (current) versus novel techniques. The standard and most widely used method involves the collection of eDNA by pumping 1-5L of water through a filter with microscopic pores. The second method is a novel sampling technique for lakes where eDNA is collected by dragging a fine mesh plankton tow net through the water (see video at <http://flbs.umt.edu/videos/default.aspx?id=1>). The third method uses a new manual water sampler for streams we designed and field tested; this method combines the two methods by using a fine mesh net with a pre-filter (to remove large suspended solids in streams) and a filter with microscopic pores (Fig. 3).

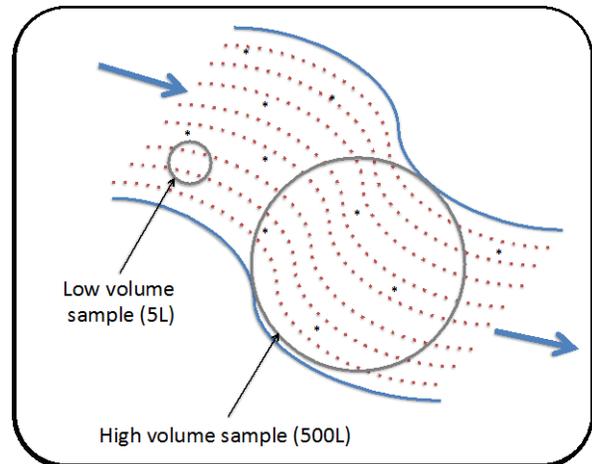


Figure 2. Stream segment showing the relationship between sample volume (grey circles), total amount of eDNA (red dots) and rare eDNA collected (black stars). High volumes detect eDNA more reliably.

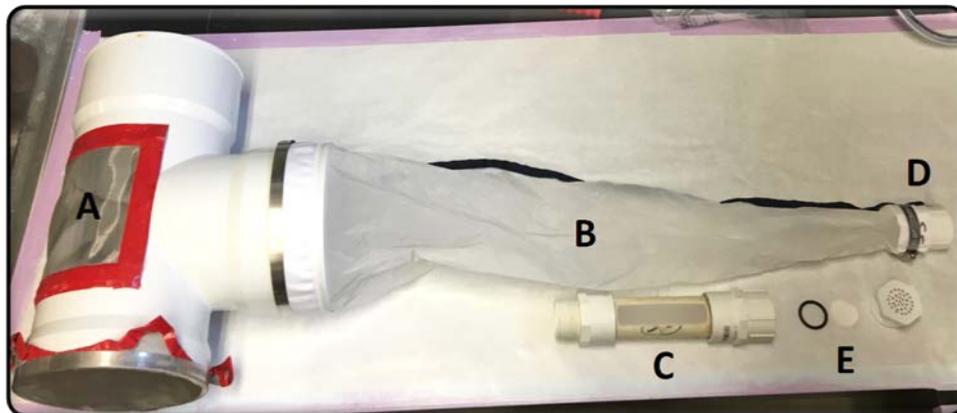


Figure 3. High volume water sampler for collection of eDNA samples from streams. Water enters the screen in the body (A) and exits through the cone shaped net (B). The collection bucket (C) is shown in the lower right hand corner, and screws onto the end of the net (D), and holds a small filter (E).

- **Develop qPCR assays for detection of invasive, rare, or threatened species**

We developed, validated, and applied seven new quantitative PCR (qPCR) assays for testing eDNA samples. These were used to compare eDNA sampling methods, but were also implemented in our ongoing efforts to monitor and protect Montana's aquatic resources, improving early detection capabilities for economically devastating aquatic invasive species and pathogens. After the recent discovery of quagga mussels in the Tiber Reservoir, we focused our qPCR assay design efforts on improving our ability to detect zebra and quagga mussels. We designed a new qPCR assay for zebra and quagga mussel eDNA, validated a second qPCR assay (developed in the Midwest) on samples collected in Montana, and began testing of a third qPCR assay developed in Canada. We also designed, validated and applied qPCR tests for the presence of eDNA from New Zealand

Mudsnails, Eurasian Milfoil, Curley Leaf Pondweed, and Rainbow trout to samples collected by our collaborators from across Montana. Finally, we also designed a qPCR assay for the myxozoan parasite (found in the Yellowstone River in 2016) causing Proliferative Kidney Disease in salmonids, often resulting in massive fish kills.

- **Analyze preliminary data**

We demonstrated that novel sampling methods for filtering large volumes of water are more sensitive than the standard widely used methods for filtering small volumes of water for detecting eDNA from invasive mussels, Eurasian milfoil, and fish. Our manual river sampler and plankton tow net sampling techniques filter 1000s of liters of more water than the current sampling method. As a consequence, we also detect an average of 2 - 10 times more DNA per sample, thereby providing more sensitive and reliable detection of invasive species (Amish et al. 2016). In addition, when sampling lakes, we showed that sampling large volumes of water improved the detection rate for eDNA from a native aquatic plant related to the invasive Eurasian milfoil (Schabacker et al. 2016).

We confirmed the specificity and sensitivity of seven of the eight assays we designed or tested. Each qPCR assay was tested for specificity to insure it only detects DNA from the target species in the presence of native species or any closely related taxa. Sensitivity of all assays was high, with minimum quantities of DNA needed to detect a species ranging from 4 - 10 copies (much less than is present in a single cell).

Samples from around Montana were used to validate qPCR assays, and as part of our ongoing efforts to monitor for aquatic invasive species. We have analyzed over 700 samples from waterbodies in both eastern and western Montana using these qPCR assays over the last 2 years. In collaboration with biologists from the US Forest Service, US Geological Survey, Glacier National Park, the Blackfoot and Salish Kootenay tribes, Missoula County Weed District, Flathead Basin Commission, Clearwater Resource Council, and the Whitefish Lake Institute we are using our new sampling techniques and these highly sensitive eDNA tests to establish an early detection network to prevent the spread of aquatic invasive species.

- **Design autonomous eDNA sampling prototype**

The autonomous eDNA prototype design provides complete automation of collection and preservation of up to 16 environmental DNA samples from a lake or stream (Fig. 4). Modular computer circuit board and sampler components facilitate expansion of sampling capabilities to 48 or more samples. LCD text display and selector switches make it easy to setup sampling programs. Sample volumes can range from 1-2000 liters and are monitored in real-time to ensure accuracy and precision. Sample volume and collection time/date are set independently for each filter unit providing incredible flexibility when setting up sampling programs. The sampler also has the ability to interface with a wide variety of external water quality sensors (pH, dissolved oxygen, temperature) that can be used to trigger sampling events. All data associated with a sample (volume, collection time, sample time, filter number, and internal and external water quality sensor readings) are logged to non-volatile memory and are available for download via a RS-232 serial data port. Compatibility with AC and DC power supplies allow the sampler to be set up in practically any location (see the Q7/8 report for further prototype details).

- **Field-test autonomous eDNA sampling prototype.**

Prototype consisting of a solenoid valve, manual valve, flow meter, turbidity sensor, vacuum sensor and the large pump was powered using a 12-volt, 33 amp-hour battery, to test electronic control circuits and power usage. The motor drive circuit was refined to give a wider speed range and run as cool and quiet as possible. The motor control software was programmed so that flow rate and volume can be used to control the pump. Electronic control circuits are currently being manufactured on printed, modular, circuit boards, which will allow us to scale up our design to 48 or more samples per unit.

Prototype consisting of the complete 8-filter design was powered using a 12-volt, 33 amp-hour battery, to test electronic control circuits and power usage. The basic sampling protocol was run, including testing water turbidity, collecting a sample while monitoring internal sensors for vacuum and flow rate, logging sample data, pumping water from the sampler, pumping sample preservative into the filter unit, and clearing sample preservative from the unit. Our field-testing results suggest the instrument will be reliable and can be deployed to sample at any desirable frequency (e.g. daily, weekly, or monthly). Field tests will continue in 2017.

- **Long-term impacts and future activities**

As part of our ongoing monitoring work to protect Montana’s aquatic resources, we will continue to expand our collaborations. We will further increase the number of species we can detect using eDNA by designing and testing additional qPCR assays. We will also continue to pursue opportunities to identify cutting-edge molecular techniques that can be applied to eDNA. We are actively researching field-deployable PCR technologies. We are currently testing digital droplet PCR (in collaboration with Cody Youngbull at the Flathead Lake Biological Station) that promises to be faster, very sensitive, and easily operated in the field. We are also collaborating with the only other zebra mussel eDNA lab in the United States (Amberg USGS, Wisconsin), investigating applications for a new molecular technique they are developing (LAMP, Loop-mediated isothermal amplification) which would allow non-specialists to do rapid testing for invasive mussels in the field with minimal training and equipment. Both of these new techniques could help prevent the spread of invasive

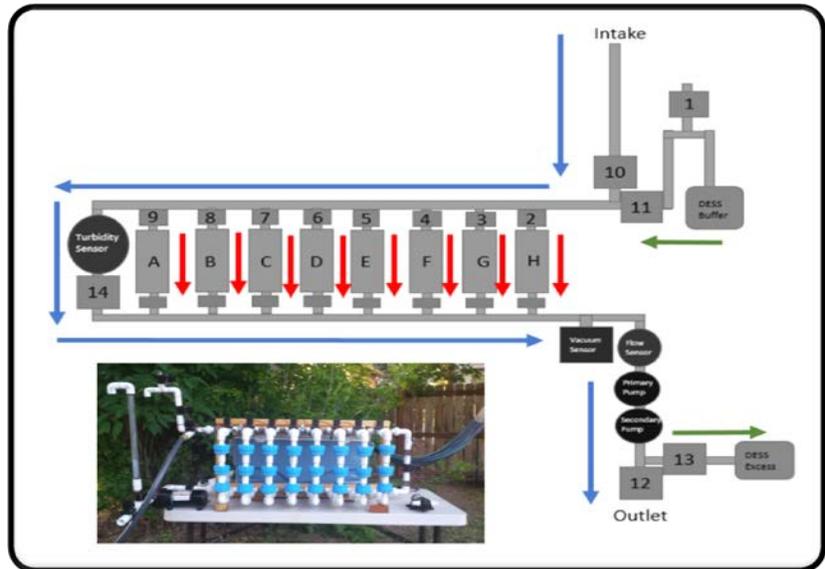


Figure 4. Schematic and photo of a prototype 8-filter design. Blue Arrows: Primary flow path for water through the instrument. Green Arrows: Flow path for eDNA sample preservative. Red Arrows: Sample flow paths. Closing and opening valves forces water to flow through a filter unit rather than the primary flow path. Samples are isolated in separate flow paths using a series of valves on the upstream and the downstream side.

species by identifying watercraft infested with zebra and quagga mussels at boat check stations and ramps.

The development of the autonomous sampler will increase the efficiency of eDNA sample collection and aquatic species detection. The most immediate benefit is the ability to collect more samples, from more time points, at a site with less manpower reducing the cost. Additionally, the ability to trigger samples based on observed water quality parameters (temperature, flow, dissolved oxygen, turbidity) allows us to leverage existing knowledge about an organism's physiological constraints to increase the probability of detection. Future work will focus on maximizing sample volume, expanding the ability of the sampler to interface with external sensors, and experimenting with long-term deployments in remote locations.

- **Grants received and in development**

MREDI funds helped us secure \$100,000 (2016-7) from anonymous donors around Flathead Lake for eDNA monitoring for invasive mussels, expand or establish contracts with USFS, USGS, Glacier National Park, Whitefish Lake Institute, Missoula County Weed district, and Clearwater Resource Council to monitor for invasive mussels, milfoil and pondweed (~\$90,000 for 2016 & 2017). We submitted a National Fish & Wildlife Fund grant proposal for detecting and monitoring bull trout in Montana streams. We also applied for an Army Corps of Engineers grant for monitoring aquatic invasive species (\$250,000, resubmission pending), and a NASA grant to combine remote sensing and eDNA data (\$300,000, resubmission pending). We also submitted a U.S. Geological Survey Innovation Fund proposal (\$50,000) for field-testing of the autonomous sampler that will be resubmitted in 2018. We are also currently developing an application for the M.J. Murdock Commercialization Initiation grant to further develop the autonomous sampler prototype.

- **Partnerships formed**

Partners and collaborators from this work include US Forest Service, US Geological Survey, Glacier National Park, the Blackfeet and Salish Kootenai tribes, Missoula County Weed District, Flathead Basin Commission, Clearwater Resource Council, the Whitefish Lake Institute, Sunburst Sensors LLC, SensorSpace, and JDM Enterprises Inc..

- **Publications & Reports**

Schabacker J., Luikart G., Amish S.J. *In prep.* Sensitive new qPCR tests for early detection of invasive species: Usefulness of eDNA and plankton net sampling.

Amish S.J., Schabacker J., Luikart G., Sepulveda, A. *In prep.* Detection of greater quantities of eDNA using high volume versus low volume stream sampling techniques.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Flathead National Forest, USFS, on water sample testing for Eurasian Watermilfoil: environmental DNA for detection of aquatic invasive species. Flathead Lake Biological Station, UM (MT). 2017. Report Number: FNF-USFS-2017-1.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Flathead National Forest, USFS, on water sample testing for Dreissenid mussels: environmental DNA for detection of aquatic invasive species. Flathead Lake Biological Station, UM (MT). 2017. Report Number: FNF-USFS-2017-2.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Flathead National Forest, USFS, on water sample testing for pondweed: environmental DNA for detection of aquatic invasive species. Flathead Lake Biological Station, UM (MT). 2017. Report Number: FNF-USFS-2017-3.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Blackfeet Tribe, on Spring water sample testing for Dreissenid mussels: environmental DNA for detection of aquatic

invasive species. Flathead Lake Biological Station, UM (MT). 2017. Report Number: BFT-2017-1.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Flathead National Forest, USFS, on water sample testing for Eurasian Watermilfoil: environmental DNA for detection of aquatic invasive species. Flathead Lake Biological Station, UM (MT). 2016. Report Number: FNF-USFS-2016-1.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Flathead National Forest, USFS, on water sample testing for Dreissenid mussels: environmental DNA for detection of aquatic invasive species. Flathead Lake Biological Station, UM (MT). 2016. Report Number: FNF-USFS-2016-2.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Blackfeet Tribe, on Fall water sample testing for Dreissenid mussels: environmental DNA for detection of aquatic invasive species. Flathead Lake Biological Station, UM (MT). 2016. Report Number: BFT- 2016-1.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Whitefish Lake Institute, on Fall water sample testing for Dreissenid mussels: environmental DNA for detection of aquatic invasive species. Flathead Lake Biological Station, UM (MT). 2016. Report Number: WLI-2016-1.

Miller, D., Amish S.J., Schabacker J., Luikart G. Report for Whitefish Lake Institute, on Fall water sample testing for Eurasian Watermilfoil: environmental DNA for detection of aquatic invasive species. Flathead Lake Biological Station, UM (MT). 2016. Report Number: WLI-2016-2.

- **Personnel supported by these funds**

Personnel supported by these funds included one undergraduate student, 3 technicians, one post-doc, one faculty member, one research scientist and one engineer.

Literature cited

Amish et al. 2016. Quantifying biomass from fish eDNA: a comparison of the sensitivity of 2 sampling methods for flowing water. *Poster*. Montana Chapter American Fisheries Society, Helena, MT.

Schabacker, J et al. 2016. Using Environmental DNA to monitor for aquatic invasive species in Lakes: variable detection rates between sampling methods and seasons. *Poster*. Montana Chapter American Fisheries Society, Helena, MT.

WRP-ANS 2010 Western Regional Panel on Aquatic Nuisance Species. 2010. Quagga-Zebra Mussel Action Plan for Western U.S. Waters Final February 2010. Submitted to the Aquatic Nuisance Species Task Force. Available at: http://www.anstaskforce.gov/QZAP/QZAP_FINAL_Feb2010.pdf

Objective 4: Lab testing of a combined pH and alkalinity system for autonomous freshwater measurements.

PI: Mike DeGrandpre, University of Montana, Department of Chemistry and Biochemistry

Summary:

The objectives of this grant were to develop and test an autonomous underwater measurement system for alkalinity and pH. Alkalinity and pH are commonly measured water quality parameters. Our goal is to develop a system that can be operated in situ (underwater) in natural or industrial water systems to provide higher quality data with increased temporal resolution. More specifically our goals were to:

1. Build three alkalinity measurement systems in collaboration with Sunburst Sensors, LLC a Missoula company.

2. Integrate an indicator-based pH analysis into the alkalinity system.
3. Extensively test these sensors in the lab and field (Clark Fork River).
4. Analyze data, prepare intellectual property documents, and prepare research publications.

We have made excellent progress on all four goals. Our efforts focused on improving the performance of the alkalinity system (SAMI-alk) and we have not yet integrated it with the pH measurement design. This will be an objective of future work. Field work was limited because we were not ready for field testing until November and the initial tests were not successful. River conditions were subsequently too risky throughout the remaining period of the grant. We are now moving ahead with field testing through support from another grant.

Accomplishments:

1. Grants received

M.J. Murdock Commercialization Initiation grant: *Commercialization of a Novel Titration System for Industrial and Environmental Monitoring* (\$120,000) August 2015.

National Science Foundation: *Long Term Research in Environmental Biology: River ecosystem responses to floodplain restoration* (co-PI with UM professor Maury Valett) (\$380,807) May 2017.

National Oceanic and Atmospheric Administration – *Field testing of the SAMI-alk*, (\$50,000 contract with Sunburst Sensors), June 2017.

2. Grants in development

National Oceanographic Partnership Program (collaboration between UM, Sunburst Sensors and NOAA)

3. Partnerships formed

We (DeGrandpre/Sunburst Sensors) are working with Steve Amish (UM Divisional of Biological Sciences) to develop an autonomous eDNA field sampling system.

We are working with NOAA scientist Jessica Cross to test the autonomous alkalinity system (SAMI-alk) in a coastal environment.

Sunburst Sensors and UM have established a collaboration with Professor David Long (Flathead Valley Community College) and William Pardis (MSU engineering student) to develop a hand-held indicator-based pH analyzer.

4. Publications

Stets, E.G., Butman, D., McDonald, C.P., Stackpoole, S., DeGrandpre, M.D. and R.G. Striegl (2017). Carbonate buffering and metabolic controls on carbon dioxide in rivers, *Global Biogeochemical Cycles*, 31, 663–677, doi:10.1002/2016GB005578. Highlighted on the cover issue, <http://onlinelibrary.wiley.com/doi/10.1002/gbc.20452/epdf> and the AGU newsletter *EOS* - https://eos.org/research-spotlights/why-is-there-so-much-carbon-dioxide-in-rivers?utm_source=rss&utm_medium=rss&utm_content=why-is-there-so-much-carbon-dioxide-in-rivers.

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, *Limnol. Oceanogr. Methods*, 14, 864–873, doi: 10.1002/lom3.10137.

5. Equipment

An automated titration system for measuring dissolved oxygen in natural waters was purchased.

6. Patents

DeGrandpre, M.D., Martz, T.R. and A.G. Dickson (2017). Tracer monitored titrations, U.S. Patent 2017_02_08_UMT-114X_15/419129.

7. Commercial products developed

In collaboration with Sunburst Sensors we have moved the autonomous alkalinity system significantly closer to commercialization. We have sold one system to NOAA for testing purposes. New software and hardware (see figures below) have been developed.

8. Personnel supported by the grant (6 directly, 4 indirectly)

The following personnel were supported by the project:

Graduate student Reba Van Beusekom
Undergraduate students Taymee Brandon and Joe Clinch
Postdoctoral Researcher Chunze Lai
Research Associate and Lab Manager Cory Beatty
Professor Mike DeGrandpre

The following personnel were supported by funds leveraged for the project:

Mechanical Engineer Jim Beck (Sunburst Sensors)
Research Scientist Reggie Spaulding (Sunburst Sensors)
Electrical Engineer Greg Leary (Sunburst Sensors)
Undergraduate Intern (from MSU) William Pardis (Sunburst Sensors)

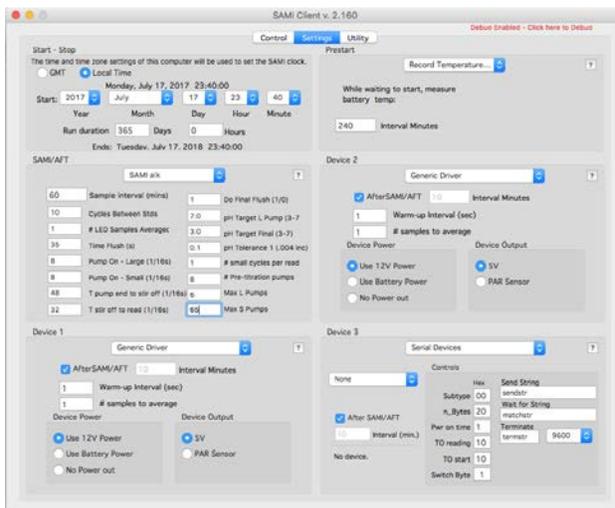


Figure 1: Screenshot of the software interface developed by Sunburst Sensors for the autonomous alkalinity system developed at the University of Montana (SAMI-alk). This new interface is a critical step to commercialization of the instrument.

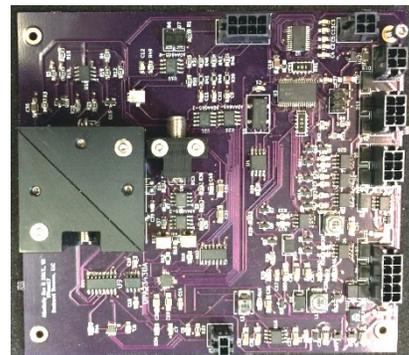


Figure 2: SAMI-alk integrated controller and optical board developed by Sunburst Sensors. The optical light source and detectors are housed in the black square at left. The new board makes the SAMI-alk optics and controller compatible with other Sunburst Sensor products greatly facilitating the software development (Figure 1). Actual size is ~4"x4".



Figure 3: SAMI-alk being deployed in the Clark Fork River in November 2016. Field tests are continuing during 2017.