Vermont Water Resources and Lake Studies Center
Annual Technical Report
FY 2017

Introduction

The Vermont Water Resources and Lake Studies Center (Water Center) facilitates water resources related research and supports faculty and students at Vermont colleges and universities. Research priorities are identified each year, determined by the Water Center Advisory Board, as well as through collaboration with the State of Vermont Department of Environmental Conservation, Lake Champlain Sea Grant, Lake Champlain Basin Program, and other programs in the state. The Director works with state, regional, and national stakeholders to identify opportunities to link science knowledge with decision making in water resource management and policy development. The Director of the Water Center is also the director of Lake Champlain Sea Grant (LCSG) and both programs share the same advisory board, which leverages the strengths of each program. The LCSG currently has limited funds available for research, but is dedicated to research extension through outreach and education. By working closely with LCSG, research extension of the Water Center is enhanced. The Director of the Water Center is also a member of the Steering Committee of Lake Champlain Basin Program (LCBP) and regularly brings information from Center-funded projects to the attention of LCBP committees. His activity on these committees also helps to inform the directions of the Water Center and has led to a number of productive partnerships.

Research Program Introduction

During the 2017-18 project year, the Water Center funded five projects, including three graduate student research projects and two faculty projects. Proposals were reviewed by external peers and the advisory board. Water resources management research, including physical, biological, chemical, social science, and engineering were solicited in the RFP. These topics are of interest to stakeholders of the Water Center, including the Vermont Department of Environmental Conservation, the Lake Champlain Basin Program, the Lake Champlain Research Consortium, and Lake Champlain Sea Grant.

The research projects supported by the 104b funds in the 2017-18 project year were:

1. Trails to remediation: the effects of seasonal variations on the acid mine drainage microbiome at Ely Copper Mine in Vershire, VT. Lesley-Anne Giddings (Middlebury College). Faculty project

2. Pharm-free surface waters: Identifying barriers and motivators that influence Vermonters' participation in pharmaceutical take-back programs. Alexandra Millar (M.S. student, Rubenstein School of Environment and Natural Resources), Christine Vatovec (Rubenstein School of Environment and Natural Resources). Graduate student project.

3. Phosphorus export from forested watersheds in the Missisquoi Basin. Vanesa Perillo (PhD student, Department of Plant and Soil Sciences), Don Ross (Department of plant and Soil Sciences), Beverley Wemple (Department of Geography). Graduate student project.

4. Global to local assessment of cyanotoxins in fish. Jason Stockwell (Rubenstein School of Environment and Natural Resources). Faculty project.

5. Application of neural networks to classify erosional and depositional stream reaches in glacially-conditioned Vermont catchments. Kristen Underwood (PhD student, School of Engineering), Donna Rizzo (School of Engineering), and Mandar Dewoolkar (School of Engineering). Graduate student project.

Additionally, a two-year project previously funded was completed and the final report is included:

System-wide rapid quantification of streambank erosion, Year 2. Mandar Dewoolkar (School of Engineering, University of Vermont), Jarlath O'Neil-Dunne (Spatial Analysis Laboratory, Rubenstein School of Environment and Natural Resources, University of Vermont), Donna Rizzo (School of Engineering, University of Vermont), Jeff Frolik (School of Engineering, University of Vermont).

System-wide rapid quantification of streambank erosion

Basic Information

Title:	System-wide rapid quantification of streambank erosion
Project Number:	2016VT79B
Start Date:	3/1/2016
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	Vermont-at-Large
Research Category:	Engineering
Focus Categories:	Sediments, Models, Geomorphological Processes
Descriptors:	None
Principal Investigators:	Mandar M. Dewoolkar, Jarlath ONeilDunne, Donna Rizzo, Jeff frolik

Publications

1. Hamshaw, S. D., Bryce, T., Rizzo, D. M., O'Neil-Dunne, J., Frolik, J., and Dewoolkar, M. M. (2017). "Quantifying streambank movement and topography using unmanned aircraft system photogrammetry with comparison to terrestrial laser scanning." River Research and Applications, 33(8), 1354–1367.
2. Hamshaw, S. D. (2018). "Fluvial Processes in Motion: Measuring Bank Erosion and Suspended Sediment Flux using Advanced Geomatics and Machine Learning." Ph.D. Dissertation, University of Vermont, Burlington, VT.
3. Hamshaw, S. D., Dewoolkar, M., Rizzo, D. M., O'Neil-Dunne, J., Rizzo, D.M., Frolik, J., & Engel, T. (2016). Quantifying streambank erosion: a comparative study using an unmanned aerial system (UAS) and a terrestrial laser scanner. American Geophysical Union 2016 Fall Meeting, San Francisco, California. Poster presentation.
4. Hamshaw, S. D., Engel, T., O'Neil-Dunne, J., Rizzo, D. M., and Dewoolkar, M. M. (2018). "Application of unmanned aircraft system (UAS) for monitoring bank erosion along river corridors." Geomatics, Natural Hazards and Risk, Revision in Review.
5. Ross, D.S., Wemple, B.C., Willson, L.J., Balling, C., Underwood, K.L, & Hamshaw, S.D. (2018) Tropical Storm Irene's Impact on Streambank Erosion and Phosphorus Loads in Vermont's Mad River. Journal of Geophysical Research: Biogeosciences. In Revision
6. Hamshaw, S. D., Bryce, T., Dewoolkar, M., Rizzo, D. M., O'Neil-Dunne, J., Rizzo, D.M., Frolik, J., & Engel, T. Quantifying streambank erosion using unmanned aerial systems at the site-specific and river network scales. Geotechnical Frontiers 2017 Conference, Orlando, Florida.

13. Evaluating Quantitative Models of Riverbank Stability

14. Regional or State Water Problem

A growing concern over the past few decades in the Lake Champlain region is the eutrophication of Lake Champlain. The Vermont Agency of Natural Resources identified sediment and phosphorus as the largest contributors to the impairment of surface water quality and aquatic habitat in the State (e.g. VTANR, 2011). Phosphorus has also been identified as the rate-limiting nutrient for the algal blooms in Lake Champlain and has been blamed for accelerating eutrophication for the past several decades (Meals and Budd, 1998). With over 7,000 miles of streams and rivers feeding the Lake, massive amounts of sediment and associated nutrients are discharged each year. High phosphorus levels allow algae to flourish because phosphorus is often the limiting nutrient for growth. Understanding where sediment and its particle-associated nutrients come from is therefore critical for informed and effective land and water management.

Streambank erosion is estimated to account for 30-80% of sediment loading into lakes and waterways (Simon and Rinaldi 2006; Evans, et al. 2006; Fox, et al. 2007). In the Lake Champlain Phosphorus Total Maximum Daily Load (TMDL) report, the Vermont and New York Departments of Environmental Conservation (VTDEC and NYSDEC, respectively), suggested that streambank erosion, such as the example shown in Figure 1, could be one of the most important nonpoint sources of sediment and phosphorus entering streams, rivers, and lakes in the state (VTDEC and NYSDEC, 2002). The Lake Champlain Basin Program (LCBP) also considers streambank erosion to be a potentially important source of phosphorus loading and has advocated the funding of streambank stabilization measures to reduce these loads (Lake Champlain Management Conference, 1996). Langendoen et al. (2012) conducted a study involving extensive field work and BSTEM (Bank Stability and Toe Erosion Model) modeling for the State of Vermont to quantify sediment loadings from streambank erosion in main stem reaches of Missisquoi River. Using the flow records between 1979 and 2010, they predicted that 36% (31,600 t/yr) of the total suspended-sediment load entering Missisquoi Bay was from streambank erosion. These estimates were based on "one-time", yet labor and resource intensive, field work performed at 27 sites that were extrapolated to 110 km of stream length. Although this study demonstrated the feasibility of obtaining estimates of streambank erosion at the watershed level, this approach requires tremendous resources. Also, this method could not provide estimates of deposition; all eroded material was considered to be transported. Here, an alternate approach of using an affordable Unmanned Aircraft System (UAS) is proposed.

Figure 1: Example riverbank failure

15. Statement of Results or Benefits

We propose to assess the capability of the low-cost UAS technology to make reliable quantification of streambank erosion and deposition at variable scales (ranging from site specific to the watershed scale). An UAS can be quickly deployed and acquire continuous images of several kilometers of streambanks, yielding orthorectified imagery and 3D topographic models. Because UAS are not subjected to the high costs and atmospheric constraints of aerial and satellite systems, data can be acquired for a given location at numerous times throughout the year, particularly in early spring and late fall when the vegetation is sparse. Multi-temporal UAS data have the potential to track streambank erosion and stream migration over a desired period of time. This will lead to a reliable and affordable way of quantifying streambank erosion and deposition. The project will capitalize on significant experience developed at UVM in applying UAS and terrestrial LiDAR technologies for characterizing built and natural environments. The proposed study site is Mad River Watershed, which has been one of the major subjects of study under the current NSF EPSCoR RACC research project (http://epscor.w3.uvm.edu/2/node/30). The study area was recently flown for airborne LiDAR by the State; these data will also be useful to the proposed project to some extent. The educational components include graduate and undergraduate researchers and incorporation of research methods and results of this project into UVM courses. Professional development workshops will be developed and conducted for Vermont state and government personnel.

16. Objectives and Timeline

The specific objectives of the proposed study are to:

(1) develop decision support tools to effectively acquire and process continuous streambank profiles using an affordable UAS;

(2) compare the results at select sites from terrestrial LiDAR-based surveys;

(3) develop and validate a methodology to reliably quantify annual system-level streambank erosion and deposition rates; and

(4) develop and incorporate related educational modules for UVM coursework and conduct professional development workshops for Vermont state and government personnel; and prepare and submit manuscripts to journals and relevant conferences.

Our testable hypotheses are summarized in Table 1 below along with the criteria for success.

Table 1 – Research hypotheses

#	Hypothesis	Performance Criterion
1	The UAS-based analysis will yield accurate measurements of streambank changes.	When compared to measurements of streambank changes measured from terrestrial LiDAR the target level of match will be within 10%.
2	UAS streambank mapping will be more cost effective and timely than field survey or terrestrial LiDAR mapping.	A single UAS flight (~40 minutes in duration) will capture 2-8 km of stream and multiple flights will capture at least 10 km of a stream in a single outing. Data processing will be largely automated.
3	UAS streambank mapping will be timely and responsive.	UAS data acquisition will be conducted at key times during the year to coincide with optimal mapping conditions. Following a major event (e.g. flooding) UAS data will be collected within 72 hours. UAS data processing will be largely automated and yield 2D and 3D products within 24 hours of data acquisition.

During the first project year, selection of sites, UAS flights, terrestrial LiDAR scans, and RTK-GPS survey were completed. In Year 2, repeat surveys of all streambank monitoring sites were completed. Initial analysis focused on detailed comparisons of the UAS and terrestrial LiDAR at select cross-sections. This allowed determination of the baseline reliability and repeatability of UAS data to capture the streambank topography and measure bank movement. In Year 3 river corridors were resurveyed under optimal early spring leaf-off conditions with UAS and RTK-GPS. UAS-derived DEMs were created and evaluated for geomorphic change detection by comparison to ground survey and existing airborne LiDAR surveys resulting in estimates of annual rates of bank movement as well as volumetric change. In Year 4, a repeat UAS survey will be collected at one study site and used to quantify longer term rates of channel migration.

The project builds on the bank stability work we have done under previous Water Center projects, which also laid the foundation for the streambanks related work currently underway for the ongoing National Science Foundation-funded EPSCoR RACC project in the Mad River Valley. The project also benefited from the recent developments of UAS technologies at UVM that are funded by an ongoing U.S. Department of Transportation-funded project on applying UAS imagery for disaster response and recovery.

17. Methods, Procedures, and Facilities

Several methods have been used to quantify streambank erosion and deposition as depicted in Figure 2. One of the most basic methods is direct measurement. For example, Lawler, et al. (1999) made use of longitudinal surveys and pins to quantify sediment loading through bank erosion. Longitudinal surveys allow the measurement of bank top retreat, while the pins allow measurement of toe erosion of laterally migrating streambanks. Direct techniques such as these have been found valuable in determining sediment loads in small watersheds; however, they are very labor intensive. Other approaches have included estimates of lateral channel movements based on the analysis of aerial photography (e.g. Reinfelds, 1997; Hughes, et al., 2006), and more recently, using remote sensing (e.g. aerial or terrestrial LiDAR) observations (e.g. De Rose and Basher, 2011), which are quite expensive.

Figure 2: Methods for quantifying streambank retreats

Analytical approaches have used slope stability analysis based on the limit equilibrium method (e.g. Osman and Thorne, 1988; Darby and Thorne, 1996) and often employ computer programs such as SLOPE/W (e.g. Dapporto, et al., 2003; Borg et al., 2014) and BSTEM (e.g. Simon, et al., 2000; Langendoen et al. 2012). The latter model includes fluvial erosion in addition to geotechnical failure. These approaches rely heavily on determination of relevant soil properties and site characterization (e.g. soil classification, unit weights, shear strength parameters, soil suction, root strengths, etc.) requiring extensive field work (e.g. Simon, et al., 2000; Borg, et al. 2014). With the exception of remote sensing applications such as airborne LiDAR, which is expensive; the above mentioned other methods (erosion pins, traditional and terrestrial LiDAR-based surveys, analytical slope stability methods) provide only site specific information requiring crude extrapolations to make watershed-level estimates of streambank erosion. Recent developments in UAS technology provide opportunities to develop methodologies for rapidly and economically determining streambank erosion at system-wide scale. The proposed research employs the following UAS system and terrestrial LiDAR. Both are owned and operated by UVM.

Figure 3. The UAS team preparing for launch of the UAS at Lareau site in Waitsfield

Figure 4. The UAS being launched at Moretown site by graduate student Scott Hamshaw

The UAS to be used for this research is SenseFly eBee, shown in Figures 3 and 4. The eBee is owned and operated by UVM Spatial Analysis Lab (SAL) in collaboration with the Transportation Research Center (TRC). The eBee was purchased under a US Department of Transportation grant. Over 300 flight operations have been conducted yielding over 20TB of 2D and 3D data products. The eBee is lightweight autonomous foam aircraft that contains an integrated 16 MP camera capable of recording aerial imagery at resolutions as fine as 2 cm/pixel. The entirety of this system's hardware can be easily transported in a flight case and rapidly assembled in the field. With a well-practiced team following a set of established standard guidelines, the eBee can be deployed in a few minutes. A field-swappable rechargeable battery provides up to 45 minutes of flight time and allows the eBee to cover areas up to 10 km^2 (3.9 mi^2) in a single flight; and the system can be used in light rain or snow and can tolerate winds as high as 10 m/s (22 mph) (Zylka, 2014). An integrated GPS unit and radio module facilitates communication between the UAS and the associated software (eMotion2) to provide real-time flight monitoring. The stream environment can sometimes be unsafe during storm events and conventional surveying often requires targets to be held in the stream; making UAS a much better alternative.

The terrestrial LiDAR used in this research is a RIEGL VZ1000 model (Figures 4 and 5), which was acquired through a National Science Foundation grant. It is capable of remote three-dimensional mapping of surfaces with very fine resolution (better than 1 cm) that are from 2.5 m to 1,000 m in distance. Each return from the laser pulse system has range and intensity values, as well as spatial position measured in three dimensions. When plotted in 3D space, these returns are referred to as a point cloud. By distributing reflective control targets around an area of interest, it is possible to combine the data collected by several scans at unique locations into a single composite point cloud. UVM also owns copies of the software RiScan and QT-Modeler, which are used to post-process the LiDAR data. The former also allows for multi-station alignment which alleviates the need for specific targets and aligns scans using environmental features. It is to be noted that control targets will not be necessary for the UAS because it is fitted with a GPS unit making it even easier to deploy and use.

A total of about 20 km of stream reaches within the Mad River, New Haven River, and Winooski River watersheds have been selected for this investigation. These include the main stems as well as some tributaries. Seven specific sites within these 20 km stream reaches have been selected for terrestrial LiDAR scans, which cover about 100 m length of the stream at each location. It is anticipated that a total of about eight sets of UAS and companion terrestrial LiDAR datasets would be collected over the 2-year project duration. Data will be gathered in early spring and late fall when the vegetation is sparse; these will total to four sets of data. Two additional data sets will be gathered following significant storm events and also when water levels in the stream are lowest. UAS and terrestrial LiDAR data will be collected concurrently to allow direct comparison and assess the accuracy of UAS-based measurements against the terrestrial LiDAR-based measurements.

The analyzed data will be compared to airborne LiDAR data. The Airborne LiDAR was recently (~May 2014) flown in the Mad River Watershed and the associated data were released in late 2016 (there is usually about a year or longer lag between data collection and dissemination owing to time-consuming data processing and QA/QC). Unfortunately, multi-date airborne LiDAR data will not be available for the study area. Nonetheless, the airborne LiDAR-based data from May 2014 when compared to the UAS and terrestrial LiDAR-based data to be collected as part of this project will allow estimation of streambank retreats between this duration of about a year. This retreat rate could then be qualitatively compared to the ones determined between 2015 and 2016 obtained using UAS and terrestrial LiDAR.

Findings from Year 1 - Year 3 of Project

Data Collection

To-date on this project the UAS was deployed to survey 21 km of river corridor in Central Vermont. Terrestrial laser scanning and RTK-GPS surveying were also utilized at 7 detailed streambank monitoring sites. River corridor and streambank sites are displayed in Figure 6; all areas were surveyed at least twice. From spring 2015 to spring 2017, a total of 21 full days of surveying were performed totaling over 50 km (30 mi) of river corridor length.

Figure 5. Mapping area along a section of the Mad River covering the MR-B site for a single flight. Yellow lines are user-selected, pre-programmed flight lines that the UAS follows automatically.

The river corridors were surveyed using an eBee (SenseFly) UAS resulting in the successful acquisition of orthoimagery, true color point clouds, digital surface models (DSMs), and digital elevation models (DEMs). Three models of the eBee were used in this study, the eBee, eBee RTK, and eBee Plus. The standard eBee UAS was utilized for the first round of flights in late spring (April and May) 2015. Fall 2015 and 2016 flights were completed using the eBee RTK which is a survey-grade system that features a more accurate GPS receiver capable of connecting to a virtual or local GPS base station. Spring 2017 flights were completed using the eBee Plus, which features a UAS optimized camera and longer flight times in addition to RTK-GPS capability. The UAS imagery was collected at a ground resolution of 3.6 cm or finer with an overlap of 60-80% to allow for creation of high resolution imagery and topographic data. An example of the flight path flown by the UAS in a single flight is shown in Figure 5.

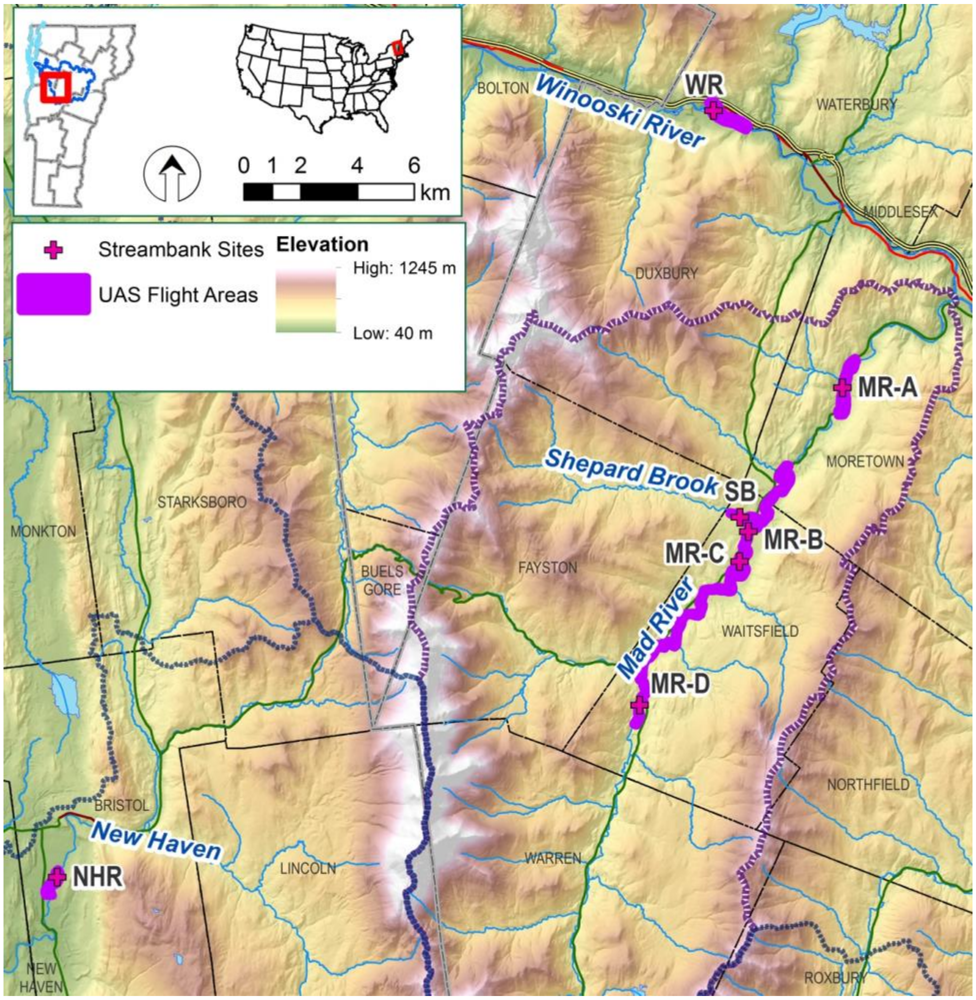

Figure 6. Map of streambank sites for terrestrial LiDAR surveying and areas where data were acquired using UAS.

All areas were flown in early spring, prior to leaf out to minimize the effects of vegetative cover while collecting topographic data. Following a moderate storm event on June 1, 2015, three 1 km reaches were re-surveyed for comparison of pre- and post-storm events. Finally, a 2 km section of the Winooski River in Waterbury was flown and scanned (Figures 7 and 8) in August to assess similar methods on taller streambanks. Repeat surveys of approximately 50% of the river corridor in the Mad River watershed (encompassing streambank sites) were conducted in November after leaf off. An active river reach in Bristol on the New Haven River was also added to the study area and flown in late December. These areas were all resurveyed in spring 2016 and spring 2017, in leaf off conditions. For the entirety of the study area, 2016 was exceptionally dry which resulted in low streamflows and no storms capable of causing bank erosion. Repeat surveys planned for fall 2016 were postponed to spring 2017 to have a greater chance of observing channel change. The one exception to this was a strong late summer storm isolated to the Shepard Brook watershed that caused significant erosion. The Shepard Brook river corridor was therefore resurveyed after the storm to measure bank movement resulting from the storm.

To assess the accuracy and repeatability of the UAS derived topographic data, seven streambank sites were identified for simultaneous data collection using all three methods - UAS, terrestrial LiDAR, and GPS survey. These seven monitoring sites (Figure 6) were selected to represent a variety of bank heights, vegetative conditions, and lateral instability. As summarized in Table 2, bank heights ranged from 1.4 m to 3.7 m and featured different soil types ranging from fine sand to silt loam. Vegetative conditions ranged from no tree cover with only grass to brush and heavy brush and tree cover.

Table 2. Streambank location and characteristics of detailed comparison sites

Site	River	Bank Height	Bank Soil Type	Channel Substrate	Vegetation	Erosion Sensitivity
WR	Winooski	3.7 m	Fine sand	Silt	Grass	High
MR-A	Mad	2.8 m	Fine sandy loam	Silt	Grass / brush	Low
MR-B	Mad	2.2 m	Very fine sandy loam	Gravel	Heavy brush & trees	Medium
MR-C	Mad	2.1 m	Fine sandy loam	Gravel	Heavy brush & trees	Medium
MR-D	Mad	2.0 m	Fine sandy loam	Gravel	Grass / brush	High
SB	Shepard	1.4 m	Silt loam	Gravel	Grass	High
NHR	New Haven	1.9 m	Very fine sandy loam	Gravel	Grass	High

Figure 7. Terrestrial LiDAR data collection along the Winooski River with graduate student Thomas Bryce and undergraduate researchers

Figure 8. Streambank site along Winooski River with active erosion being scanned by the terrestrial LiDAR

Short sections (50 - 100 m) of streambanks were scanned using the Riegl VZ-1000 terrestrial laser scanner to acquire true color point clouds of the bank surface (e.g. Figure 9). A Topcon HiperLite+ RTK GPS System was used to capture a bank profile and ground control points. Table 3 summarizes the timing of data collection at each site. During data collection river flows were not above normal, but sometimes high enough to not be safe for wading and performing ground survey at all sites. Where the TLS and GPS could be utilized safely, ground survey and scans were completed on the same day as UAS flights.

Table 3. Survey dates

SITE	SPRING '15	SUMMER '15	FALL '15	SPRING '16	SUMMER '16	SPRING '17
WR	--	8/3/15	--	4/27/16	--	4/24/17
MR-A	5/6/15	--	11/9/15	5/4/16	--	4/24/17
MR-B	4/29/15	--	11/10/15	5/18/16	--	4/20/17
MR-C	4/29/15	--	11/10/15	5/18/16	--	4/24/17
MR-D	4/22/15	6/22/15	11/10/15	5/18/16	--	--
SB	4/27/15	8/26/15	11/9/15	5/4/16	8/24/16	4/20/17
NHR	--	--	12/22/15	4/27/16	--	4/18/17

Table 4. Summary of 2015 – 2017 UAS flights and survey coverage

Year	Number of flights	Total Length of River Surveyed (km)	Mean Length of River per Flight (m)	Total days** in field for surveying	Number of days impacted* by weather
2015	55	21.7	395	12	3
2016	18	13.7	760	5	5
2017	17	14.3	843	4	1

* Impacted survey days refer to those that were either cancelled and rescheduled due to rain or wind or those days cut short due to wind or rain.
** A field day was considered 8 hours in the field, with approximately 6 hours available for survey efforts given 2 hours for travel accommodation.

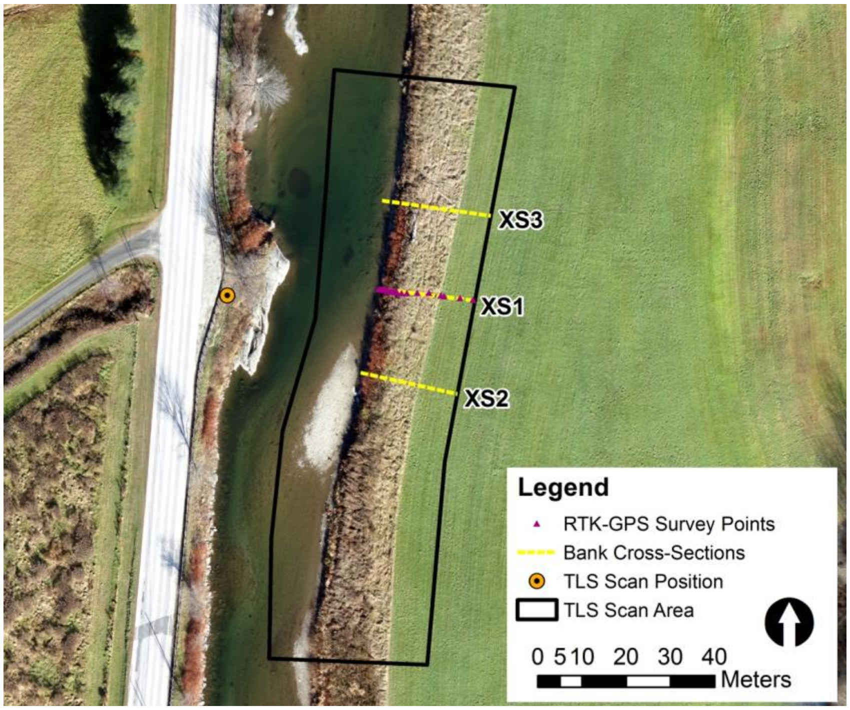

Figure 9. MR-A field site on the Mad River showing UAS imagery, area of terrestrial laser scanning, and location of cross section survey with GPS

UAS-derived bank topography accuracy

Analysis initially focused on assessing the accuracy of the UAS data through comparison of the UAS, LiDAR, and GPS datasets at the seven streambank sites. To do this, a cross-section approach was used. Point cloud data from both the UAS and LiDAR methods were georeferenced using surveyed ground control points (GCPs) to allow for direct comparison and assessment of accuracy of the UAS data except when using the eBee RTK UAV which is capable of directly georeferencing at high accuracy. Figure 10 shows an example of this raw point cloud data for both survey methods which was compared along selected cross-sections.

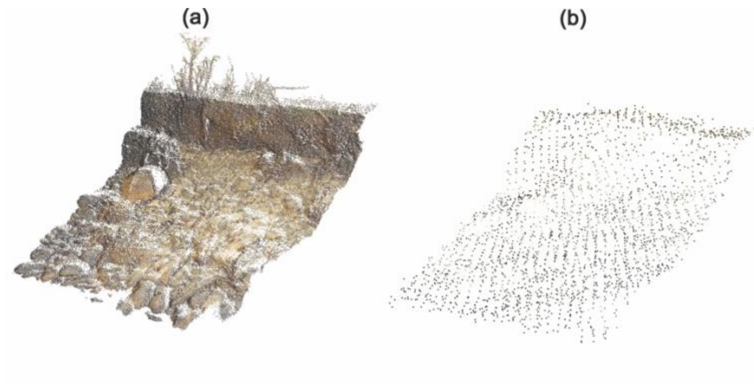

Figure 10. LiDAR (a) and UAS (b) point clouds from the SB site

Because all study sites featured some amount of vegetation, filtering of both the TLS data and UAS data was desired to estimate the bare-earth surface. Two approaches were developed to compare the UAS-derived streambank topography to the TLS and GPS survey: one using a vertical reference plane and the other a horizontal plane. This allowed analysis of the capability of UAS data for both obtaining horizontal bank retreats and for measuring change in ground surface elevation.

The repeatability and reliability of the UAS to capture the streambank topography was analyzed in depth across all seven streambank monitoring sites. Figure 11 shows a bank cross section from two flights flown within an hour of each other. The median difference between the flights was 0.03 m which is approximately equal to the target resolution of the UAS imagery indicating strong agreement in subsequent flights and repeatability in image processing.

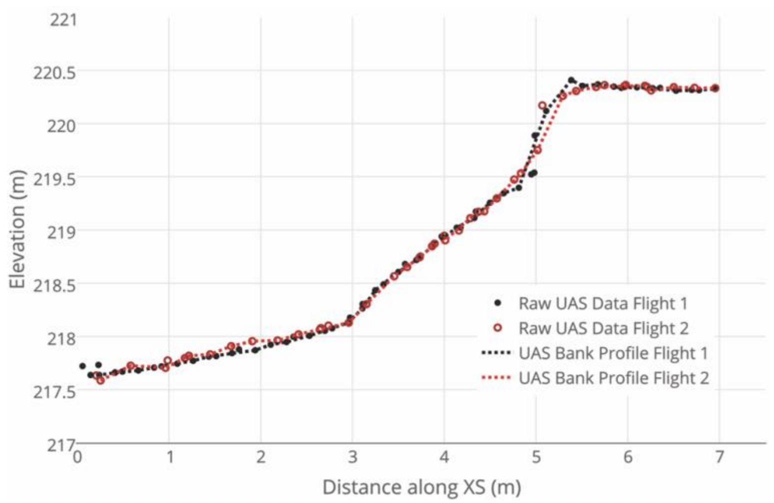

Figure 11. Comparison of data from two UAS flights at streambank site at MR-D site from April 22, 2015, flown within an hour of each other

Using the TLS data as ground truth, the UAS-derived bank topography was analyzed along three cross-sections at each site for all surveys (56 total paired UAS and TLS cross sections). Results showed that the UAS could reliably capture the bank topography under a variety of streambank settings. The overall median vertical error along all the cross sections was 0.11 m.

Several trends in the UAS-derived bank profiles were observed. All median errors in UAS data were positive and errors were largest in summer conditions indicating a strong effect of vegetation on the UAS data. This corresponds well to other studies and expectations of a photogrammetry data product. It was also observed that horizontal differences (errors) were larger than vertical ones indicating that care needs to be taken when using UAS data to extract horizontal streambank retreat rates that proper error metrics are calculated.

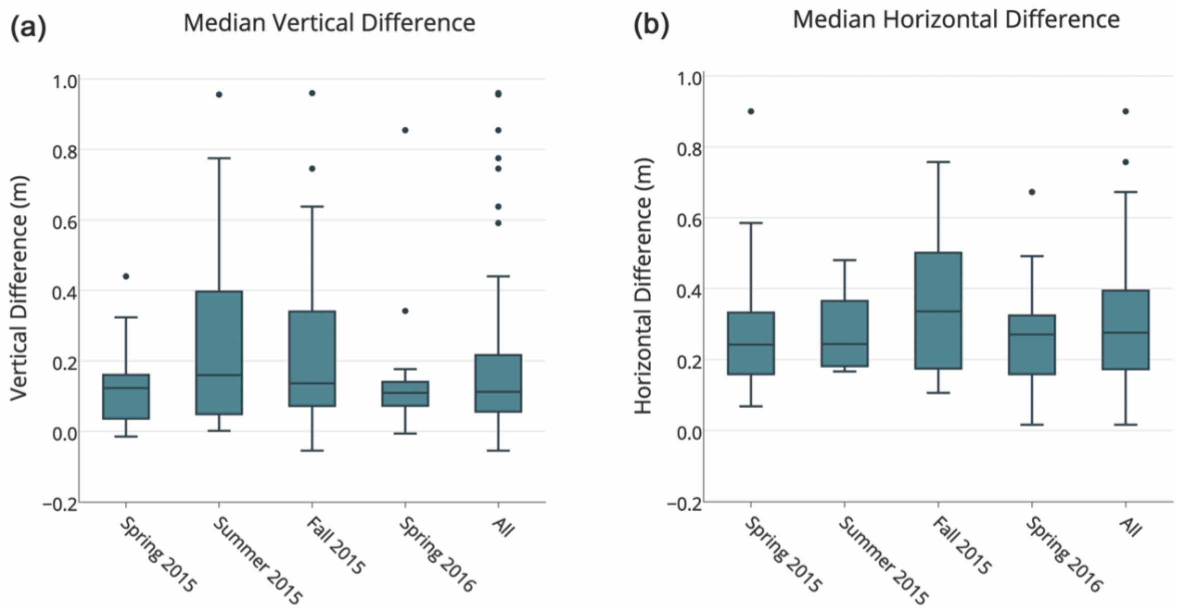

Figure 12. Box plots of (a) median vertical and (b) median horizontal differences between UAS and TLS bank profiles across all sites and cross sections

In addition to evaluation of cross-sections, river corridor topography was generated from UAS imagery as digital elevation models (DEMs). UAS-derived DEMs were assessed for their accuracy in comparison to existing airborne LiDAR surveys at two of the streambank sites (SB and NHR). Error metrics of RTK-surveyed GCPs showed that UAS surveys had comparable accuracy to airborne LiDAR derived DEMs (Table 5).

Table 5. Assessment of accuracy of DEMs based on comparison to GCPs.

New Haven River Site (n = 16)				
	2012 ALS	**2015 UAS**	**2016 UAS**	**2017 UAS**
Mean Error (m)	0.02	0.25	0.12	0.04
Median Error (m)	0.02	0.23	0.08	0.02
Standard Deviation Error (m)	0.09	0.09	0.15	0.12
RMSE (m)	0.09	0.26	0.19	0.12
Shepard Brook Site (n = 10)				
	2014 ALS	**2017 UAS**		
Mean Error (m)	0.04	-0.09		
Median Error (m)	0.00	0.03		
Standard Deviation Error (m)	0.20	0.36		
RMSE (m)	0.19	0.35		

Geomorphic Change Detection

Geomorphic change detection and estimation of bank erosion from UAS survey data was assessed through two approaches. First, by quantifying the net change in bank cross-sectional area at specific locations and comparing the UAS-derived results to that obtained by GPS and terrestrial LiDAR data. Second, by comparing differences in sequential UAS-derived DEMs and existing airborne LiDAR DEMs. During the study period, the most significant bank erosion was observed at the New Haven River site and therefore that site was used as the primary evaluation site for change detection methods.

Cross-sectional Bank Erosion Measurement

A snowmelt event on February 26, 2016 caused significant streambank erosion at two locations along the NHR site (Figure 13); cantilever bank failures were observed along the channel resulting in bank retreats up to ~13 m. This event provided an opportunity for direct comparison of bank erosion between the UAS and TLS data at multiple cross-sections. Net change in bank cross-sectional area between the December 2015 and April 2016 surveys were determined for each cross-section. Vegetation conditions on the two dates were very similar allowing for comparison of UAS and TLS to detect change over time.

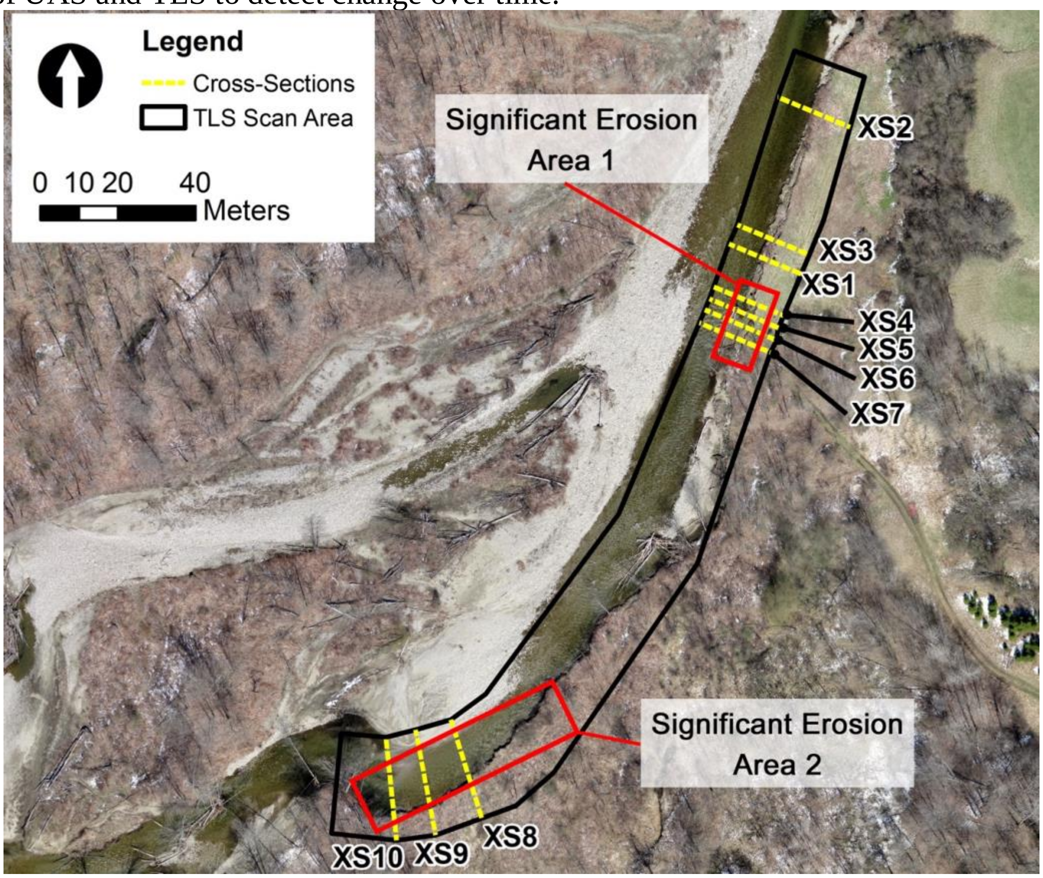

Figure 13. NHR Site with cross sections and area of TLS scan acquisition.

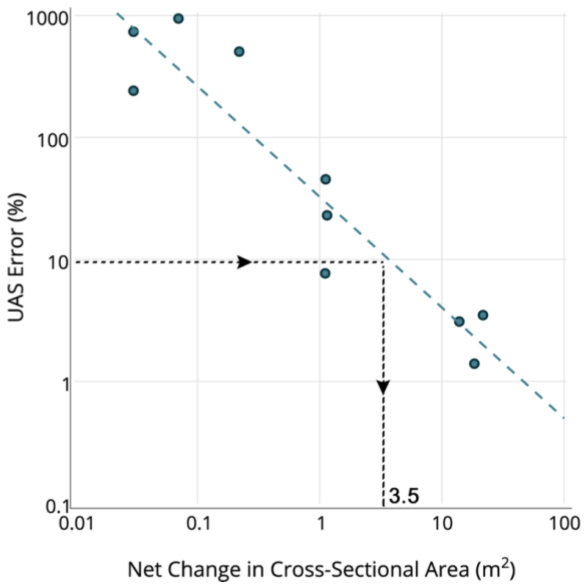

Figure 14. Percent error in UAS measurement of net change in cross sectional area as a function of net change measured by TLS at the NHR site across ten cross sections. Dashed line indicates power law fit of data.

The analysis of the NHR site results indicate that the UAS reliably estimates large amounts of bank movement within 10% of the change captured by TLS surveys along a typical streambank. This error threshold of 10% was observed for net changes in bank cross-section greater than 3.5 m^2 (Figure 14). Translating this threshold to measures of horizontal bank retreat means that for streambanks with typical heights (e.g. 2 m), about 1.8 m of bank retreat provides the 10% level of error, assuming a slab failure. Quantifying erosion or deposition in areas with smaller rates of retreat is more sensitive to the effects of vegetation and other sources of error. An additional source of error is bank under-cutting which creates a challenge for estimating bank erosion amounts using fixed-wing UAS data.

Change Detection from DEMs

A 1.2 km section of the New Haven River (NHR) and 1.5 km section of the Shepard Brook (SB) were utilized to evaluate the capability of UAS for monitoring reach-scale geomorphic change. The study section of the NHR has a history of active lateral migration and this was evident in comparisons of UAS surveys as well as previously collected airborne LiDAR. Between the November 2012 LiDAR survey and the December 2015 UAS survey, extensive channel movement was evident as the likely result of a number of high flow events. Continued erosion along portions of the streambank was apparent from subsequent UAS surveys in April 2016 and April 2017.

Automated DEM generation of the 2016 and 2017 UAS surveys produced high quality topographic data with few obvious vegetation errors and little missing data (Figure 15). Difference of DEMs (DoDs) were generated from multiple date DEMs which allowed for a spatio-temporal analysis of topographic change within the river corridor area. Between the April 2017 UAS and November ALS 2012 surveys, a net volumetric change of -19,920 m^3 occurred over the 15.2 ha area. The changes included isolated areas of both deposition and erosion (Figure 16). In all, an estimated 31,509 m^3 of erosion occurred and 11,589 m^3 of deposition or aggradation was evident over the nearly five-year period. The average annual rate of volumetric erosion was ~6,300 m^3/year. If an average bank height of 1.9 m (based on field measurements) is assumed over the entire 1,200 m long river reach, the average annual rate of bank retreat is estimated at 1.4 m/yr/m across each bank which equates to an average annual lateral migration of the river channel of 2.8 m/yr.

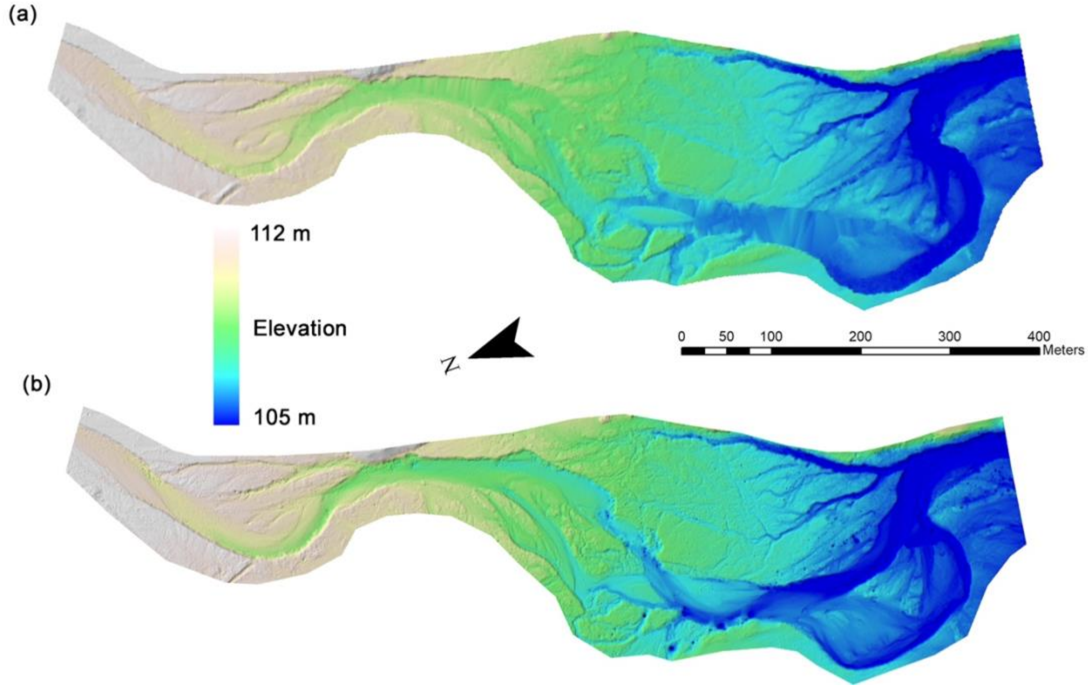

Figure 15. Digital elevation model (DEM) of New Haven River produced from (a) 2012 ALS survey and (b) 2017 UAS survey.

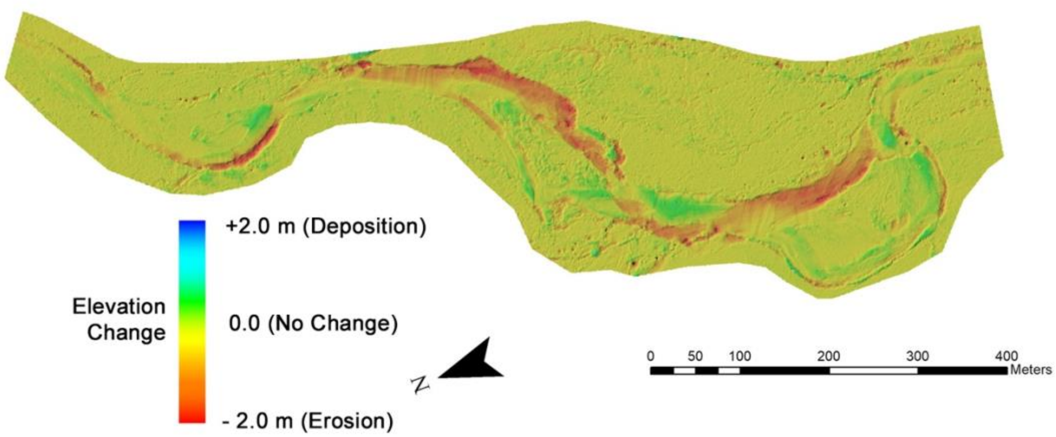

Figure 16. Elevation change between surveys along a section of the New Haven River as visualized by DEMs of difference (DoDs) between 2012 ALS survey and 2017 UAS survey.

Differences in water surface and vegetation growth were potential sources of error that were identified in the DEMs. Errors due to vegetation in the 2016 and 2017 UAS DEMs were not significant as evidenced by only small changes in elevation observed in areas where significant vegetation growth can be observed during the summer. When comparing water surface elevations across a relatively stable portion of the river, differences of ~0.2 m in the DEMs derived from the UAS and the ALS surveys were observed.

In contrast to the New Haven River area, Shepard Brook has different characteristics (i.e., denser vegetation and greater tree cover in the river corridor); it also is a smaller river with shorter streambanks (~1.2 m high) and is less susceptible to channel movement and bank erosion. Between the May 2014 ALS survey and April 2017 UAS survey, several medium size storm events caused minor observable erosion in isolated locations. A short duration flash flood event in summer 2016 caused the greatest amount of bank erosion, but still only along short sections (e.g., site shown in Figure 17) and with less than 1 m of retreat over 3 years. Errors in the UAS DEMs were more prevalent at the Shepard Brook site than the New Haven River site. Large areas of smoothed/interpolated data occurred in locations with thick tree cover where the UAS imagery could not reliably capture the ground surface (Figure 17). Similarly, missing data resulting from smoothing can be observed along much of the streambank. Shepard Brook has greater tree cover along the streambanks compared to the New Haven River, which may explain the poorer performance. The results of DEM generation from Shepard Brook indicates that in densely vegetated river corridors, including those with a number of evergreen trees, a greater erosion threshold is necessary for UAS surveys to be reliable.

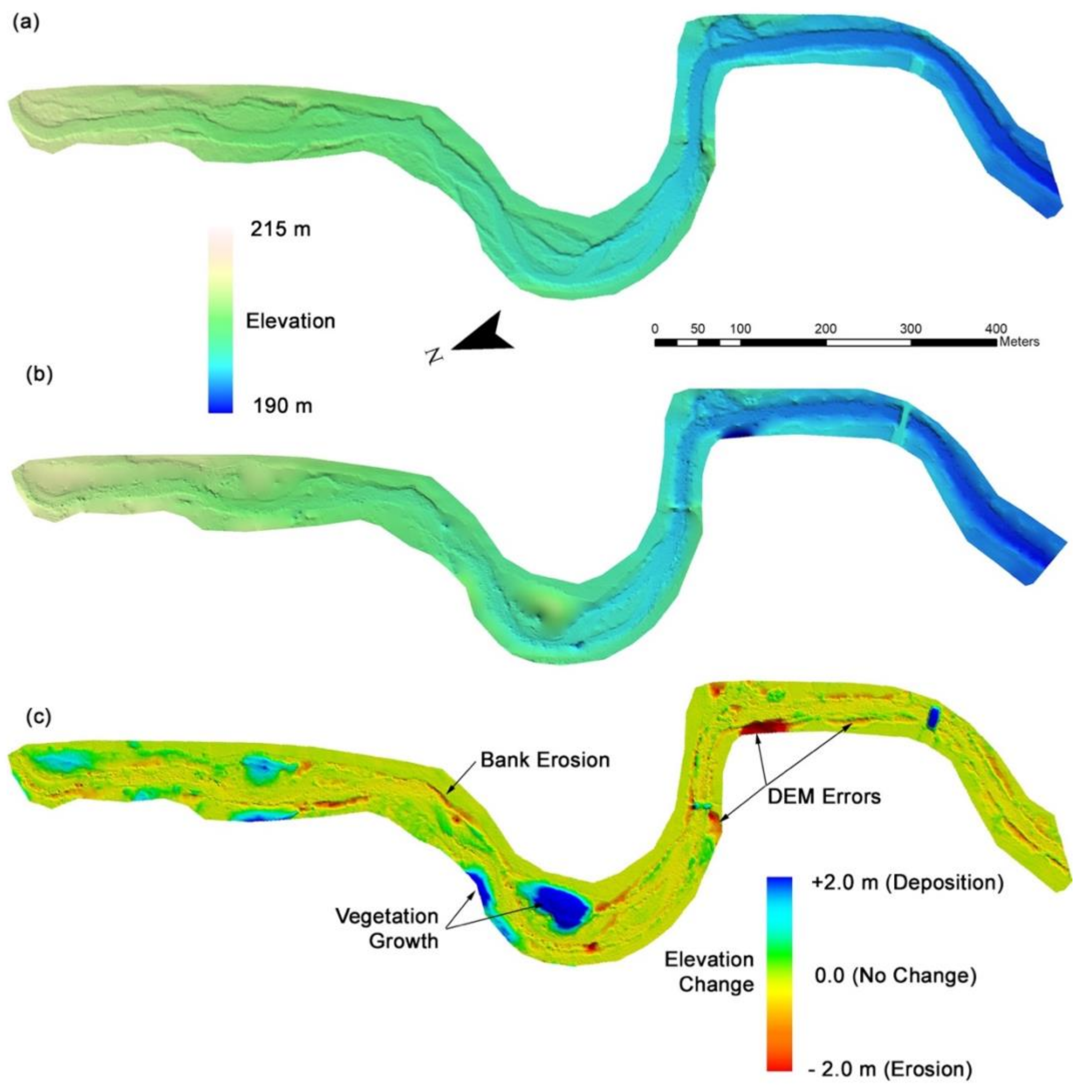

Figure 17. Digital elevation models (DEMs) of New Haven River produced from (a) 2014 ALS survey and (b) 2017 UAS survey and (c) DEM of difference (DoD) calculated from 2017 UAS survey – 2014 ALS survey.

Integration of Research and Education:

So far, four undergraduate students, one MS student, and one PhD student have had direct involvement in the data collection and analysis in this project. Of these, only the PhD student, Scott Hamshaw, was supported by the VT Water Resources and Lake Studies Center grant. The MS student, Thomas Bryce, performed majority of the work for academic credits. The four undergraduate students participated in the fieldwork and research efforts through a variety of summer internships. Two of the undergraduate students (Nathalie Simoes and Wimara Sa Gomes) used VT EPSCoR Research on Adaptation to Climate Change (RACC) summer internships for participating in this research. The other two undergraduate students, Anna Waldron and Kira Kelley, were supported on Richard Barrett Foundation Scholarships. Additional students (PhD student Kristen Underwood, MS student Jordan Duffy, undergraduate intern Alex Morton) were able to participate in some elements of the fieldwork and analysis. The UAS work involved six staff and ten undergraduate students from UVM's Spatial Analysis Laboratory.

18. Discussion

Data collection and processing workflows and methods were successfully implemented during the first year of the project. This has allowed for analysis of the UAS technology to reliably capture topographic data along streambanks and to be able to detect bank retreat over time. During the 2015-2017 project period, widespread, significant erosion of streambanks did not occur; however, one site (NHR) saw significant bank movement. Other sites featured moderate erosion of banks at very localized sites. This allowed for using the NHR site for analysis of change detection in support of testing Hypothesis #1 (Table 1) that suggested UAS-derived surfaces would be able to detect change within 10% of that detected by TLS. Results showed that UAS can reliably estimate bank erosion within 10% of that measured by TLS for banks that experience a net cross-sectional change greater than 3.5 m^2. This was tested on a section of river that featured typical vegetated streambanks. On banks with minimal vegetation, higher accuracies could be expected. Results also showed that UAS-derived DEMs were comparable to the accuracy of airborne LiDAR data (Table 5).

The efficiency of UAS data collection is an important criterion in assessing Hypothesis #2 described in section 16. Single UAS flights performed in the Mad River have covered on average ~600 meters of river length and include the entire river corridor with flight times ranging from 25 – 35 minutes. With this length of river covered in a single flight, it was feasible to cover a 5 km reach during a single field outing of about 8 hours. In general, due to suitable landing and take-off locations and visibility of the UAS from the landing/take-off area, two to three flights could be made from a single setup location. This translated to requiring a setup location for approximately each 1.5 - 2 km of river length. While this is less than the proposed performance criterion that Hypothesis #2 hoped, the high ground resolution and overlap did allow for complete data coverage in the area and higher accuracy. Expanding the coverage during a single flight to 2 km would be feasible with a crew that had additional spotters to be deployed upstream or downstream and also if the UAS target ground resolution is reduced allowing flights at higher altitudes. However, currently FAA regulations limit the use of UAS at higher altitudes.

During Year 3 of this project, a new model of the eBee UAS (the Plus) that features a longer flight time and UAS optimized camera sensor became available for use on the project. Additionally, software updates to the photogrammetry software included automated generation of "bare-earth" DEMs from the UAS data. The rapid advancement of UAS platform and photogrammetry software could be observed in the results of this project. For example, coverage per flight (Table 4) and ground surface elevation accuracy (Table 5) both increased with each subsequent survey campaign (project year). In particular, the advancement of direct geo-referencing capability of the eBee RTK and eBee Plus UAS which feature RTK-GPS capability greatly simplified field data collection because they virtually eliminated the need for ground control points. The automated generation of DEMs by the Pix4D software used in this study was quite robust in filtering out the noise associated with sparse vegetation and trees.

In support of testing Hypothesis #3, post processing of the UAS data from a single outing in under 24 hours has been successfully accomplished. While some additional filtering or post-processing may be desired, complete coverage of orthoimagery, DSM, DEM, and point cloud are easily completed in under 24 hours. The current requirements for UAS operation have a 72-hour approval process for air space which makes it practical to be on site and collecting data within 72 hours following a major event. There is also a rapid approval process if the situation is time sensitive.

The rates of channel movement and bank erosion from the study area were compared to previous studies of channel migration in the Lake Champlain basin. Jordan (2013) measured areas of channel migration along 13 main stem reaches of the Mad River between 1995 and 2011 using aerial imagery and found mean rates of lateral channel movement to range between 0.6 m/yr and 2.1 m/yr with an overall mean of 1.0 m/yr across all reaches. Another study estimated mean lateral channel migration along ten Vermont rivers between 2004 and 2007 using a slightly different method by making use of aerial imagery and single airborne lidar survey and found mean lateral migrations between 0.2 m/yr and 0.5 m/yr with individual stream reaches as high as 0.8 m/yr (Garvey 2012; Ishee et al. 2015).

The 2.8 m/yr of channel migration observed along the 1.2 km section of the New Haven River was greater than all the estimates found in previous bank erosion studies in the Lake Champlain basin. However, this result is certainly reasonable and likely represents the upper end of rates of expected channel movement in the region given the reach's susceptibility to bank erosion due to its geologic setting and past upstream channel alterations (Underwood 2004) and that a large avulsion occurred during the study period. In contrast, the Shepard Brook reach, which had less extensive bank erosion (estimated lateral channel migration of 0.2 m/yr), is similar to the low-end of rates of channel movement observed in the other studies.

Summary of Planned work for Year 4

Year 4 effort will include a final flight of the New Haven River site in early spring 2018 prior to leaf-out. Analysis of multi-year channel migration and bank erosion along the site will be continued with the repeat survey. A case-study of the application of UAS for monitoring geomorphic change will be prepared using the New Haven River site, which has been subject to river conservation and restoration activities over the last five years.

Project Leverage of Additional Funding Sources

This project leveraged several additional sources of funding during the first two years. Funding from the U.S. Department of Transportation Office of the Assistant Secretary for Research & Technology provides additional support for UAS operations and processing resources (OASRTRS-14-H-UVM). Funding from the National Science Foundation (NSF) (VT EPSCoR Grant No. EPS-1101317 and Grant No. OAI-1556770) provides additional support for undergraduate internships and graduate student and faculty support. Additional NSF support

through the graduate research fellowship program (Grant No. DGE-0925179) provided additional resources for the full time graduate student on the project. The lead graduate student was also supported from the Department of Civil and Environmental Engineering at UVM for part of the academic year 2016-17. The Robert and Patricia Switzer Foundation provides additional funding support to the lead graduate student on the project. Finally, the Richard Barrett Foundation provides support for undergraduate internships assisting with the project.

19. Training Potential

This research has a strong educational and mentoring component at a variety of levels. A number of graduate and undergraduate researchers have already been engaged in the project and the PIs will continue this effort of integrating research into education. These students are and will gain experience operating UAS and processing UAS data as well as with TLS. That the PIs are from different backgrounds (Civil, Environmental, and Electrical Engineering and Natural Resources), further enriches both the students' experience as well as the potential for the research to make significant gains. The methodology and results have been integrated into educational modules in the CE010 geomatics course at UVM. The data and methodologies will also be integrated into the VermontView Remote Sensing Workshop. This workshop, offered annually at UVM, trains geospatial professionals from federal, state, and local government agencies throughout the state on cutting-edge technologies. The results of this research are being presented at relevant on-campus and off-campus conferences (e.g. AGU 2015, AGU 2016, Geocongress 2017, Lake Champlain Research Conference, Northeast GSA 2018, AGU 2018, Geocongress 2019) and will be submitted to proceedings and refereed journals, thus educating a wider group of individuals, researchers and agencies interested in riverbank behavior.

Additional outreach to engage stakeholders and the general public have and will include presentations of the project to different non-profit/community organizations and governmental agencies. To-date these have included presentations to the Vermont Society of Professional Land Surveyors, Vermont Agency of Natural Resources, Lake Champlain Basin Program Technical Advisory Committee, and members of the Bristol Conservation Commission. A presentation is planned with the Friends of the Mad River organization for Fall 2018. Additional offers for presentations will be made to the Vermont chapter of American Society of Civil Engineers (ASCE), and the Agency of Transportation.

20. Investigators' Qualifications

Mandar Dewoolkar is a Professor in Civil and Environmental Engineering. Through his graduate and post-doctoral research work and industry experience, he has developed significant expertise in the fields of *in situ* and laboratory soil testing, equipment and instrument development and computer-aided slope stability and flow analyses among other types of analytical methods. He has been the PI on two previous Water Center projects.

Jarlath O'Neil-Dunne is the Director of the Spatial Analysis Laboratory (SAL) at the University of Vermont. His research focuses on the application of geospatial technology to a broad range of natural resource issues ranging from water quality to urban ecosystems to land cover change. For the past two years he has served as the principal investigator on a US Department of

Transportation grant that pioneered techniques for using unmanned aerial systems to rapidly map and measure transportation and hydrologic networks.

Donna Rizzo is a Professor in Civil and Environmental Engineering. She is a surface and groundwater hydrologist whose research focuses on the development of new computational tools to improve the understanding of human-induced changes on natural systems and the way we make decisions about natural resources. Her involvement using advanced GIS and remote sensing technologies in the above-mentioned research project funded by NSRC in coordination with the USDA Forest Service most closely relates to the proposed work.

Jeff Frolik is an Professor in Electrical Engineering at UVM. His expertise is in sensor networks and he was PI on the NSF Major Research Instrumentation award (CMMI-1229045) that acquired the RIEGL VZ-1000 Terrestrial LiDAR. He has led the use of the terrestrial LiDAR for characterizing a wide range of built and natural environments including streambanks, snow packs, historical structures, and civil infrastructure. In this project, he will train students on the use of the LiDAR system and supervise its use.

Publications and Outreach

Publications

Hamshaw, S. D., Bryce, T., Rizzo, D. M., O'Neil-Dunne, J., Frolik, J., and Dewoolkar, M. M. (2017). "Quantifying streambank movement and topography using unmanned aircraft system photogrammetry with comparison to terrestrial laser scanning." *River Research and Applications*, 33(8), 1354–1367.

Theses and Dissertations:

Hamshaw, S. D. (2018). "Fluvial Processes in Motion: Measuring Bank Erosion and Suspended Sediment Flux using Advanced Geomatics and Machine Learning." Ph.D. Dissertation, University of Vermont, Burlington, VT.

Presentations:

Dewoolkar, M. M. (2018). Stability Assessment of Streambanks in Vermont, Gund Tea Seminar, March 9.

Hamshaw, S.D. and Dewoolkar, M.M., (2018). *Monitoring fluvial geomorphic change using unmanned aircraft system (UAS) photogrammetry and laser scanning.* Northeast Geological Society of America 2018 Meeting, Burlington, Vermont

Hamshaw, S.D. and Dewoolkar, M.M. (2018). *Using Unmanned Aircraft System (UAS) Photogrammetry to Monitor Bank Erosion along River Corridors.* Lake Champlain Research Conference, Burlington, Vermont

Hamshaw, S.D. (2017) *Fluvial Processes in Motion: Measuring Streambank Erosion and Suspended Sediment Flux with Advanced Geomatics and Machine Learning*. Invited presentation to the Lake Champlain Basin Program Technical Advisory Committee, Grand Isle, Vermont.

Hamshaw, S. D., Dewoolkar, M., Rizzo, D. M., O'Neil-Dunne, J., Rizzo, D.M., Frolik, J., & Engel, T. (2016). *Quantifying streambank erosion: a comparative study using an* *unmanned aerial system (UAS) and a terrestrial laser scanner.* (Poster), American Geophysical Union 2016 Fall Meeting, San Francisco, California.

Hamshaw, S.D. and Dewoolkar, M. (2016, Oct 4) *Use of unmanned aircraft systems (UAS) to monitor streambank erosion in Vermont.* Presentation to VT Agency of Natural Resources, Montpelier, Vermont.

Hamshaw, S.D. (2016, Jun 10) *Terrestrial Laser Scanning Introduction and Demonstration*. Presentation at Historic Preservation Conference, Waterbury, Vermont

Press and Outreach:

Hamshaw, S.D. (2017) Interview and filming with Vince Franke for VT PBS *Saving Our Waters* documentary on Lake Champlain.

Education:

Class module on UAS and terrestrial-LiDAR technologies incorporated in to UVM CE010 Geomatics course, Fall 2016 & Fall 2017 semester

Conference papers:

Hamshaw, S. D., Bryce, T., Dewoolkar, M., Rizzo, D. M., O'Neil-Dunne, J., Rizzo, D.M., Frolik, J., & Engel, T. *Quantifying streambank erosion using unmanned aerial systems at the site-specific and river network scales.* Geotechnical Frontiers 2017 Conference, Orlando, Florida.

Peer-reviewed manuscripts in progress:

Hamshaw, S. D., Engel, T., O'Neil-Dunne, J., Rizzo, D. M., and Dewoolkar, M. M. (2018). "Application of unmanned aircraft system (UAS) for monitoring bank erosion along river corridors." *Geomatics, Natural Hazards and Risk*, Revision in Review.

Ross, D.S., Wemple, B.C., Willson, L.J., Balling, C., Underwood, K.L, & Hamshaw, S.D. (2018) Tropical Storm Irene's Impact on Streambank Erosion and Phosphorus Loads in Vermont's Mad River. *Journal of Geophysical Research: Biogeosciences*. In Revision

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Rizzo, D.M., S.D. Hamshaw, H. Anderson, K.L. Underwood and M.M. Dewoolkar (2013), "Estimates of Sediment Loading from Streambank Erosion Using Terrestrial LIDAR sediment in rivers using artificial neural networks: Implications for development of sediment budgets", EOS Transactions, American Geophysical Union, Abstract H13D-1353, Fall Meeting, San Francisco, CA, December.

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Trails to remediation: the effects of seasonal variations on the acid mine drainage microbiome at Ely Copper Mine in Vershire, VT

Basic Information

Title:	Trails to remediation: the effects of seasonal variations on the acid mine drainage microbiome at Ely Copper Mine in Vershire, VT
Project Number:	2017VT85B
Start Date:	3/1/2017
End Date:	2/28/2019
Funding Source:	104B
Congressional District:	Vermont-at-Large
Research Category:	Biological Sciences
Focus Categories:	Climatological Processes, Water Quality, Acid Deposition
Descriptors:	None
Principal Investigators:	LesleyAnn Giddings

Publication

1. Morillo, K. 2018, The Effects of Seasonal Variations on the Acid Mine Drainage Microbiome at Ely Brook in Vershire, VT, Undergraduate Thesis, Department of Chemistry & Biochemistry, Middlebury College, Middlebury, Vermont.

13. Title: Trails to remediation: the effects of seasonal variations on the acid mine drainage microbiome at Ely Copper Mine in Vershire, VT

14. Statement of regional or State water problem.

The mining industry exploited the state of Vermont's copper belt in Orange County during the 19^{th} and 20^{th} centuries, after which several copper mines were abandoned. Toxic levels of copper, iron, zinc, and lead, in addition to massive pyrrhotite-rich, Besshi-type sulfide deposits [11] at mining sites have adversely affected the water quality in this region. Ely Copper Mine is one of three major abandoned mines in the Vermont copper belt. This site encompasses 350 acres, including areas contaminated with approximately 172,000 tons of waste rock, tailings, ore roast beds, slag heaps, smelter wastes, and 3,000 ft of underground mine workings with shafts and adits that open into the flooded mine. Although heavy metals (e.g., iron, manganese, and copper) play a central role in cellular metabolism, high doses can cause oxidative damage, ultimately resulting in cell death. For example, degraded surface-water quality from copper has been found to be acutely toxic to the crustacean *Hyalella Azteca* and minnows *Pimephales promelas* [12, 13]. Thus, in 2001, the Environmental Protection Agency (EPA) placed Ely Copper Mine in Vershire, VT on the Superfund National Priorities List. AMD has formed for at least 50 years at this location and had deleterious effects [12] on the aquatic ecosystem, as Ely Copper Mine is drained by Ely Brook, which has several tributaries, one of which drains into six ponds in the area [12].

Microbes contribute to changes in the pH of this environment by actively oxidizing iron sulfides. To better understand how to restore the water quality at Ely Copper Mine to preserve Vermont's natural resources, we aimed to characterize the AMD microbiome and identify genes involved in transforming copper, manganese, aluminum, and iron [12], the most abundant metals reported, that can potentially be used to bioremediate AMD. The effects of seasonal changes on AMD biodiversity were also examined to *identify sulfate-reducing bacteria, heavy metal resistant microbes, and functional genes selected for in these unique, acidic environments*. Seasonal changes affect the pH and temperature of environments, which affect microbial enzymatic activities as well as nutrients available for their growth. Temperature affects the growth rates of microorganisms as well as modifies the pH of the environment. Warm and prolonged dry periods change the concentrations of metals, sulfate, and acidity due to a reduced flow of ground water [14]. Several studies have identified tractable genes that can be used to facilitate the sequence-wide discovery of other heavy metal resistance genes in environmental DNA [15]. Furthermore, AMD is a carbon-limited environment [16] and some microbes may aid in enriching this environment with carbon and act symbiotically with other microbes that alter AMD acidification rates. Similarly, the results of this study reveal how these AMD microbial communities adapt to seasonal changes and provide insight into the genes selected for adaptation to AMD sites.

15. Statement of results or benefits.

The current study is the first genomic <u>and</u> metabolomic study conducted at any site in the Vermont copper belt. Statistical analyses of our metagenomic data provided 1) a phylogenetic analysis (*Eubacteria*, *Archaea*, and *Eukaryota*) of AMD communities at Ely Mine to elucidate the roles of these microbes in this extreme environment; and 2) led to the identification of novel genes for the bioremediation of heavy metals as well as other important functional genes important for adaptation (e.g., carbon assimilation and energy generation). AMD communities are typically characterized by *Proteobacteria*, Gram-positive microbes, flexibacteria, *Thermoplasmatales*, *Sulfolobales*, and very few eukaryotes [4, 17]. More molecular studies are finding that more rare organisms, such as *Ferroplasma* spp. [18] and those belonging to the iron-oxidizing chemolithoautotropic *Leptospirillum* group III[19], are dominant in mine environments and likely play a role in the oxidative dissolution of iron sulfides.

Temperature affects many biological and abiotic processes in different ways. Lower temperatures can increase the dissolved oxygen levels, accelerating the oxidation reaction. Conversely, water evaporates faster at warmer temperatures, lowering its pH and the enzymatic rates of oxidation also increase. The pH of Ely Brook was previously monitored in 2002 and found to increase as the from winter to spring months [20]. As the temperature changes due to seasonal variations, the biodiversity of AMD changes [21] and the microbes that thrive contribute to changes in pH. This study revealed the microbial ecology and nutrient preferences between summer and winter at Ely Brook.

The characterization of major metabolites has also provided some insight into how changes in temperature affect the metabolomes of some of these microbes. Previous studies have identified novel ectoine-, lipid-, and taurine-related molecules as well as polyamines and amino acids from these extreme environments [22, 23]. Our research can also lead to the identification of new chemical entities, such as the sesquiterpenes found in the Berkeley Pit in Butte, Montana [24]. Importantly, our data can be used to correlate novel metabolites to expressed genes in these environments to understand how microbes adapt to AMD or other specific environmental conditions.

16. Nature, scope, and objectives of the project, including a timeline of activities.

AMD effluent is one of the most impactful byproducts of the mining industry that has long-term, detrimental effects on aquatic ecosystems worldwide. It forms as the result of the exposure of mineral pyrite (i.e., iron disulfide, FeS_2) or, in the case of Ely Copper Mine, pyrrhotite [i.e., $Fe_{(1-x)}S$] to oxygen and water during the process of mining: $FeS_2(s) + 14\ Fe^{3+}(aq) + 8\ H_2O(l) \rightarrow 15\ Fe^{2+}(aq) + 2\ SO_4^{2-}(aq) + 16\ H^+$. AMD acidification increases the solubility of heavy metals, erosion, and leaching. Atmospheric changes also transport heavy metals throughout natural aquatic systems. This process is quite slow as the oxidation of iron is the rate-determining step [25]; however, autochthonous chemolithotrophs, microbes that depend on the oxidation of sulfides for energy primarily, accelerate the rate of acidification [25, 26], especially in low pH environments [25]. The distribution of these microbes affects the rates of iron and sulfur cycling, impacting the pH of these environments.

This proposal aimed to address the impact of seasonal variations on the AMD microbiome at Ely Brook (EB-90M) and a nearby Vadose area of the Lower Waste Area (at a depth of 10 cm) at Ely Copper Mine, a Superfund site. The biodiversity may vary across the entire brook; however, the same location will be sampled and used as a starting point for characterizing microbial communities at Ely Copper Mine. This environment is likely characterized by a limited number of distinct species due to its extreme ecological conditions, such as pH (3.2), specific conductivity (447 μS/cm), iron concentration (6370 μg/L or 6.37 ppm), and copper concentration (1560 μg/L or 1.560 ppm) [12]. The mean summer temperature is 15 °C (55 °F) and the overall mean temperature is 7.7 °C (46 °F), a significantly lower temperature [1]. Changes in temperature in an extreme environment, changes the chemistry of a number of pollutants [27], changing the toxicity of the water.

Seasonal variations affect the water quality of AMD environments as well as its microbiome based on alternating wet and dry seasons [21, 28]. The primary environmental factors that affect AMD-microbial communities are pH, concentrations of solutes, such as sulfates, heavy metals, total organic carbon, and dissolved oxygen [28]. Temperature is an important factor as it increases the rate of iron sulfide oxidation [29], changes AMD chemical composition [30], and has an effect on species distribution and phenology [31]. The levels of oxygen transport increase within AMD due to warm weather, resulting in weathering and the dissolution of sulfidic metals, which ultimately increase heavy metals and acidity. The rates of a number of other processes, such as biodegradation, uptake and metabolism, volatilization, adsorption, photodegradation, and photo-enhanced toxicity, increase with increasing temperature [32]. Increases in acidity also occur with increased evaporation [33], which commonly occurs in hot, dry months of the year. On the other hand, the oxygen levels increase, increasing oxidation reactions and changing from the winter the spring seasons have resulted in increases in pH. We will not understand the effects of seasonal changes unless we identify the microbes in these sites, as they mostly contribute to the pH of this environment. Thus, we hypothesized that the AMD microbiome must change to adapt to these significant changes in growth conditions [34].

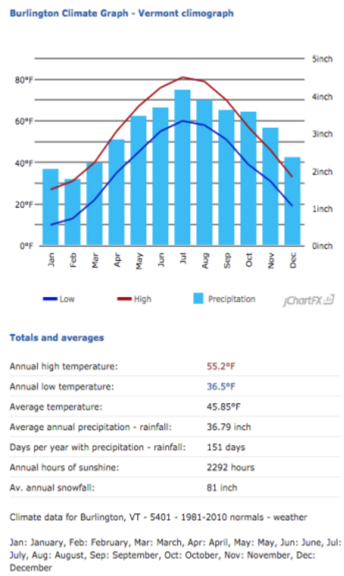
The AMD microbiome consists of microbes that can withstand toxic conditions and increasing temperatures could possibly promote the attenuation of AMD and toxic elements. Microbes aid in the precipitation of heavy metals via mechanisms involved in either mobilization or immobilization. For example, microbes mobilize metals via chelation, bioleaching, oxidizing metals (e.g., ferrous iron to ferric iron, which is insoluble at low pH), changing the pH (e.g., converting strong acids to weak acids or accelerating acidification), and other chemical transformations [35]. Microbes can immobilize metals via precipitation, such as reducing sulfate to make insoluble sulfides [36], sorption, uptake, and intracellular sequestration, as well as the crystallization of insoluble organic or inorganic compounds. Microbes and corresponding exudates can also act as nucleation sites from which cations are attracted [37]. Furthermore, microbes have genes involved in heavy metal sensing (e.g, TRASH [38]) and resistance genes, such as Cu^{+}/Fe^{2+} permeases and P-type ATPases (e.g., CopA [39]), which are common to some microbes [40] living in mining environments. Some genes, such as *cueO*, assist in oxidizing Cu(I) to a less toxic Cu(II)[41]. Thus, microbes have the genetic blueprints that can be exploited for bioremediation purposes, as magnetic filtration can be used to scavenge metal pollutants [42].

Research in the Giddings laboratory has shown that temperature affects the metabolites produced in microbes, specifically bacteria (Fig. 2B). Figure 2B shows the changes in the metabolite profile of *Streptomycetes sviceus* when the growth temperature is varied. Changes in the ecosystem, related to temperature, also affect metabolites produced by AMD microbes. For example, some of the metabolites that have been isolated from AMD communities are phosphatidylethanolamine lipids, taurine, and hydroxyectoine at concentrations that most likely vary due to changes in temperature [22].

We hypthesized that seasonal variations change the abundance of a select taxa of microorganisms as well as their metabolomes and genes required for adaptation. To begin to characterize the microbial ecology at Ely Copper Mine, we pursued the following specific aims:

1) Characterize the AMD microbiome in the surface water of Ely Brook (EB-90M; previously reported USGS study site [12] and nearby unsaturated soil (10 cm deep), and determine how these microbial communities change with temperature. Shotgun metagenomic sequencing was used to quantify the relative abundance of bacteria, fungi, and archaea in AMD collected in the coldest winter month of January and the warmest summer month of July (Fig. 2A). Additionally, metal resistance and functional genes were identified in this metal-rich environment.
2) Determine the effects of temperature on the metabolites of some of the most abundant, culturable bacteria in AMD at Ely Copper Mine. Culturable microbes were grown in sterilized AMD or other media at increasing temperatures to gain insight into how seasonal changes may affect the metabalome. Abundant metabolites were characterized using analytical methods such as LC-MS.

A.



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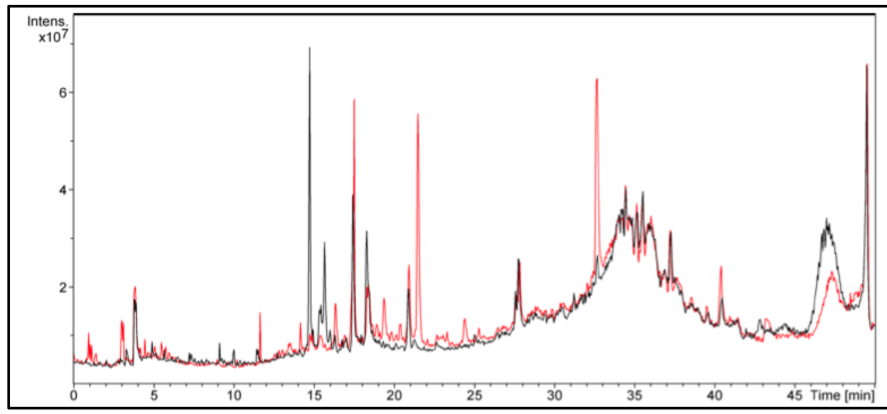


Figure 2. A. Average changes in temperature throughout the month in Burlington, VT from 1981 to 2010 [1]; B. LC/MS total ion current chromatogram of *Streptomyces sviceus* grown in ISP2 liquid media at 27°C (red) and 37°C (black) demonstrating the changes in the metabolites produced with temperature.

These aims are directly responsive towards Section 104(b) of the Water Resources Research Act of 1984 requiring Institutes of Centers to “plan, conduct, or otherwise arrange for competent applied and peer reviewed research that fosters (A) improvements in water supply reliability; (B) the exploration of new ideas that – (i) address water problems; or (ii) expand understanding of water and water-related phenomena; (C) the entry of new research scientists, engineers, and technicians into water resources fields; and (D) the dissemination of research results to water managers and the public [9].” Very few metagenomic studies have been done on the AMD microbiome in the state of Vermont; none have been done at any of the copper mine Superfund sites. Our sequence data facilitates the identification of microbes and genes that are critical for microbial adaptation. Ultimately, this research provides insight into how seasonal variations affect AMD microbiomes and their corresponding metabalomes, increasing our knowledge on water quality at Ely Copper Mine.

Timeline of activities

Student 1:
March to June 2017- Develop methodology for DNA isolation from water and soil
July 2017- Access site (Vadose zone and EB-90M) by the lower waste area on a representative July day to collect samples; isolate genomic DNA from samples and prepare library for sequencing
August to December 2017- Obtain and analyze metagenomic sequence data. Quantify and identify metabolites in cultures of some of the most abundant, culturable microbes
January 2018- Access the same sites on a representative January day to collect samples and isolate genomic DNA and create metagenomic library
February 2018- Submit samples for sequencing followed by data analysis

Student 2:
March to June 2017- Develop methods for dereplicating metabolome via high resolution liquid chromatography/mass spectrometry (HR-LC/MS)

July 2017- Collect AMD, grow microbial isolates, and confirm identity by 16S rDNA analysis
August to November 2017- Grow cultures in triplicate and scale-up cultures for the structural characterization of major metabolites
December to January 2018- Isolate compounds via chromatography and use nuclear magnetic resonance (NMR) and mass spectrometry (MS) to structurally elucidate key metabolites
January 2018- Collect AMD, grow microbial isolates, and confirm identity by 16S rDNA analysis
February 2018- Isolate compounds via chromatography again and structurally elucidate key metabolites

Based on our progress, we have collected enough data for at least one publication. We are currently writing a paper on the effects of seasonal variations on the phylogenetics and genes expressed in the AMD microbiome. We hope to also write a paper characterizing culturable microbes in the sediment of EB-90M and the effects of temperature on the metabolomes of culturable microbes.

17. Methods, procedures, and facilities.

The landowner, Dwight Hill LLC has granted us permission to access Ely Copper Mine. Ely Brook originates northwest of the mine site and west of the mine workings and tailings. Ely Brook is 90 m and has a latitude of 43.91924 and a longitude of -72.28629. The Vadose area of the Lower Waste Area, a few yards away from EB-90M, was also sampled at a depth of 10 cm to access a microbial metagenome undergoing more active oxidation. In order to characterize AMD microbiomes at Ely Copper Mine, both culture-independent and -dependent approaches were taken to ensure the success of this study. Both approaches provided insight into the effects of seasonal variations on the microbiome. The Giddings laboratory is fully equipped to do metabolomics work (see "Facilities" at the end of this section) and had the support of major sequencing and bioinformatics facilities.

A. Culture-independent methods

The culture-independent method of shotgun metagenomic sequencing was used to determine changes in the microbiome. In general, less than 1% of microbes are actually culturable under standard laboratory conditions. AMD communities commonly have a limited number of distinct species with a small number of metabolic reactions, making these systems ideal for genomic-based analyses. Shotgun sequencing of environmental samples provides genetic data that can be used to measure biodiversity, identify taxa to the species and sub-species level, and identify the metabolic capabilities of the entire AMD microbiome. Shotgun sequencing was necessary, in particular, to provide information on highly diverse metabolic genes, which can play a role in the bioremediation of copper and determine the impact increasing temperatures have on the total microbial community and constituent functional capabilities. In order to determine how AMD microbiomes at Ely Copper Mine change, used shotgun sequencing to analyze genomic DNA extracted from environmental samples collected from the same location during the summer and winter. Three samples from two different locations (EB-90M and a nearby Vadose zone) were studied to determine how microbes in the same environment vary with seasons.

a. Sample collection

Samples were collected from two locations for AMD (a total of six samples per season examined): EB-90M (three water samples) and a nearby Vadose zone (three sediment samples) and stored at 4 °C. EB-90M was the sampling site based on its acidic pH (pH 2.9) and acute toxicity [12]. Using a peristaltic pump, AMD was pumped through Qiagen Sterivex filter units to retain genetic material and microbes for DNA analysis. Three samples of AMD were filtered at the site for sequencing. A few yards away, a 10-cm hole was dug with a shovel to collect soil containing microbes actively oxidizing iron. Samples were collected in triplicate in January (one of the coldest months) and July (one of the warmest months) and placed on dry ice and stored long term at -80 °C. The temperature, pH, and conductivity of the AMD, were measured using a Hanna HI98194 portable multiparameter probe. The pH of the soil from winter and summer was measured using a Thermo Fisher Orion Versa Star Pro pH Benchtop Meter. Soil was solubilized in autoclaved ultra-purified water water (1:4) and incubated for 30 min measured. The total soluble copper and iron in samples were assessed in samples immediately acidified with trace metal grade nitric acid and filtered for analysis by inductively coupled argon plasma mass spectrometry (ICAP/MS). Total organic carbon was obtained by acidifying water collected in a combusted amber bottle with sulfuric acid for analysis via a carbon-nitrogen-sulfur analyzer. Sulfate, orthophosphate, and alkalinity were determined using Hach SulfaVer® 4 Sulfate Reagent Powder Pillows, Hach Phosphorus, Orthophosphate Test kit, and Hach Alkalinity Reagent set as well as a DR900 multiparameter portable colorimeter.

b. DNA extraction

Total DNA was extracted using the PowerSoil® and PowerBiofilm® DNA Isolation kits (Mo Bio Laboratories, Inc.; Carlsbad, CA). DNA quality was assessed by gel electrophoresis and through fluorometric quantitation using a Qubit 3.0 fluorometer (Thermo Fisher Scientific).

c. Illumina® Library Preparation and Shotgun Metagenomic sequencing

Genomic DNA libraries for shotgun metagenomic sequencing was prepared using Nextera XT DNA library preparation kit (Illumina, CA) after shearing of DNA using a Covaris S2 acoustic shearing device. Sheared libraries were assessed for quality and size distribution using a 2200 TapeStation instrument (Agilent). Final size selection of library fragments (400–600 bp) was performed using a PippinPrep device (Sage Scientific). Libraries were sequenced at the DNA Services Center at the University of Illinois at Chicago (see letter of support from Stefan J. Green) on an Illumina NextSeq500 instrument, implementing 1 × 150 base pair reads. At least 6 Gb of data (20 M clusters) were produced for each sample.

d. Bioinformatic analysis

One Codex, a bioinformatics platform for microbial genomics, was used to perform preliminary metagenomic analyses. This tool was used to analyze the microbial composition in sediment and water samples from summer and winter. The data is also normalized using this software. For all statistical analyses conducted, only groups with an abundance >1 % were considered. For in-depth metagenomic analyses, raw sequence reads were analyzed by the Research Informatics Core at the University of Illinois at Chicago (see letter of support from Neil J. Bahroos). The high-throughput search algorithm DIAMOND [43] was used to blast raw Illumina sequencing reads for functional analyses against protein reference sequences (e.g., NCBI-nr database). MEGAN (MEtaGenome ANalyzer) was used to identify the taxa to which each DIAMOND alignment belongs. This software places reads to the SEED [44], COG (Clusters of Orthologous Groups of Proteins) [45], and KEGG (Kyoto Encyclopedia of Genes and Genomes) [46] classifications. In addition, the tool SUPER-FOCUS (Subsystems Profile by database Reduction using FOCUS) was used to perform faster functional profiling of large volumes of unannotated metagenomic data [47]. Differential analyses of sequence data from colder and warmer seasons are currently being performed using statistical programs (e.g., edgeR [48]) to determine the microbes and genes selected by this extreme environment.

e. Mining the genome to find metal resistance genes

Based on our sequence data, an overabundance of functional genes involved in defense and repair mechanisms was found in the acidic heavy-metal-contaminated environment. Some of these genes have novel DNA binding and C-terminal TRASH domains, which are important for metal sensing [49], as well as conserved residues involved in transporting metals (e.g., those in P-type ATPases [39]) and activating the transport of key metabolites (organic permeases [50]). Thus, we are using edgeR to look at the differential expression of genes in AMD metagenomes to begin to identify these genes involved in transforming metals, such as copper, manganese, iron, and aluminum, and understand how they change with temperature. EdgeR is suitable for measuring the best estimation of the effect size [48].

B. Culture-dependent methods

Culture-dependent methods were used to demonstrate how the AMD microbiome changes with seasons. Metabolomics can provide more information about the small molecules produced and their roles [23]. The liquid cultivation of some of the most abundant, culturable microbes facilitated the characterization of metabolites produced and provided insight into how their concentrations vary with temperature. Furthermore, microbes with metal resistance genes were selected for by varying metal (e.g., pyrrotite) concentrations in growth media to select for more metal-resistant organisms. These microbes can then be compared to the same microbe grown under conditions of lower metal concentrations.

a. Isolating microbes

Microbes were grown from water and sediment samples collected in part A of this proposal. To extract microbes from the sediment, samples were placed on Stovall Life Science, Inc Belly Dancer at low speed for approximately 2.5 hrs. Afterwards, 100 µL and 200 µL volumes of the supernatant were plated on ferrous tryptic soy broth (Fe-TSB) agar containing 1% w/v ferrous sulfate and tryptic soy broth agar containing 1% w/v of pyrrhotite then cultured at 4 °C and room temperature. The Fe-TSB medium was used to select primarily for *Leptospirillum ferrooxidans* and *Acidiothiobacillus ferrooxidans*, typically the most abundant in AMD [51]. Microbial DNA was isolated from pure isolates using a Qiagen Blood & Tissue kit (Germantown, MD). 16SrDNA will be amplified with standard 27F and 1492R primers. 16S amplicons were sequenced and 3–4 isolates were selected for fermentation studies.

b. The effect of increasing temperatures on metabolomes

The growth of microbial isolates was studied at (4 °C, 16.5 °C, 24 °C, 30 °C, and 40 °C) to identify optimal growth temperatures. Microbes will be cultured in sterilized AMD or liquid Fe-TSB at the temperatures they were isolated as well as other temperatures to determine an optimal growth temperature.

c. Structural characterization via LC/MS and NMR

Liquid cultures were homogenized and then extracted with equal volumes of LC/MS-grade ethyl acetate. Ethyl acetate will be evaporated and the resulting residue will be reconstituted in LC/MS-grade methanol. Samples (5 containing an internal standard (reserpine) were analyzed by high resolution ultra performance liquid chromatography (UPLC, Waters I-Class™ UPLC system) in tandem with a mass spectrometer (Waters Xevo™ Q-TOF) using a C18 column and a mobile phase consisting of 0.1% formic acid water (solvent A) and acetonitrile (solvent B) [23]. The following gradient will be used with a flow rate of 0.6 ml/min: 5% B for 1 min, 5–100% B over 8 minutes, 100% B for 3 min, 100% to 5% B over 2 min, and 5% B for 1 min (total run time = 15 min). The metabolic profiles of microbes grown at different temperatures were compared using Progenesis QI software and the major components were partially characterized via liquid chromatography, mass spectrometry (MS), including MS/MS.

Facilities

Middlebury College has state-of-the-art science facility to complete this project. The Department of Chemistry and Biochemistry has a Bruker 400 MHz NMR spectrometer, Thermofisher ICP/MS (iCAPQ), carbon-nitrogen-sulfur analyzer, as well as a Waters LC/MS (I-Class LC coupled to a Xevo Q-TOF), a highly sophisticated piece of equipment that very few undergraduate institutions possess. In addition, the Giddings laboratory is equipped with a Biosafety cabinet, pH meter, homogenizer, ComBiFlash system for automated purification, as well as incubators for culturing microbes. This laboratory also contains a chemical safety hood, flammable cabinets for solvent, as well as 4 °C fridges and -20 °C freezers for sample storage. Our science technical support staff consists of 14 employees, including four technicians who maintain and build scientific equipment, are available on an as-needed basis for routine maintained and repair, as well as special needs projects. McCardell Bicentennial Hall also houses a stockroom with routine chemicals, solvents, supplies, and glassware.

18. Findings [52].

Geophysical characterization

A geophysical characterization of Ely Brook was conducted in both July 2017 and January 2018 by measuring environmental factors, such as temperature, pH, dissolved elements in the water, and sulfate concentrations. We observed an increase in pH of 0.50 units from July to January, indicating hydrogen ion activity is 5 times greater in the summer (Table 1). These data also show an increase in sulfate levels and total organic carbon from January to July. We also determined the pH of soil to be 3.59 in January 2018 and 3.79 in July 2017.

Table 1. Geophysical characteristics of the water at the Ely Brook site EB-90M during the summer and winter

Properties	**July 2017**	**January 2018**
Water pH	3.36	3.86
Temperature	16.4 °C	-0.36 °C
Total Organic Carbon	3.13 ± 0.22 mg/L	1.35 ± 0.23 mg/L
Sulfate	126 mg/L	95 mg/L

Orthophosphate was not detected below 0.15 mg/L and alkalinity was not detected below 0.25 mg/L in filtered water samples. The amount of select dissolved elements in water measured by ICP-MS during the summer and winter are shown in Figure 3 below. The data show a significant decrease in Fe, Cu, Al, and Si concentrations from July to January (Fig. 3), which is consistent with lower sulfate levels and higher pH shown in Table 1 as sulfate and iron ions are formed in a one-to-one ratio. The levels of Cu, Fe, and Al exceed the EPA health criteria of 4.8, 1000, and 87 ppb, respectively [53].

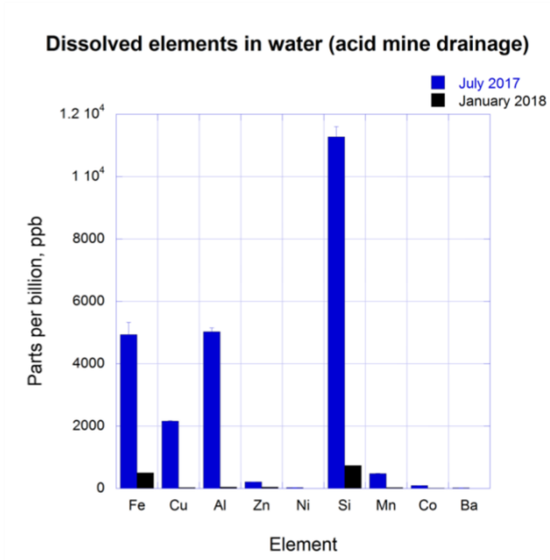
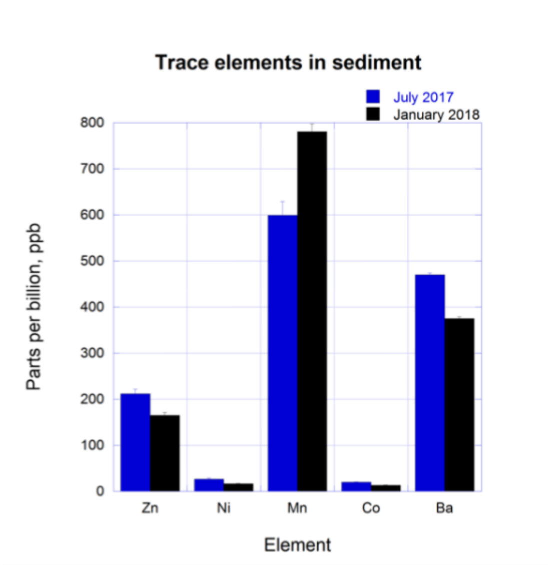


Figure 3. Dissolved elements in the water at Ely Brook during July 2017 (blue) and January 2018 (black). Data obtained via ICP-MS.

The elements in sediment were also quantified by ICP-MS (Fig. 4). With the exception of a significant increase in Mn from the summer to winter, the levels of Ni, Zn, Ba, and Co decrease from the summer to the winter. The percentage of metal oxides was determined by X-ray fluorescence spectrometry. With the exception of the increase in the levels of SiO_2, the levels of Fe_2O_3 and Al_2O_3 decreased from summer to winter in sediment.



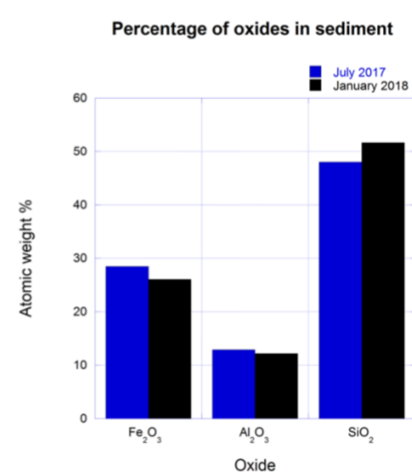


Figure 4. A) Amount of trace elements in sediment and B) percentage of metal oxides in winter and summer sediment.

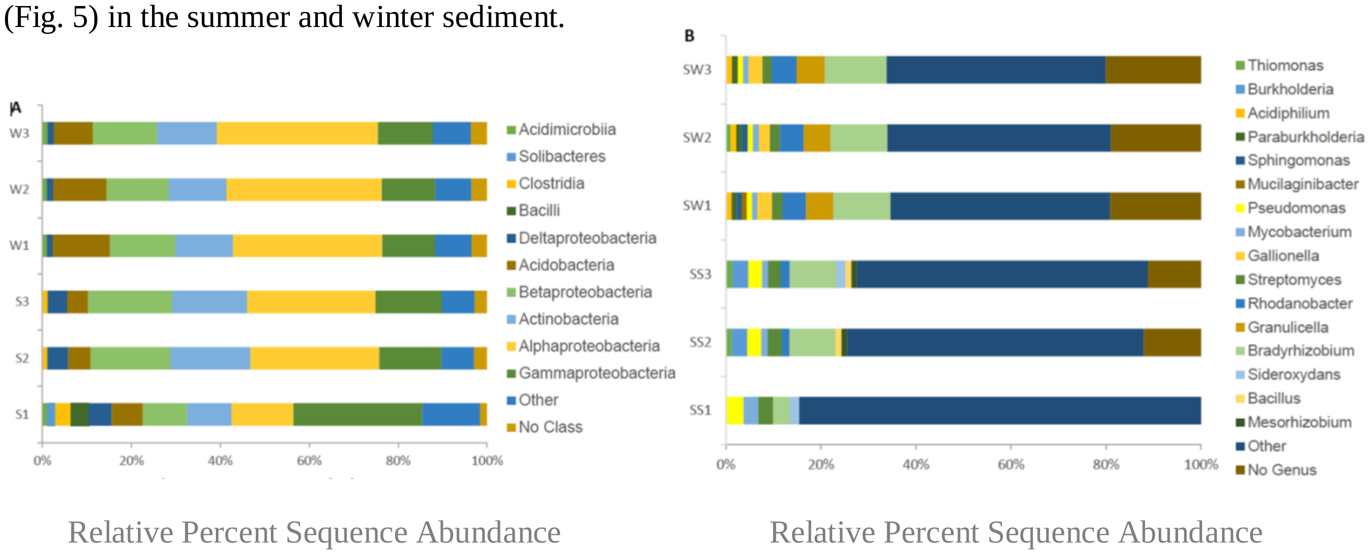
<u>Metagenomic analysis</u>

Using culture-independent approaches, data were obtained to characterize the microbiome at EB-90M. Illumina shotgun metagenomic sequencing was performed on DNA isolated from on water and sediment samples collected in July 2017 and January 2018. Annotations were made to a certain percentage of reads of preliminary sequence data (Table 2) using OneCodex as well as DIAMOND and MEGAN. More sequence reads were obtained for summer sediment and water samples than those obtained from winter samples. Sequence data were not obtained for winter water samples, presumably due to the low abundance of microbes.

Table 2. Sequence reads obtained using Illumina shotgun metagenomic from sediment and water samples. SS = summer sediment, WS = winter sediment (each 1/8th of sequence data), and SW = summer water. All data were collected in triplicate.

Sample	Reads	% of Reads Classified
SS1	63,800,019	0.25
SS2	56,454,845	10.49
SS3	59,911,963	10.88
WS1	2,481,723	7.37
WS2	3,174,263	7.51
WS3	2,041,336	6.92
SW1	51,445,129	5.37
SW2	45,008,412	5.26
SW3	61,252,966	3.77
SW4	57,940,652	4.00

We are currently working on normalizing and analyzing our metagenomic sequence data to be able to make thorough comparative analyses. However, preliminary data show that there is an abundance of *Betaproteobacteria*, *Actinobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* (Fig. 5) in the summer and winter sediment.



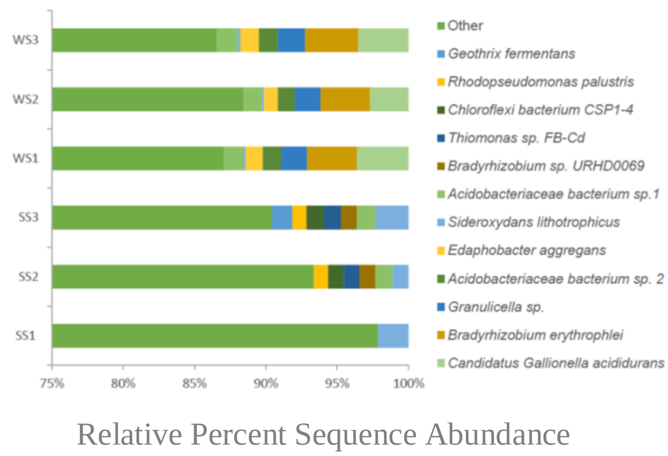


Figure 5. Ely Brook sediment microbial community profile by class (A), genus (B), and species (C) from winter (SW) and summer (SS) as depicted by relative percent sequence abundance. All data were collected in triplicate.

Based on normalized summer water data in Figure 6, prokaryotes dominate the water at EB-90M, specifically *Betaproteobacteria* and *Alphaproteobacteria*. Archaea, such as *Candidatus micrarchaeum* (Fig. 6) and species from the genus *Nitrosophaera* (not shown in Fig. 6), were identified. There are also a number of methanotrophs (e.g., *Methylobacterium* sp., *Methylotenera* sp, and *Methylobacter* sp.) and methanogens (e.g., *Methanomassiliicoccus* sp., *Methanocella* sp., *Methanobacterium* sp., and *Methanosarcina* sp.) present in these samples. There are also a number of amoebae and associated bacteria as well as diatoms. Furthermore, microbes from the water in the summer have a number of genes involved in virulence, RNA metabolism, DNA repair, protein folding, protein metabolism, ammonia assimilation, fatty acid biosynthesis, and responses to stress. Interestingly, the virulence genes in this community are mostly those involved in cobalt-zinc-cadmium-lead-mercury resistance (*cusA* and P-type ATPase), resistance to fluoroquinolones and acriflavin, and copper homeostasis (e.g., copper P-type ATPase and multicopper oxidase), as predicted. These microbes also have genes dedicated to the biosynthesis of siderophores, which are small, metal-chelating molecules that are known antimicrobial agents [54].

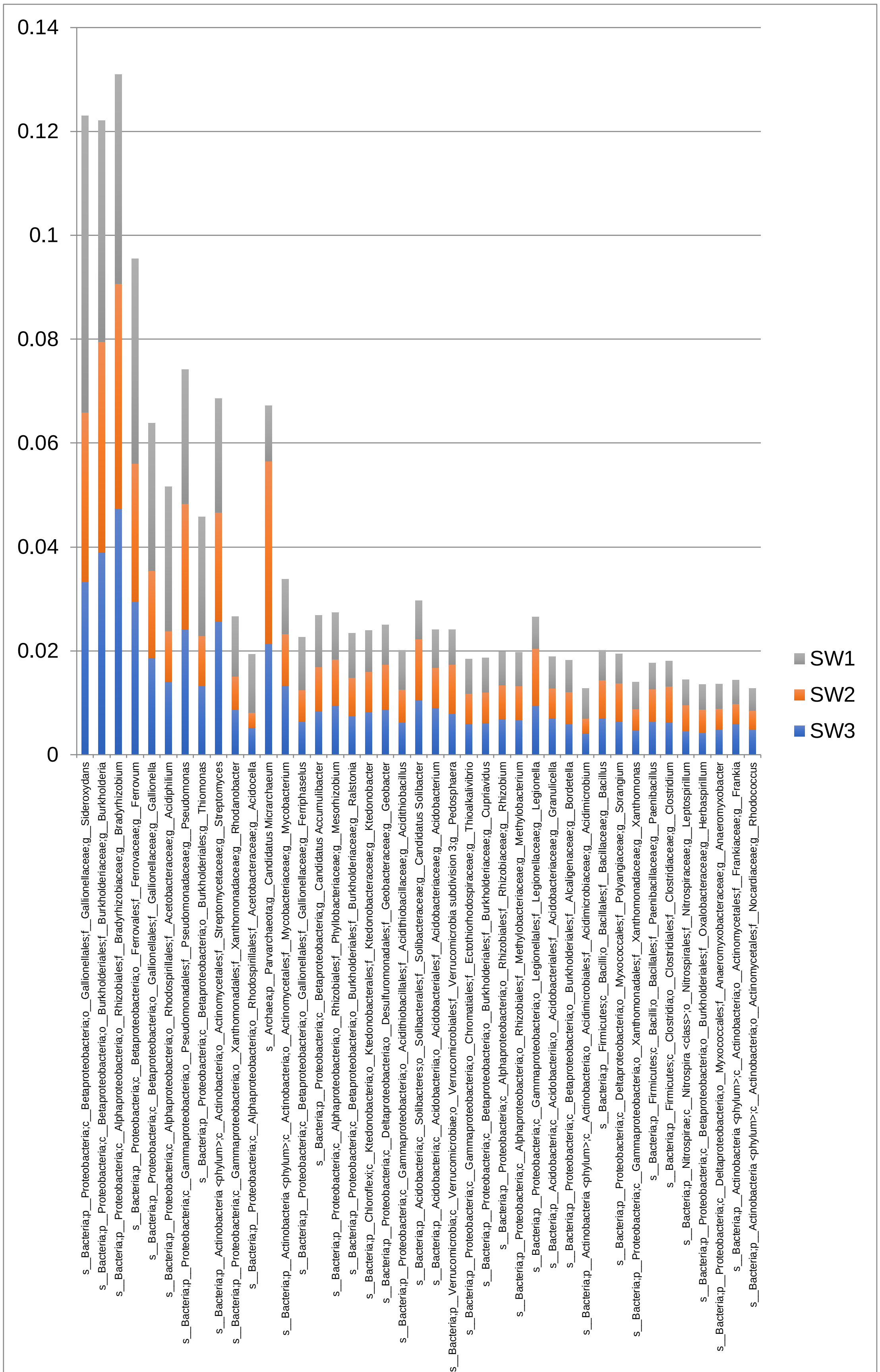


Figure 6. Normalized relative abundance of sequence reads from summer ware samples corresponding to domain, phylum, class, order, family and genus.

Microbial identification

The 16S rRNA region of three isolates was successfully amplified and sequenced. One isolate was from summer sediment and two isolates were from winter sediment, EBS001 and EBW 001-2 (Fig. 7). Post-sequencing, microbial identification was obtained by comparing the data with sequences deposited in the National Center for Biotechnology Information database. No microbe was isolated from water samples, likely due to the low density of microbes.

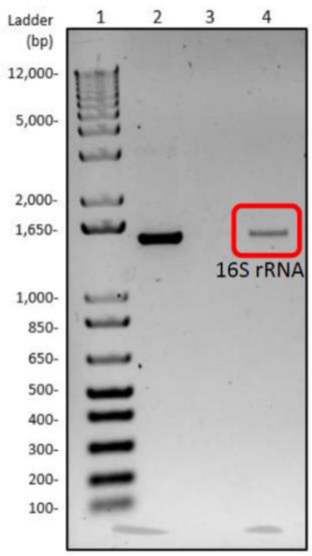


Figure 7. *Lane 1.* Invitrogen 1 kb plus ladder (bp = base pairs). *Lane 2.* Positive control. *Lane 4.* Successful amplification of the 16S rRNA gene of EBW001. EBW= Ely Brook Winter Sample. A representative example of the PCR amplicon obtained for 16S rDNA.

Microbial isolates EBS001 and EBW001-2 have a 99% similarity with a strain from the genus *Alicyclobacillus* (Table 3). The sequences for EBS001 and EBW002 showed 100% similarity when aligned using NCBI BLAST, suggesting both microbes are the same strain. However, since the 16S rRNA region is so highly conserved and the entire 1,500 bp of the sequence was not obtained, it is possible that these are two distinct species.

As for EBW001, the data show 90% similarity with both EBS001 and EBW002, suggesting this is a different strain. Additionally, the sequence of the 16S rRNA region amplified of EBW001 has 99% similarity with the amplified region of *Alicyclobacillus ferripilum.* This species has been shown to oxidize iron and are species of interest in heavy metal bioleaching studies to develop ways to clean contaminated system. However, one cannot confidently say EBW001 is identical to *A. ferripilum.*

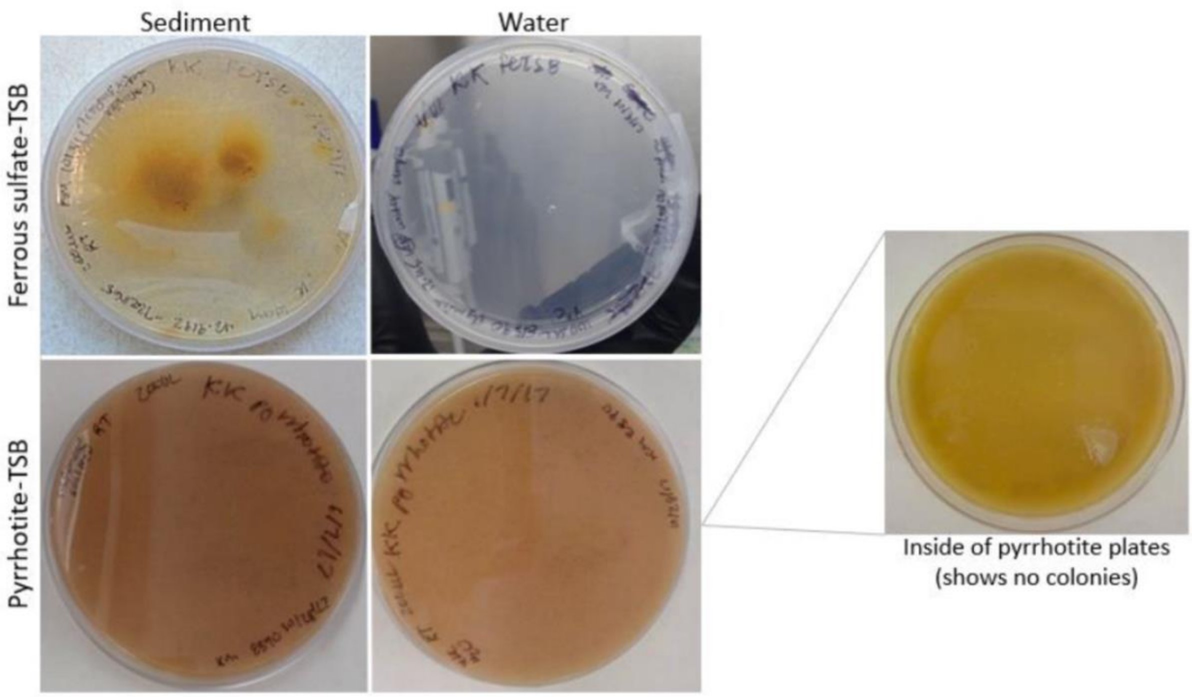
Table 3. Identification of unknown microbial isolates from Ely Brook site EB90-M using NCBI BLAST.

Organism	Identification	% Query Cover	% ID	E-value
EBS001	*Alicyclobacillus sp.* strain 1 (99%)	100	99	0.0
EBS002	Unidentified	n/a	n/a	n/a
EBW001	*Alicyclobacillus sp.* strain 2 (99%)	100	99	0.0
EBW002	*Alicyclobacillus sp.* strain 1 (99%)	100	0.0	99

Microbial cultivation studies

Microbes from the sediment were generally able to grow in liquid Fe-TSB medium and water from site EB-90M. However, microbes from the water samples were unable to grow in these media. Microbes from both sediment and water samples were not able grow on solid Fe-TSB medium supplemented with pyrrhotite (Fig. 8).

A.



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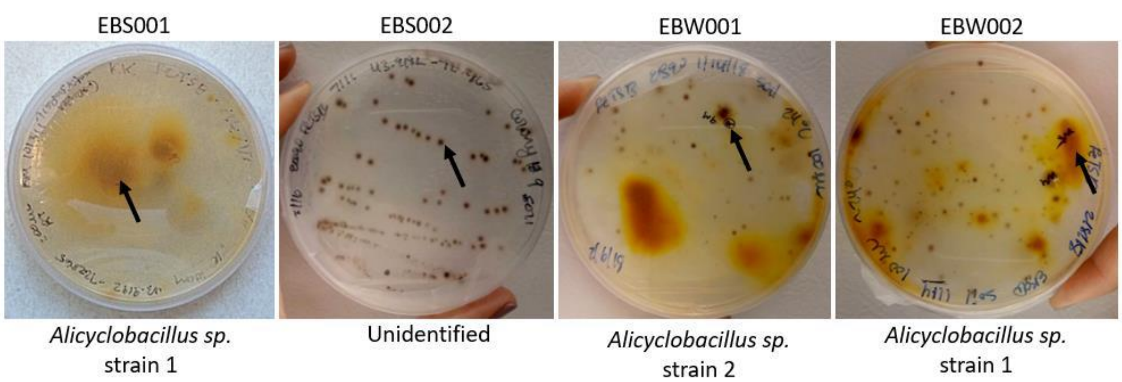
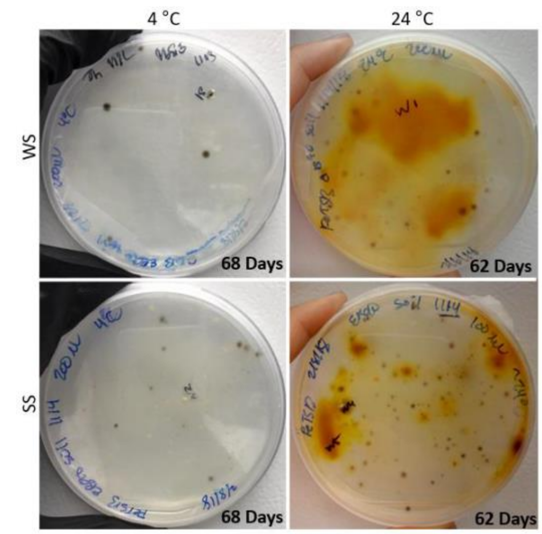


Figure 8. A) Microbes from Ely Brook site EB-90M summer sediment and water samples grown on different types of media. B) Colonies of microbes isolated from sediment samples growing on Fe-TSB. EBS = Ely Brook Summer sample, EBW= Ely Brook Winter Sample.

Colonies for EBS001 and EBW002 are very similar (Fig. 8), supporting the conclusion that these two organisms are the same species. While there is some variation with the colony of EBW0001, which appears to be both black and orange, there could possibly be two types of organisms present. However, the DNA of the *Alicyclobacillus* sp. was the only one successfully amplified.

The *Alicyclobacillus* species was a large focus of this study, not only due to its identification, which alluded to the organism's iron-oxidizing potential, but also the abundant growth of this organism on the Fe-TSB medium. By varying temperature, we can not only mimic summer and winter conditions to observe variations in microbial growth, but can also potentially identify organisms that produce novel metabolites to facilitate adaption to the extreme conditions found at Ely Brook. To this end, microbes from summer and winter sediment samples were plated on Fe-TSB and incubated at two different temperatures 4 °C and 24 °C (RT). Microbes exhibited significantly more growth at 24 °C and a more diverse group of colonies (Fig. 9A). While the plates shown were grown over a period of 62–68 days, the extensive growth observed on the plates incubated at 24 °C is apparent after a minimum of three weeks. No colonies were observed before 10 days.

A.



B.

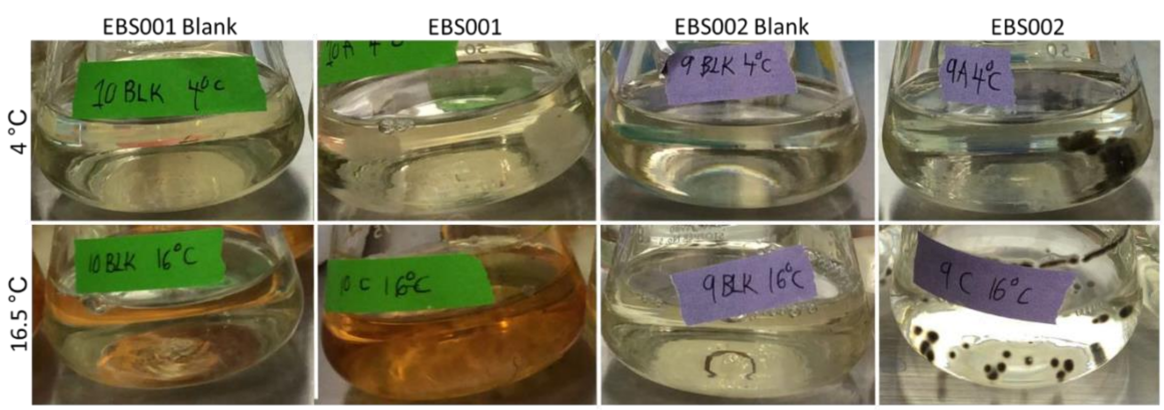


Figure 9. A. Ely Brook summer and winter sediment samples cultured on ferrous sulfate-tryptic soy broth at 4 °C and 24 °C. WS = winter sediment, SS = summer sediment. B) EBS001 and EBS002 cultured at 4 °C and 16.5 °C in liquid ferrous sulfate-tryptic soy broth. Growth period = 28 days.

Microbial isolates EBS001 and EBW002 were cultured on Fe-TSB liquid media at 4 °C and 16.5 °C, which more closely mimics the conditions at site EB-90M in both summer and winter (Table 2). This would not only allow one two observe distinctions between the two isolates but would shed light on the differences and similarities between EBS001 and other *Alicyclobacillus* species as well as the metabolites they produce. For both organisms, growth was more abundant at the higher temperature among other significant variations (Fig. 9B). Both EBS001 and EBS002 appear to aggregate at the colder temperature.

Interestingly, there was a visible color change in the media of EBS001 when grown at 16.5 °C.

The color of the media changed from the light-yellow color of the Fe-TSB to a deep orange (Fig. 9B). Yet, this deep orange color, which is indicative of iron oxidation, was only observed after 3 weeks. This variation suggests there is iron-oxidizing activity being mediated by EBS001, which we will refer to as *Alicyclobacillus* sp. from here onward. To confirm iron-oxidizing activity, a chemical test was conducted to assess the presence of ferric iron (III) using potassium ferrocyanide. Potassium ferrocyanide reacts with ferric iron to form a deep blue precipitate, more specifically Prussian blue [55]. However, a light blue precipitate is only formed when the compound reacts with ferrous iron (II), which is initially present in the media. This confirmatory test showed ferrous iron was oxidized in cultures grown at 16.5 °C but not in those grown at 4 °C (Fig. 10). Thus, *Alicyclobacillus* sp. has iron oxidizing capabilities but may not be as metabolically active at colder temperatures, preventing detectable iron-oxidizing activity.

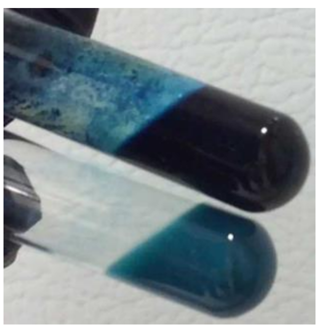


Figure 10. Reaction with potassium ferrocyanide confirms presence of ferric iron in cultures grown of *Alicyclobacillus* sp. grown at 16.5 °C (top), but not 4 °C (bottom).

It is possible that the iron-oxidizing activity of *Alicyclobacillus* sp. is unique compared to other *Alicyclobacillus* species. It may also have the unique ability to thrive at higher temperatures of up to 65 °C [56]. To assess its optimal growth temperature, *Alicyclobacillus* sp. was cultivated at 40 °C to determine optimal growth and the species' potential to oxidize iron at higher temperatures. However, while efficient growth and iron oxidation were observed at temperatures up to 25 °C, this was not observed at 40 °C (Fig. 11). There was little to no growth at 40 °C. Furthermore, there was no indication of iron oxidation marked by the deep orange color observed in cultures grown at 25 °C. These data suggest that the optimal growth range for this species is 1625 °C.

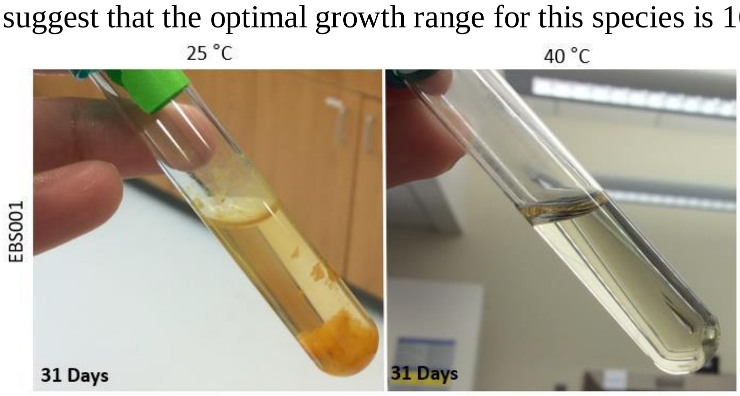


Figure 11. *Alicyclobacillus* sp. cultured at higher temperatures. Iron oxidation marked by deep orange color.

<u>Microbial cultivation and metabolomics</u>

Liquid cultures of *Alicyclobacillus* sp. grown in Fe-TSB at 4 °C and 16.5 °C were harvested and extracted analysis by liquid chromatography-mass spectrometry (LC-MS) to identify metabolites produced by the organism under both conditions. The data were then analyzed using Progenesis QI Software, created by Waters Nonlinear Dynamics to facilitate LC-MS data analysis. Using the raw LC-MS data in tandem with the results from the Progenesis QI software we were able to obtain some interesting preliminary data. There were clear variations in abundance of metabolites produced by EBS001 when grown at the two different temperatures (4 °C and 16.5 °C).

Data from the QI Progenesis software revealed a compound with an *m/z of* 481.2979. This mass was then extracted from the LC-MS data from the samples grown at 4 °C and those grown at 16.5 °C. A notable peak was observed at retention time of 5.97 min. This peak at 5.97 min was present in samples from cultures grown at 16.5 °C, but not in those from the lower temperature (Fig. 12). This suggest that *Alicyclobacillus* sp. is producing compounds at higher temperatures that it is not producing at lower temperatures, which may contribute to its ability to oxidize iron at higher temperatures or have other functionality. This peak with an *m/z* of 481.2979 was detected by LC-MS, but the structure of this compound remains to be elucidated. While there were some interesting variations when *Alicyclobacillus* sp. was cultured at two different temperatures, all of the data is not presented in this study and warrants further investigation due to various challenges with culture extraction that we are currently troubleshooting.

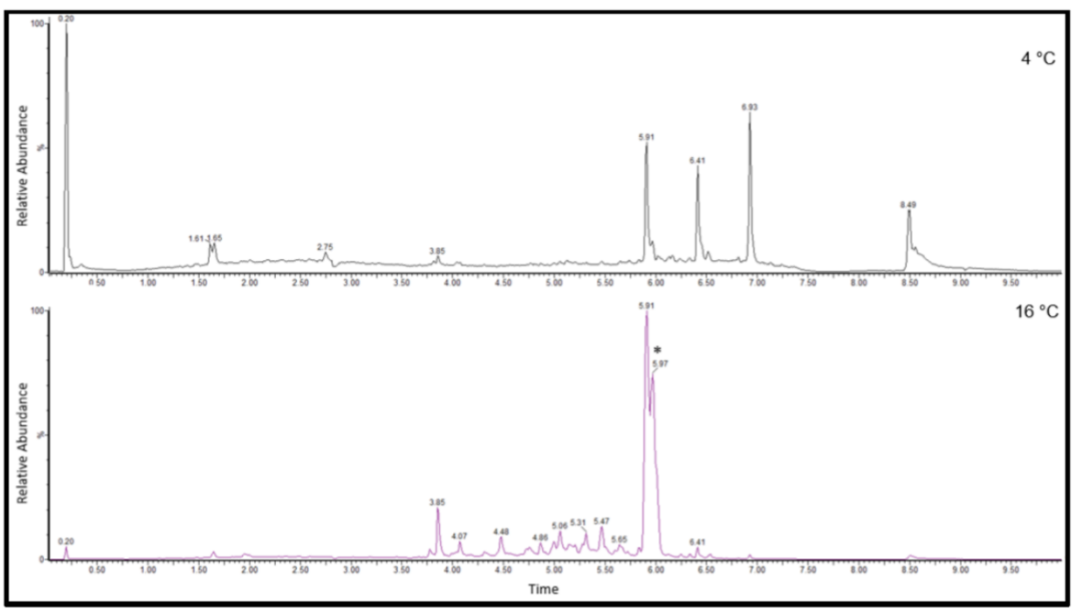


Figure 12. Extracted ion chromatogram for *m/z* 481.2979 from *Alicyclobacillus sp.* cultured at two different temperatures. The asterisk (*) represents the *m/z* 481.2979 peak.

19. Discussion [52].

The proposed study used culture-dependent and culture-independent methods to characterize the acid mine drainage microbiome at Ely Brook (Vershire, VT). Ely Brook (EB-90M) was geochemically characterized to observe changes in parameters, such as temperature, pH, sulfate concentrations, total organic carbon, and metal concentrations, and how these may affect organism metabolism, particularly of organisms which may be involved in the formation of AMD. Moreover, Illumina shotgun metagenomic sequencing was used to obtain an overview of microbial diversity at Ely Brook in July 2017 and January 2018 by extracting DNA directly from environmental samples. Using this approach, we obtained metagenomic sequence data from winter and summer sediment samples, as well as, summer water samples. However, results for the winter water samples were inconclusive.

As for the culture-dependent approach, samples were incubated in various media: Fe-TSB, water from site EB-90M, and pyrrhotite-TSB. Microbes were able to grow mainly on Fe-TSB, making this the primary medium utilized in this study. Isolates grown on this medium were identified using PCR-based 16S rDNA amplification. Isolates EBS001 and EBW001-2 were successfully identified as species from the genus *Alicyclobacillus*; however, the identity of EBS002 remains to be elucidated. The genus *Alicyclobacillus* consists of Gram-positive, rod-shaped, spore-forming bacteria. They are strictly acidophilic, moderately thermophilic, aerobic, soil bacteria, which have been shown to grow at temperatures of up to 65 °C. Additionally, they have a growth pH range of 2.2–5.8, respectively [57]. Despite only growing this species at temperatures up to 65 °C, *Alicyclobacillus* species can survive standard pasteurization conditions where liquids are heated up to 95 °C for a maximum of 2 min [57]. The difference between the species of *Alicyclobacillus* identified at EB-90M can also be further elucidated through microbial cultivation.

An analysis has yet to be conducted on other isolates and methods still need to be optimized to obtain entire 16S rDNA sequences for microbial identification. To better facilitate the identification of our isolates would be to use wider amplicons, such as 8F and 1542R [58], to obtain more of the 1,500 conserved rRNA region. For complete 16S rRNA sequences, we can amply the 16S rDNA region with internal primers. To identify eukaryotes, such as fungi, we plan to use primers specific for amplifying 18S rDNA.

EBS001 and EBS002 were cultured at two temperatures mimicking summer and winter (4 °C and 16.5 °C, respectively). Both species grew better at higher temperatures. A metabolomic analysis of these cultures was conducted using LC-MS to determine whether there were any variations in the metabolites produced by EBS001 when grown under one condition over another. We also wanted to identify compounds, some of which may have novel chemical structures or bioactivities.

Our culture-dependent and -independent methodology provided some interesting insight into how to study microorganisms found at Ely Brook and their functional capabilities. The geophysical characterization of Ely Brook in both summer 2017 and winter 2018 showed significantly higher concentrations of dissolved elements in the water in summer than winter, suggesting higher rates of AMD formation in summer months, potentially mediated by the acidophilic organisms present at the site. This hypothesis was further supported by an increase in pH and decrease in sulfate levels from summer to winter. Interestingly, there are high concentrations of silicon, which is heavy supported by the presence of diatoms in water and sediment. Furthermore, our preliminary data show a number of eukaryotes and archaea present in our samples, which have been reported in other AMD studies [59, 60]. However, acidophilic bacteria still appear to dominate this microbial community.

Metagenomic sequencing analyses showed a high abundance of iron-oxidizing microbes present in water samples, specifically *F. myxofaciens* and *Ferrovum* sp., supporting conclusions made from the geochemical characterization. *F. myxofaciens* and other species from this genus have been shown to accelerate the dissolution of pyrite, the most common metal sulfide found in AMD environments [61]. Iron-oxidizing microbes have also been shown to play an important role in nitrogen metabolism by using a variety of fixed nitrogen sources [62]. Yet, isolation and cultivation studies have only been successful for *F. myxofaciens* [62], thus studies have heavily relied on genomics to better understand the capabilities of this group. Future studies could explore varying methods to cultivate the *Ferrovum* species found at site EB-90M to further examine their metabolic response to changes in temperature and nutrient availability. Additionally, we could try growing these microbes immediately from the site or in inorganic medium, which contains only ferrous sulfate as an energy source [61].

Other microbes of interest were found in the sediment, such as *S. lithotrophicus* and *G. fermentans*. *Sideroxydans* species can grow by coupling the oxidation of ferrous iron to the reduction of oxygen, which would explain a decrease in their abundance from summer to winter [63]. Notably, this species contains a unique porin-cytochrome complex protein, MtoAB, that functions as an electron conduit. As for *G. fermentans,* this sulfate-reducing bacteria (SRB) and others alike are ideal targets for bioremediation technologies. These may be targeted using specific primers for SRBs and culturing methods that favor the growth of these microorganisms. *Deltaproteobacteria* can also be targeted in this way and are a source of interest due to the high abundance of sulfate-reducing and Hg-methylators that part of this group and are still highly understudied. Some of these species, such as *G. fermentans,* may be anaerobic organisms, thus future sampling should include appropriate handling for the collection of anaerobic organisms as to limit the organisms one can study under laboratory conditions.

Our preliminary data show that there are several genes involved in siderophore biosynthesis and antibiotic resistance. Future studies aim to perform further statistical analyses on the metagenomic sequence data and use metatranscriptomics to determine the genes that are actively expressed. Metatranscriptomics involved sequencing RNA to identify and quantify the expression of genes currently expressed under a set of growth conditions.

Our culture-dependent studies revealed that microbes cultured at two different temperatures have distinct growth characteristics. Notably, EBS001, *Alicyclobacillus sp.* oxidized ferrous iron at temperatures between 1625 °C, respectively, but not at lower temperatures. When these cultures were analyzed by LC-MS, there was an *m/z* 481.2979 peak present at 5.97 min in cultures grown at 16.5 °C but not those grown at 4 °C. There was also a peak at 5.67 min in cultures grown at 4 °C. This *m/z* 485.1170 peak was not present in the samples from cultures grown at higher temperatures. These preliminary data suggest that there are compounds produced only under one condition. The structures of these compounds and others remain to be elucidated and may be novel compounds. We are currently working to optimize our metabolomic analyses as follows: a) obtain higher sample concentrations for analyses through improved extraction methods b) ensure equal cell density in all replicates; and c) ensure proper concentration and dilution with the reserpine internal standard.

In conclusion, this is the first study of the AMD microbiome at this site as well as in the copper belt of Vermont. This study provides a preliminary overview of the microbial diversity at Ely Brook in both summer and winter. Our data suggest that there are significant differences in the microbial community, which likely have an effect on the geophysical characteristics of the site. Furthermore, several genes involved in antibiotic- and metal-resistance genes were identified. Our preliminary data demonstrate that the microorganisms at EB-90M likely have a plethora of functional capabilities that could be exploited for industrial and medicinal purposes, such as bioleaching, bioremediation, improvement of agricultural productivity, and the production of new antibiotics.

20. Plans for the second year of funding.

While our data provide insight into the microbes present in the water and sediment of EB-90M, they do not provide a picture of the *metabolically active* microbes present in our samples, which were collected under specific environmental conditions. Thus, to better understand how to restore the water quality at Ely Copper Mine, this proposal aims to 1) use metatranscriptomic sequencing to study gene expression in the active AMD microbiome in Ely Brook and characterize how gene expression changes seasonally; 2) identify genes that are *actively* expressed in this environment and involved in adaptation and heavy metal sequestration (i.e., siderophores and biosurfactants) and transformation (e.g., P-type ATPases, multicopper oxidases, and *cus* determinants [6]) to gain insight into AMD metal cycling; and 3) study changes in metabolome and bioleaching of metal sulfides using scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM-EDS) to understand seasonal affects on the cycling of heavy metals, as some microbes get their sole energy from reducing iron or secreting leaching agents that can remove heavy metals. Metatranscriptomic and bioleaching studies will be used to identify and characterize microbes that may play key, season-specific roles in biogeochemical cycling, AMD metabolism, and bioremediation; identify functional genes involved in metal sequestration and adaptation to AMD in different seasons. Lastly, we are currently preparing a manuscript to report our findings obtained with our first year of funding.

21. Training potential.

Two undergraduate students were hired to complete this work. Students were trained to identify and culture microbial isolates, isolate genomic DNA extract and quantify metabolites, and mine metagenomic data. In the fall of 2017 to May 2018, my student Katherine Morillo worked on the culture-independent component of this project, isolating DNA for sequencing, performing bioinformatics analyses of sequence data, cataloguing the bacteria present. Katherine also started to isolate, culture, and obtain preliminary data on how temperature changes in the metabolites produced by the most abundant microbes. This was a rich, interdisciplinary experience in which my student learned how to visualize an environmental problem and connect species-to-functional-genes-to-metabolites to understand the effect of increasing temperature on local AMD environments in the state of Vermont. Katherine wrote an undergraduate thesis in the spring of 2018 entitled "The effects of seasonal variations on the acid mine drainage microbiome at Ely Brook in Vershire, VT". This work was presented before the Departments of Geology, Biology, and Chemistry & Biochemistry, and defended before representatives of the aforementioned departments in May 2018.

Over the summer of 2018, another student will continue isolating microbes from samples, culturing microbes, and performing metabolomics studies. This student will be paid from our 2016 funding, as we filed for a no-cost extension due to delays in funding. We will also continue the work proposed in 2017 with two additional summer students well into 2019, again due to delays in the disbursement of grant funding.

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Undergraduate thesis based on this proposal:

Morillo, K. *The Effects of Seasonal Variations on the Acid Mine Drainage Microbiome at Ely Brook in Vershire, VT, in Department of Chemistry & Biochemistry.* Undergraduate Thesis, Middlebury College (2018).

Invited presentations:

- **Invited Oral Presentation**: "Trails to remediation: characterization of the acid mine drainage microbiome at Ely Copper Mine in Vershire, VT" Department of Universite de Poitiers, Poitiers, FR. March 27th, 2018.
- **Invited Oral Presentation**: "Secondary metabolism in the acid mine drainage microbiome at Ely Copper Mine in Vershire, VT" Marine Natural Products Gordon Research Conference, Ventura, CA. March 7th, 2018.

Phosphorus export from forested watersheds in the Missisquoi Basin

Basic Information

Title:	Phosphorus export from forested watersheds in the Missisquoi Basin
Project Number:	2017VT86B
Start Date:	3/1/2017
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	Vermont-at-Large
Research Category:	Water Quality
Focus Categories:	Nutrients, Non Point Pollution, Water Quality
Descriptors:	None
Principal Investigators:	Donald Ross, Beverley Wemple, Vanesa Perillo

Publications

1. Ryan, Sophia. 2018. Quantifying stream phosphorus dynamics and total suspended sediment export in forested watersheds in Vermont. A thesis in partial fulfillment of the Bachelor of Arts degree with Honors in Geography, College of Arts and Sciences, University of Vermont, Burlington, Vermont 36 pp.
2. Ross, Donald S., Beverley C. Wemple, Lindsay J. Willson, Courtney Balling, Kristen L. Underwood, and Scott D. Hamshaw. Impact of an extreme storm event on river corridor bank erosion and phosphorus mobilization in a mountainous watershed in the northeastern USA. Journal of Geophysical Research, Biogeochemistry. In revision.
3. Perillo, V.L., D.S. Ross, B.C. Wemple, C. Balling and E.E. Lemieux. Stream corridor soil phosphorus availability in a forested/agricultural mixed land-use watershed. Journal of Environmental Quality. Submitted.

Phosphorus export from forested watersheds in the Missisquoi Basin

14. Statement of regional or State water problem.

Excess phosphorus (P) from a variety of sources, including forest land, has impaired water quality in Lake Champlain to the point where it does not meet Vermont's water quality standards. To address this problem, the EPA recently released a total maximum daily load (TMDL) that will require the State of Vermont to reduce P within the Vermont portion of Lake Champlain (US EPA 2016a). These include reductions in P loads from forested reaches of tributaries feeding Lake Champlain, yet empirical data on forest P dynamics for this area are lacking. While it is well established that forests are a small contributor on a per area basis relative to other land uses, the large extent of forest in the Lake Champlain Basin (LCB) translates into a significant estimated load of P—estimated to be as much as 21% of total P from NY and VT forests (LCB Program, 2015) but variable across lake segments (US EPA, 2016a). Furthermore, plans under the TMDL call for reductions to these forest contributions that are quite high in several lake segments, including a mandated reduction of 50% of the loading from forested lands in the Missisquoi Bay segment of Lake Champlain (US EPA 2016a). Whether these load estimates are realistic, and whether reductions can be met through proposed management practices are outstanding questions. This project aimed to improve the information base for managing phosphorus contributions, and reductions in those contributions, from forested lands in Vermont by developing the first-ever database of forest P yield dynamics across a set of watersheds that represent a gradient in management conditions.

15. Statement of results or benefits.

The data on sediment and total P loads from a set of forested watersheds under varying management intensities is unique for this region of the Northern Forest and will help inform implementation of management practices for forests. The output from this project have already been delivered to our colleagues in the VT Dept. of Forest, Parks and Recreation and their counterparts in the VT Dept. of Environmental Conservation to aid in the formulation and adoption of better AMPs and BMPs that will lead to a reduction in the P load from forested land.

16. Nature, scope, and objectives of the project, including a timeline of activities.

Our project goal was to provide a more comprehensive understanding of the potential for P loading along forested reaches of Lake Champlain tributaries, which include approximately 42% of Vermont forestland. We focused our efforts in the Missisquoi watershed, targeted due to unacceptable P concentrations, where ongoing work by the PIs and the Ph.D. student complemented this effort. Our objectives were to quantify temporal dynamics in P fluxes during storm events and baseflow periods in streams draining forested watersheds. By selecting sites that span a gradient of management practices, we aimed to capture a range of conditions that reflect variability in the Vermont landscape.

17. Methods, procedures, and facilities.

Subwatershed sediment and phosphorus loads: In the spring of 2017, we continued monitoring two managed forested watersheds located in Bakersfield, Vermont within the Missisquoi basin, to quantify streamflow and sediment and P loads (Figure 1). Kings Hill Brook (8.5 km^2) is actively managed for timber harvesting as part of a timber investment management corporation (TIMO), a practice common across the northern forest. The last timber harvesting operations in the watershed took place roughly two years ago. Ross Brook (2.9 km^2) is managed intensively for maple sugar production by a small family-owned business. Management includes regular entry via all-terrain vehicles and foot traffic to access and service taps. Some recreational use of the watershed also occurs. During the summer of 2017, we instrumented one additional unnamed watershed near Richford that had recent logging operation

Each of these watersheds was instrumented to continuously monitor stream stage (pressure transducer) and intermittently sample storm events (ISCO autosampler) to quantify continuous discharge levels and concentrations of total suspended sediment (TSS), total phosphorus (TP) and soluble reactive phosphorus (SRP). Installations at Kings Hill Brook and Ross Brook also included continuous turbidity monitoring using Forestry Technology Services DTS-12 digital turbidity sensors.

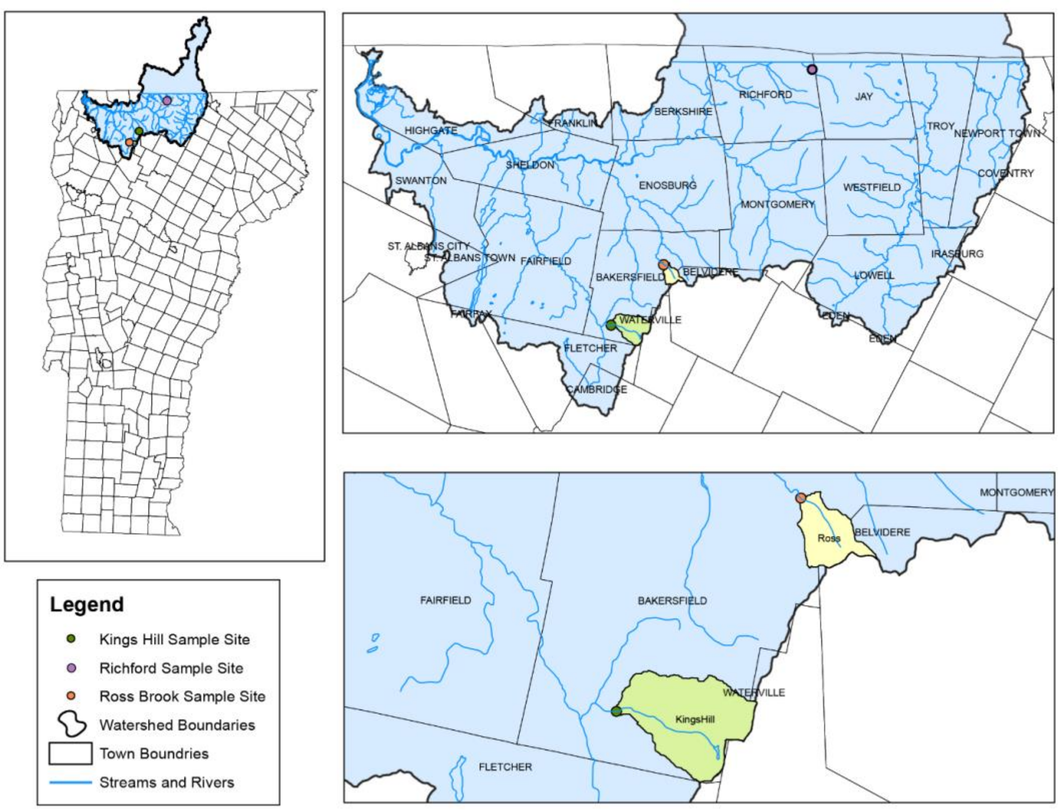

Figure 1. Sample sites located within the context of Vermont and the Missisquoi Basin. Ross Brook is in yellow and Kings Hill Brook is in green. The Richford stream site is noted in purple in the Vermont and Missisquoi maps.

Discharge rating curves were developed using empirical cross section measurements during selected site visits. For each discharge estimate, we measured channel dimensions (width, depth) and velocity in sections, for between 3 and 10 sections at each site on each measurement date. Velocity measurements for each section were taken using a Marsh-McBirney Flo-Mate 2000 Portable Water Flow Meter. Section measurements for a given date were summed to determine a cross section total discharge, and water level (stage) associated with the discharge estimate was recorded. We used the statistical software package SPSS to fit non-linear models to stage and discharge in order to extrapolate continuous stage measurements to discharge estimates.

Findings

The sampling period, May-October, fell within a relatively wet season. Of the six months studied, 2017 rainfall exceeded long-term averages by 0.32 inches to 2.05 inches for all months but September, which had below average rainfall. June was the most above average within the sampling period, with 2.05 inches of rainfall above the long-term average recorded throughout the month. Streamflow data from nearby USGS gauging stations show that the largest runoff events of 2017 occurred outside the study period, in February and April, with more modestly sized runoff events in June and July and relatively low flow conditions between August and October.

Stage-discharge relations at all three study sites exhibited non-linear relationships over the range of measurements taken. The logistic model proved to be the best fit for all three sites with R2 values ranging from 0.814-0.982 (Figure 2). Measured stage values over the May

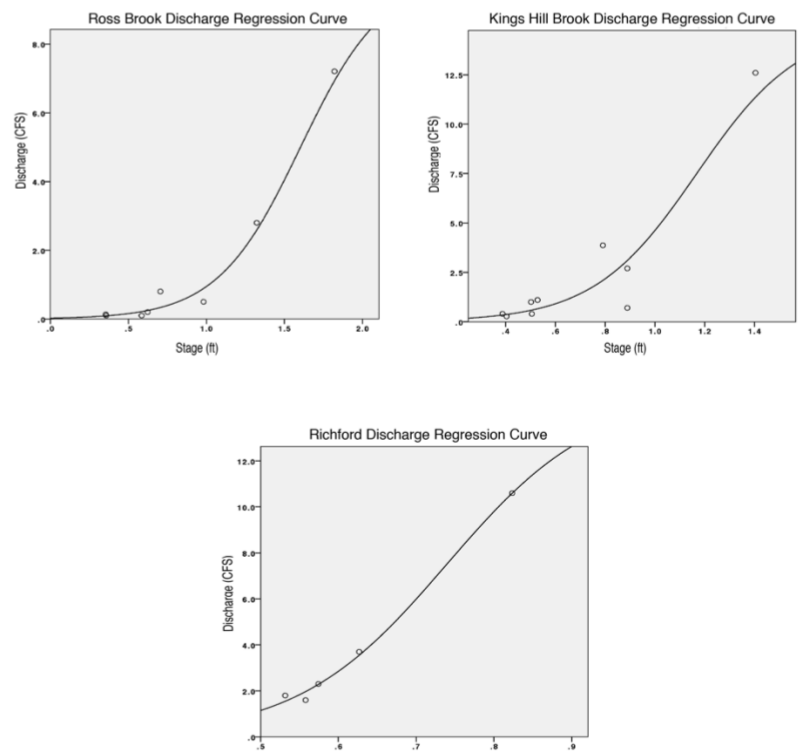

Figure 2. Discharge Rating Curves for Ross Brook, Kings Hill Brook and Richford. The logistic model was used to create all three rating curves.

through November study period ranged from 0.3-2.9 feet (0.09-0.89 m) at Ross Brook, from 0.3 -1.9 feet (0.1-0.57 m) at Kings Hill Brook, and from 0.4-1.3 feet (0.13- 0.39 m) at Richford. Thus field measurements of discharge used to derive rating curves were made over nearly the entire range of stage measurements recorded at each site during the study period.

Ross Brook recorded the lowest discharge of all three sites and one of the highest turbidity readings. (Figure 3). Discharge during precipitation events reached almost 35 CFS and turbidity reached a high of almost 500 NTU. During baseflow periods discharge was typically between 0-3 CFS and turbidity remained between 0-100 NTU, with readings typically closer to 0-10 NTU. During precipitation events discharge values ranged from 1-10 CFS and turbidity readings ranged from <100 to 500 NTUs. The turbidity sensor at Ross Brook malfunctioned during leaf fall, when the sensor installation was regularly clogged by leaves. For this reason, loads were not calculated for the period of October 1st, 2017 to October 31st, 2017 at the Ross Brook sample site.

Kings Hill Brook recorded turbidity values between 0-200 NTU and calculated discharge values ranging from 0-15 CFS (Figure 3). During precipitation events discharge ranged from 7-14 CFS and turbidity typically ranged from 100-200 NTU. During baseflow periods discharge was typically between 0-2 CFS and turbidity readings fell within 0-50 NTU, with a couple of readings reaching 100 NTU.

Richford turbidity readings ranged from 0-500 NTU and calculated discharge values fell between 0-15 CFS (Figure 3). During precipitation events turbidity readings typically fell between 0-300 NTU with some events recording turbidity values of 500 NTU. Discharge during storm events typically ranged from 3-15 CFS. During baseflow periods turbidity readings were between 0-100 NTU and discharge ranged from 0-5 CFS.

Storm event plots show varying temporal patterns in discharge, turbidity and concentrations for the three study sites (Figure 4). At Ross Brook the concentration data (TP and TSS) appear to closely follow turbidity except during the storm event on July 11th. During this specific storm event both TP and TSS appear to peak during a slight increase in discharge and turbidity, but before either of them reach their maximum. In all three storm events turbidity, TP and TSS all illustrate a relatively “flashy” response to precipitation.

At Kings Hill Brook the relationship between variables is similar, but discharge does not rise and fall as sharply as it tends to in Ross Brook (Figure 4). There is also a greater frequency of rapid fluxes in turbidity. However, overall concentration data appear to closely follow turbidity concentrations. Similarly to Ross Brook, TP, TSS and turbidity show a “flashy” response to precipitation, however at Kings Hill Brook turbidity continues to flux in the falling limb.

At the stream at Richford concentration data tend to follow turbidity (Figure 4). This relationship is weaker in the precipitation event on September 8th. Similarly to both Kings Hill Brook and Ross Brook, turbidity, TP and TSS have a “flashy” response in the stream at Richford. Turbidity appears to rise and fall in fluxes that continue through the falling limb, which is also seen at Kings Hill Brook.

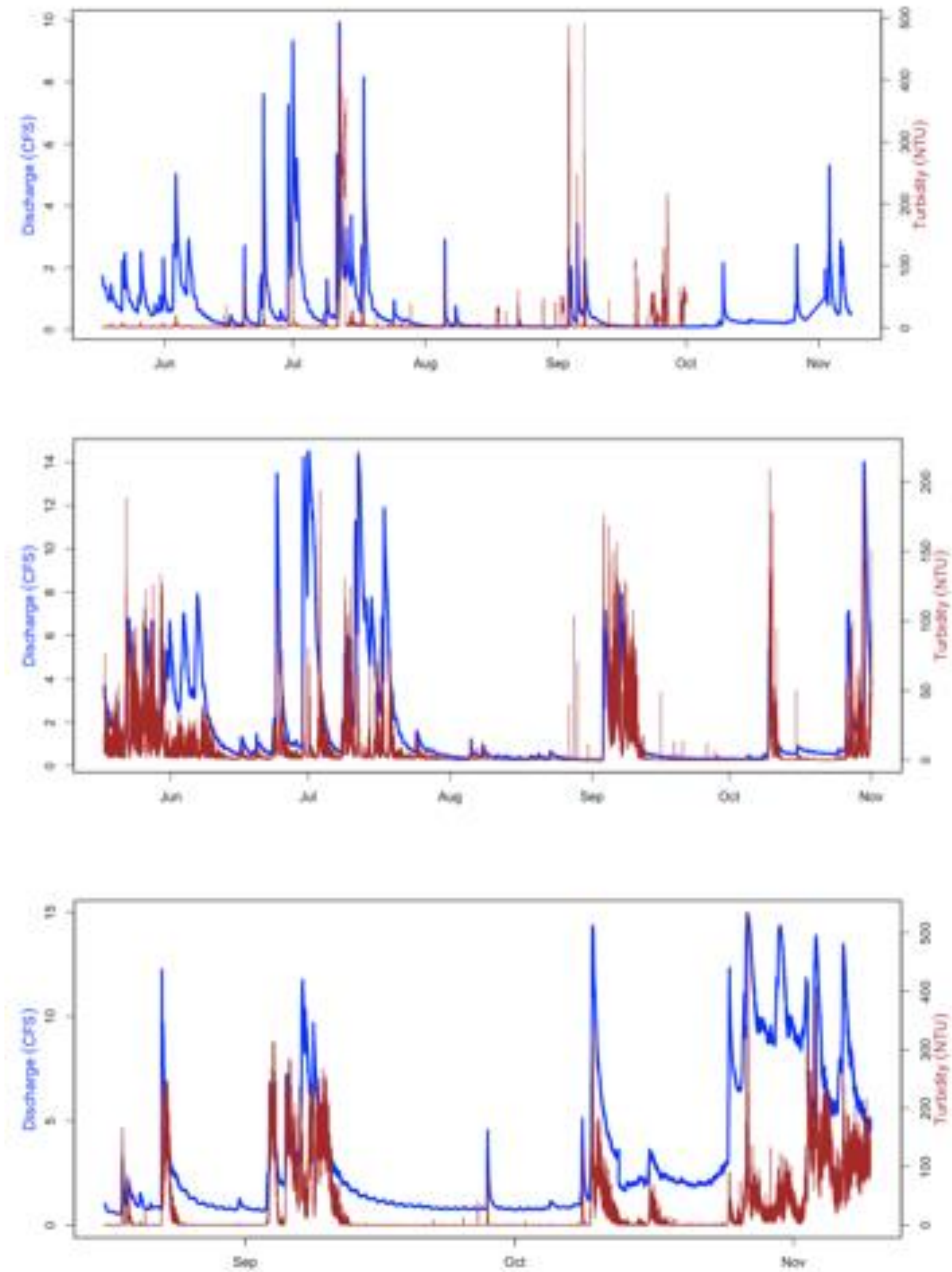

Figure 3. Sampling period discharge and turbidity for Ross Brook (upper), Kings Hill Brook (middle) and Richford (lower) Stream discharge is in blue and turbidity is plotted in red. Note apparent correlations of these two parameters.

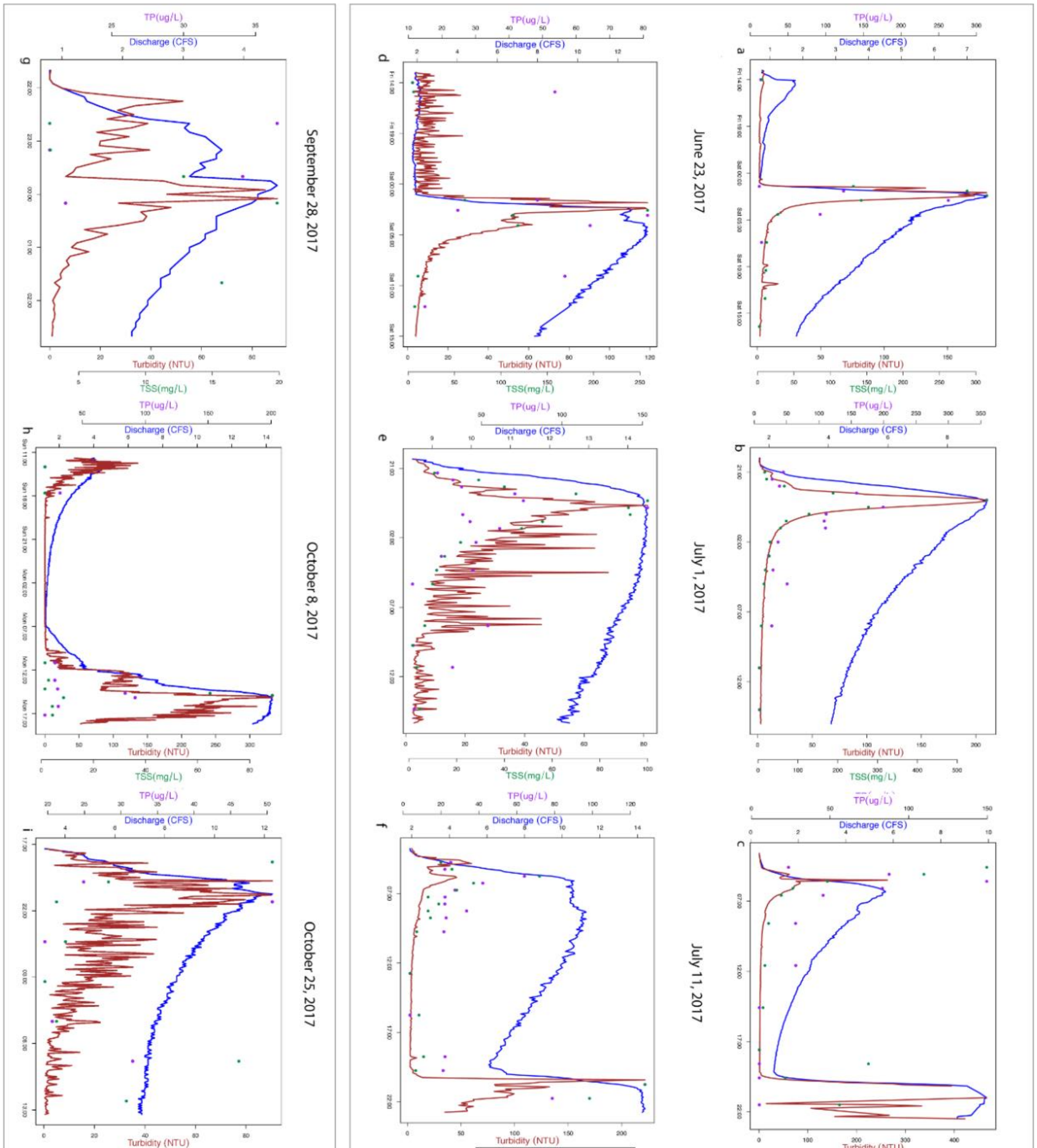

Figure 4. Individual storm events at Ross Brook (upper panel), Kings Hill Brook (middle panel) and Richford (lower panel). Ross Brook and Kings Hill Brook storm events are from June 23, July 1 and July 11, 2017. Richford storm events are from September 28, October 8 and October 25, 2017.

SRP at all three sites was quite low. The highest SRP occurred at the beginning of the sampling season, ranging from ~5 µg/L up to just above 20 µg/L (Figure 5). However, concentrations were significantly lower throughout the rest of the sampling season, not rising above ~1 µg/L.

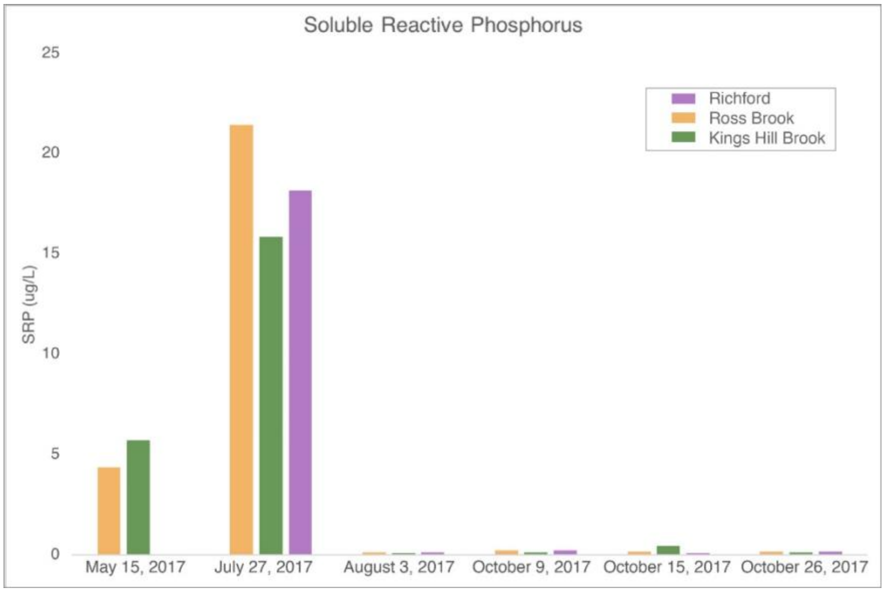

Figure 5. Soluble reactive phosphorus results. Samples were taken periodically throughout the sampling season.

Predictive power of various variables for concentrations of TP and TSS varied among sites (Figure 6). Turbidity proved to be the best predictor for both TSS and TP at Ross Brook (R^2 = 0.83 and 0.77 respectively). At Kings Hill Brook turbidity was the best predictor for TSS (R^2 = 0.69) whereas the best predictor for TP was TSS (R^2 of 0.64). At Richford all R^2 values were below 0.30 and therefore neither turbidity nor discharge were used to predict TSS and TP concentrations.

Calculated loads of sediment and TP showed varying patterns in Ross Brook and Kings Hill Brook (Figure 7). Ross Brook had a higher predicted sediment yield in June and July but Kings Hill Brook was predicted to release and carry more sediment in every other month during the sampling period. Ross Brook yielded more P during the months of June and July, whereas Kings Hill Brook yielded more in May, August and September. Monthly loads were not calculated for Ross Brook in October due to interference with the turbidity sensor throughout the month, therefore it is unclear which site is predicted to release more sediment or phosphorus, however Kings Hill Brook released very little P in October compared to the other monthly loads

Figure 6. Regressions to determine best predictor for TP and TSS concentrations in Ross Brook (left column), Kings Hill Brook (middle column) and Richford (right column).

.

The seasonal predicted P load at Ross Brook is 12.47 kg and at Kings Hill Brook it was 16.00 kg (Table 1). For both sites predicted annual loads (kg/ha/yr) are low, <1 kg/ha/yr of P. Ross Brook is predicted to export more P annually by area than Kings Hill Brook by an order of magnitude.

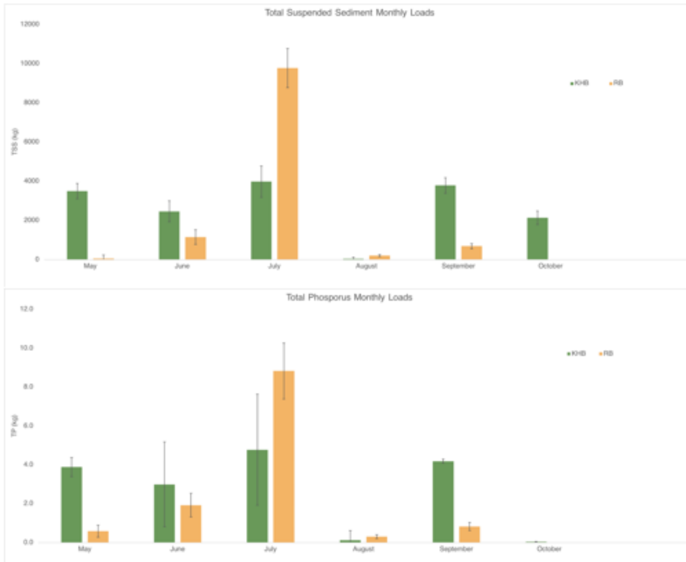

Figure 7. Predicted monthly TSS (kg) and TP (kg) loads from Ross Brook and Kings Hill Brook. Error bars incorporate 95% confidence intervals on prediction of TSS and TP for each five minute monitoring time step, subsequently multiplied by water volume to estimate load, summed over the month.

Table 1. Calculated seasonal loads, unit area seasonal loads and prorated annual load estimates for Ross Brook and Kings Hill Brook.

Sample Site	Seasonal Load 2017[1] (kg)	Record length for load estimation (no. months)	Unit area seasonal load (kg/ha)	Sampling season percent of annual discharge	Prorated annual load estimate[2] (kg/ha/yr)
Ross Brook (2900 ha)	12.47	5	0.004	0.26	0.02
Kings Hill Brook (8500 ha)	16.00	6	0.002	0.27	0.01

[1]Sum of the monthly loads presented in Figure 7.
[2] Prorated by dividing unit area seasonal load by sampling season percent of annual discharge. Assumes load for record length is representative of annual load.

18. Discussion.

We found that the forested watersheds studied exported very low concentrations of P and TSS. The relatively low export was highly responsive to precipitation events. Given that the watersheds included in this study are managed for logging and maple sugaring, the use of trails or dirt roads associated with these practices could introduce some sediment and nutrients, however not to the same degree as precipitation events. In the time series (Figure 3) for all three sites it is clear that turbidity rises with discharge and remains relatively constant throughout periods of baseflow. Due to the composition of these watersheds, there is little disturbance aside from precipitation that would result in the transport of sediment and nutrients into nearby streams.

Furthermore, it is likely that the sources of sediment are located in close proximity to the streams. Based off the individual storm event plots (Figure 4) it is clear that turbidity, TSS and TP all demonstrate a "flashy" response to precipitation, whereas discharge has a more gradual falling limb, illustrating hysteresis. The rapid rise and fall of turbidity, TSS and TP suggest that the source of sediment is located near the stream, allowing sediment concentrations to increase with the rising limb of discharge, but that the source is limited or easily exhausted, which would explain the rapid decrease in sediment concentration while discharge decreases more gradually. The exact sources of sediment and nutrients within these specific landscapes are unknown. However, it is likely that the exposed trail and gravel road surfaces in these watersheds contribute to the overall sediment and nutrient concentrations (EPA, 2016b). Given that the sediment source is likely located near the stream, it is possible that gravel roads crossing the stream or other nearby trails and dirt roads release sediment that is quickly transported into the stream. Additional proximal sources of sediment include the streambank and adjacent riparian zones that run alongside the streams (EPA, 2016b). Due to the inherent stability of forested soils (Cardoso et al., 2013) it is possible that the sediment released into the streams during precipitation events is a result of natural weathering and the limited supply of sediment released can be explained by the stability of these soils and riparian zones.

This study was conducted during a relatively wet period compared to long-term average conditions as measured at a regional precipitation station. Without a longer term study, there are uncertainties associated with annual variability in precipitation and variability in the concentration-discharge relationship. More sampling and discharge measurements during high flow events, such as snow melt, would also help create more robust predictions of annual loads.

In summary, our findings support these conclusions:

- These forested watersheds exhibited "flashy" response to storm events with high temporal variation in flow, turbidity and concentrations of sediment and P.
- In general, concentrations of sediment and P were relatively low, leading to relatively low seasonal and annual estimates of loads.
- Sources of sediment and P were not separately measured, but event plots showing rapid rise of sediment and P during an event suggests that sources are likely close to or well connected to streams. Likely sources may be stream bank erosion or connected roads/trails in the watershed.

Products:

Ryan, Sophia. 2018. Quantifying stream phosphorus dynamics and total suspended sediment export in forested watersheds in Vermont. A thesis in partial fulfillment of the Bachelor of Arts degree with Honors in Geography, 36 pp.

In revision (partially supported by past Water Center funding):

Ross, Donald S., Beverley C. Wemple, Lindsay J. Willson, Courtney Balling, Kristen L. Underwood, and Scott D. Hamshaw. Impact of an extreme storm event on river corridor bank erosion and phosphorus mobilization in a mountainous watershed in the northeastern USA. Journal of Geophysical Research, Biogeochemistry.

Submitted (fully supported by past Water Center funding):

Perillo, V.L., D.S. Ross, B.C. Wemple, C. Balling and E.E. Lemieux. Stream corridor soil phosphorus availability in a forested/agricultural mixed land-use watershed. Journal of Environmental Quality.

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Application of neural networks to classify erosional and depositional stream reaches in glacially-conditioned Vermont catchments

Basic Information

Title:	Application of neural networks to classify erosional and depositional stream reaches in glacially-conditioned Vermont catchments
Project Number:	2017VT87B
Start Date:	3/1/2017
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	Vermont-at-Large
Research Category:	Water Quality
Focus Categories:	Geomorphological Processes, Water Quality, Floods
Descriptors:	None
Principal Investigators:	Donna M. Rizzo, Mandar M. Dewoolkar, Kristen Underwood

Publications

There are no publications.

13. Title. Application of Neural Networks to Classify Erosional and Depositional Stream Reaches in Glacially-Conditioned Vermont Catchments

14. Statement of regional or State water problem.

Sediment movement in watersheds is a complex and dynamic process that exhibits high variability across spatial and temporal scales (Fryirs, 2013; Walling, 1983). The nonlinear nature of sediment erosion and deposition within a watershed and the variable patterns in sediment export from that watershed over a defined timeframe of interest are governed by many interrelated factors, including geology, climate and hydrology, vegetation, and land use (Benda & Dunne, 1997; Fryirs, 2013). Glacially-conditioned mountainous watersheds of humid temperate climates – such as Lake Champlain Basin and the Connecticut River Basin - are particularly vulnerable to sediment export, due to their topographic setting, close coupling of hillslope and channel processes, and reworking of proglacial and paraglacial sediments (Ballantyne, 2002; Church & Ryder, 1972). The geologic setting and recent glacial history have imparted longitudinal and lateral variations in valley setting and network position, as well as discontinuities in channel form and process (Toone *et al*., 2014) that influence the dynamics of sediment erosion, transport and deposition (Fryirs *et al.*, 2007, Nanson & Croke, 1992). Human disturbances to the landscape and river networks over the last 250 years have also altered patterns of water and sediment routing through the landscape (Kline & Cahoon, 2010; Noe & Hupp, 2005). Increasing development has led to channelization, berming, armoring, impoundment and diversion of rivers, that can disconnect river channels from the adjacent floodplain (Poff *et al.*, 1997, Kline & Cahoon, 2010). A nonstationary climate introduces additional motivation to understand the complex dynamics of sediment (and associated pollutant) movement through catchments, as sediment yields are expected to increase in regions of the world that will experience increased frequency, persistence, and intensity of storm events (IPCC, 2014), including the northeastern US (Guilbert *et al*. 2015; Guilbert *et al*. 2014; Hayhoe *et al*., 2007; Collins, 2009).

Watershed-level and reach-level processes that alter flow and sediment inputs, combined with reach-scale modifiers of stream power and boundary resistance, govern reach-scale adjustments in channel dimensions, profile and planform over time. These lateral and vertical adjustments, in turn, influence how the river channel transports its sediment and water inputs. Stages of lateral and vertical channel adjustments in response to natural and human perturbations have been described in terms of channel evolution models (Schumm, 1984; Simon and Hupp, 1986; Simon and Rinaldi, 2006). Based on these models, and the classification schemes of Montgomery & Buffington (1997) and Rosgen (1996), rapid assessment protocols have been developed and applied in Vermont to identify reach-based dominant adjustment process, stage of channel evolution, and sensitivity to future adjustment (Kline *et al.*, 2009; Kline & Cahoon, 2010).

To integrate these reach-based data into a sediment transport regime classification system, water resource managers are in need of computational tools and predictive models that can work well with multi-dimensional data. A given sediment transport regime is the manifestation of various governing variables operating in nonlinear, complex ways. Artificial neural networks (ANNs) are well-suited to model nonlinear processes, and handle nonparametric data of varying types (e.g., continuous, ordinal, nominal) and scales. A particular type of ANN, the Self-Organizing Map, is especially valuable for visualizing patterns in multi-dimensional data.

Classification of reach-based bedload sediment transport regime could help to identify bridges, culverts and road segments at enhanced risk of failure from channel adjustment during extreme flood events. For example, Vermont river reaches progressed rapidly through channel evolution stages as a result of extreme flows and bedload movement sustained during Tropical Storm Irene in 2011, resulting in the loss of several hundred bridges and culverts (Anderson *et al.*, 2016).

A better understanding of sediment transport dynamics at the reach and network scales would also help to identify critical reach locations and time periods ("hot spots" and "hot moments"; McClain, 2003) responsible for disproportionate fluxes of suspended sediment (and associated nutrients). The VTANR has identified sediment and phosphorus as the largest contributors to the impairment of surface water quality and aquatic habitat in the State (e.g. VTANR, 2011). Reduction in sediment (and nutrient) loading are stated objectives in the Lake Champlain Basin Program management plan ("Opportunities for Action") (LCBP, 2015).

15. Statement of results or benefits.

- A better understanding of spatial variability in sediment (and associated nutrient) transport regimes at the reach scale and linkage to topographic and geomorphologic characteristics.

- A computational framework that can be used to estimate sediment transport regimes of Vermont rivers at the reach scale, and which can be linked to GIS for visualization.

- A computational framework that can be used to automate the prediction of sediment transport regimes in response to various channel and catchment modifications, supporting adaptive management of rivers.

- Support for river corridor planning efforts of the VT Agency of Natural Resources in which river reaches are classified by their sensitivity to future vertical and lateral adjustment, with classifications considered in a tactical basin planning context to identify and prioritize channel and watershed restoration and conservation projects.

- Support for hazard management activities of VTrans and towns relating to infrastructure (i.e., bridges, culverts, roads) at risk from fluvial erosion.

16. Nature, scope, and objectives of the project, including a timeline of activities.

Stipend resources from this graduate research grant have supported PhD candidate Kristen Underwood for development of an additional dissertation chapter, incorporating stream power data sets alongside topographic and geomorphologic data for several river reaches in Vermont.

The objective of the research was to develop an existing set of computational tools to model and predict sediment erosion, deposition and transport within mountainous river networks.

The following research tasks have been completed:

1 Compiled and developed reach-scale stream power and network-scale data.

2 Compiled stream geomorphic assessment data from the VT SGA Data Management System (https://anrweb.vt.gov/DEC/SGA/Default.aspx) and derived additional parameters with possible relationships to sediment transport regime.

3 Developed a Self-Organizing Map (SOM) to cluster river reaches into sediment transport regimes categories (i.e., process domains) on a continuum from supply-limited to transport-limited, and erosion-dominated to deposition-dominated, based on inputs variables compiled in Tasks 1 and 2.

4 Evaluated the SOM performance relative to sediment regime classifications that had been assigned by practitioners based on expert judgement.

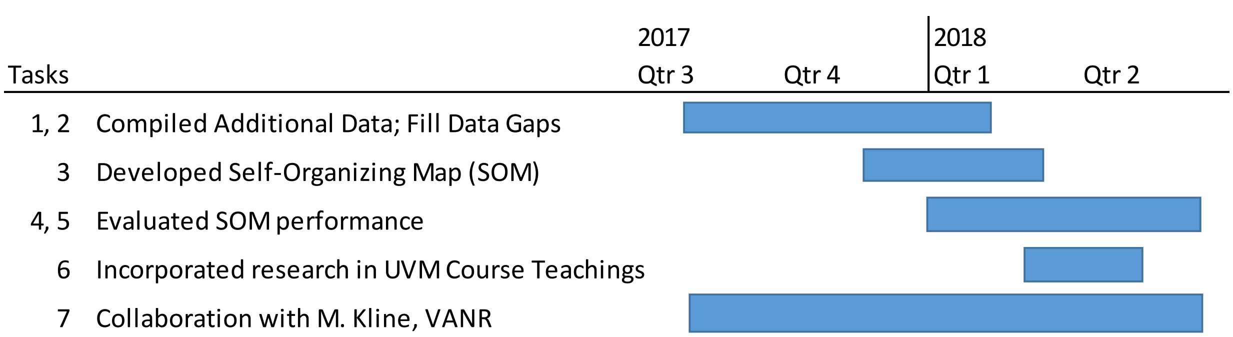
5 Examined linkages between clusters and topographic and geomorphologic variables using parametric statistical techniques.

6 Incorporated related educational modules on stream geomorphic assessment and sediment transport capacity within UVM coursework taught by Kristen Underwood during Spring semester 2018 (CE 295C Applied River Engineering); and

7 Shared modeling results with project partner, Mike Kline, VT Department of Environmental Conservation River Program Manager, during multiple working meetings on four separate occasions over the grant period.

Research was completed over a 1-year duration (Figure 1).

Figure 1. Proposed project timeline



17. Methods, procedures, and facilities.

As part of her dissertation, Underwood has investigated the application of a SOM to cluster reach-based sediment regimes based on remote-sensing-derived and field-measured data developed and catalogued in the VTANR Stream Geomorphic Assessment (SGA) database (https://anrweb.vt.gov/DEC/SGA/Default.aspx). The SOM has been applied to test the reproducibility of sediment regime classifications proposed in the VTANR River Corridor Planning Guide (Kline, 2010; Figure 2). Mike Kline, Program Manager of the River Section of VTANR Watershed Management Division, and primary author of the SGA protocols and River Corridor Planning Guide, has participated as a project advisor.

<table>
<tr><th>Valley Confinement</th><th>Sediment Transport Regime</th><th>Slope</th><th>Valley Confinement Ratio</th><th>Incision Ratio</th><th>Entrenchment Ratio</th><th>Width/Depth Ratio</th></tr>
<tr><td rowspan="2">Confined</td><td>Transport</td><td rowspan="4">≥ 2 %</td><td rowspan="3">< 6</td><td>< 1.3</td><td rowspan="5">< 1.4 (< 2.2)</td><td rowspan="4">< 12
(A, G)

> 12
(F, B)</td></tr>
<tr><td rowspan="3">Confined Source & Transport</td><td rowspan="6">> 1.3</td></tr>
<tr><td rowspan="2">Partly Confined</td></tr>
<tr><td>≥ 1</td></tr>
<tr><td rowspan="7">Unconfined</td><td rowspan="2">Unconfined Source & Transport</td><td rowspan="2">< 4 %</td><td rowspan="5">≥ 4</td><td rowspan="2">< 30
< 12 (E)</td></tr>
<tr><td rowspan="6">> 2.2</td></tr>
<tr><td>Fine Source & Transport and Coarse Deposition</td><td rowspan="4">< 2 %</td><td rowspan="2">> 30 (B, C)

> 12 (E)

> 40 (D)</td></tr>
<tr><td rowspan="2">Coarse Equilibrium & Fine Deposition</td><td rowspan="2">< 1.3</td></tr>
<tr><td>< 30 (C)
< 12 (E)</td></tr>
<tr><td rowspan="2">Deposition</td><td rowspan="2">≥ 6</td><td rowspan="2">1.0</td><td>> 30</td></tr>
<tr><td>> 1 %</td><td>> 40</td></tr>
</table>

Figure 2. Reach-scale sediment regime classifications after Kline, 2010

Study Area

Reach-scale topographic, hydrographic, geologic, and geomorphic data were compiled for study catchments from remote-sensing derived and field-based assessment data stored in the VTANR SGA Data Management System for 193 reaches located in six catchments in central and southern Vermont (Figure 3; Table 1). The data set includes unconfined to confined, steep- to shallow-gradient, mid-to-high order, channels that are predominantly alluvial in nature although characterized by occasional bedrock grade controls and valley pinch points. The study reaches are dispersed across a mix of physiographic regions, including the Northern and Southern Green Mountains, Champlain Valley, Taconics, and Northern Piedmont.

Table 1. Characteristics of Study Area Reaches.

	Elevation	Length	Drainage Area	Slope	Valley Confinement	D50
	m (ft)	m (ft)	km^2 (mi^2)	%	(ratio)	mm
Min	29 (96)	95 (312)	0.93 (0.36)	0.03	1.1	0.06
Max	573 (1,880)	4,724 (15,500)	302 (117)	10.7	104	303
Mean	203 (666)	997 (3,272)	83.5 (32.2)	1.5	11.5	75

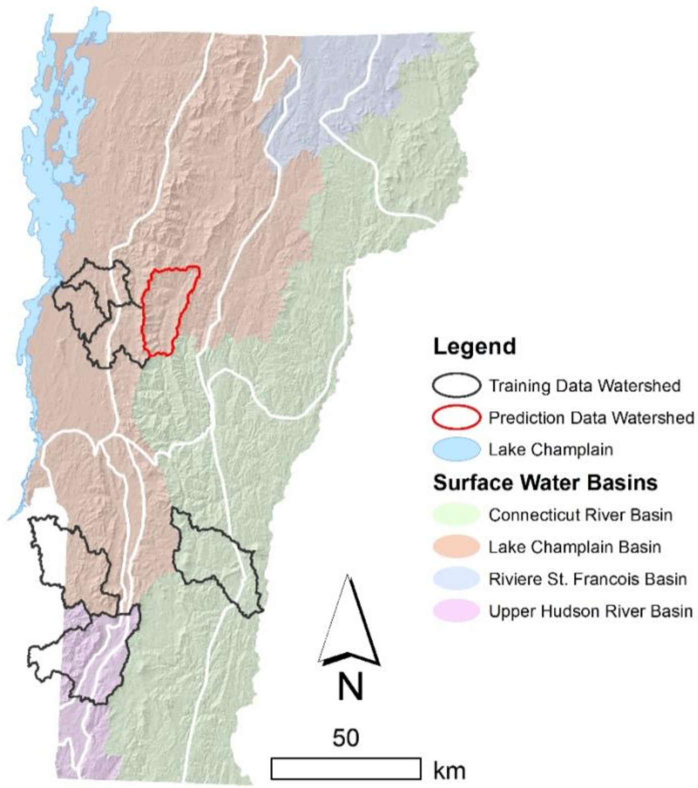


Figure 3. Location of Vermont Study Area Catchments and Major Drainage Basins.

Data Compilation

Geomorphic variables compiled for each reach have included elevation, drainage area, reach length, slope, valley confinement ratio, incision ratio, entrenchment ratio, bankfull channel width, width/depth ratio, percent bank armoring, number of depositional bars (normalized to reach length) and number of flood chutes (normalized). We derived additional parameters with possible relationship to sediment transport regime, including percentiles from cross-sectional pebble count data (D95, D84, D50, D16), mean cross-sectional flow velocity, hydraulic radius (Rh), relative roughness (Rh/D85 ratio), and various measures of stream competence.

We have also compiled and developed reach-scale stream power and network-scale data including total stream power (TSP), specific stream power (SSP), critical SSP, subject-reach-to-upstream-reach ratio of SSP; subject-reach-to-upstream-reach ratio of valley confinement (VC), as well as specific stream power balance (SSPbal) defined as the ratio of subject-reach SSP to upstream-reach SSP (after Parker *et al.*, 2014). An SSPbal < 1 would indicate a downstream reduction in stream power, with an expected inducement of sediment deposition. Conversely, an SSPbal > 1 would indicate a downstream increase in stream power, and the associated likelihood for stream bed and bank erosion.

Self-Organizing Map

The Self-Organizing Map (SOM) (after Kohonen, 1990) is an unsupervised learning algorithm, which means that the number of clusters is not defined *a priori*, but instead is data-driven. The SOM links multidimensional data to a low-dimensional feature map, usually a 2-dimensional plane or lattice (Figure 4). During unsupervised processing within the SOM, a competitive ("winner-takes-all") algorithm ensures that the unit (node) whose weight vector is most similar to the input vector is selected. This Best Matching Unit (BMU) – along with a user-defined neighborhood of nodes around the BMU - is made more similar to the input vector by adjusting the weights. In this way the BMU and its neighborhood of units "learn" the input vector (Ultsch, 1993). SOMs have demonstrated advantages over other methods for data visualization and interpretation, and have demonstrated superior performance over parametric methods where data contain outliers or exhibit high variance (Mangianeli *et al.* 1996).

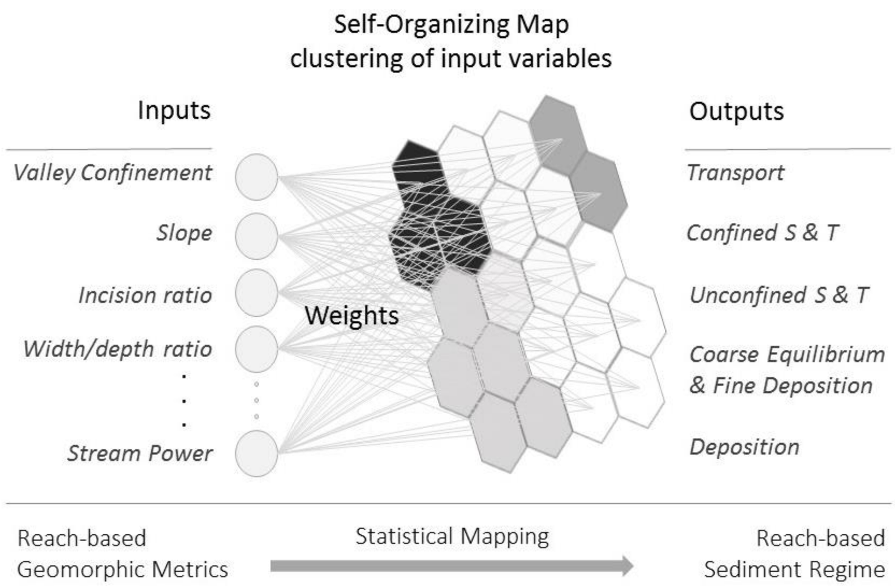


Figure 4. Generalized architecture of the Self-Organizing Map (SOM) to cluster/classify reach-based sediment regime.

18. Findings

Input data were mapped to seven clusters, broadly corresponding to the six sediment regime classifications proposed by Kline (2010). Multivariate input data (Table 1) for the 193 training reaches were reduced to a two-dimensional 6 x 13 lattice for visualization (Figure 5). The column-to-row ratio for this lattice approximated the ratio of the first two principal components of the (transformed) input data, as per *Cereghino and Park* [2009]. For each input variable, the intra-cluster mean (on a normalized scale) was plotted against the overall mean, and the magnitude and direction relative to the overall mean was examined to better understand variables driving the clustering.

Three clusters (3, 4 and 5) were characterized by steeper-than-average slopes, greater-than-average SSP, and coarser bedload. Reaches in Clusters 4 and 5 were confined by valley walls; Cluster 4 reaches comprise much coarser bed material (bedrock); and Cluster 5 reaches represented bedrock-controlled knickpoints at a transition from a lesser-gradient upstream reach. One reach comprised Cluster 3 which was a special case of a post-glacial alluvial fan trench in a moderate-gradient, unconfined setting. At the opposite end of the sediment transport continuum, two clusters (6 and 7) in unconfined settings were characterized by larger-than-average valley confinement ratios and entrenchment ratios. Cluster 7 reaches, however, were distinguished by their lower-than-average width/depth ratios, lower slopes and finer-grained bed material (dune-ripple bedforms). The Cluster 6 reaches were moderately-steep, comprised of coarser bedload and had much higher width/depth ratios (braided channels).

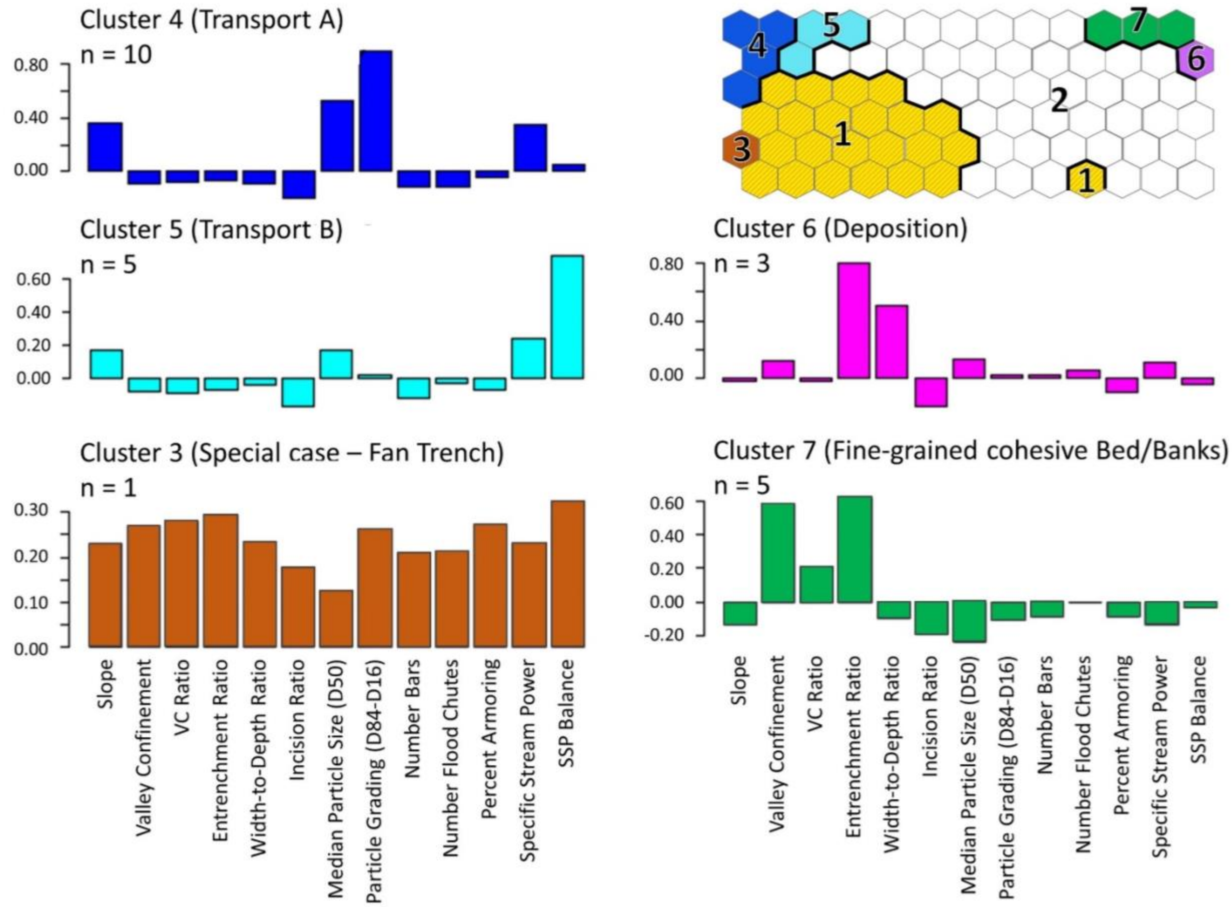


Figure 5. SOM clustering outcomes for study area reaches, including **(**a**)** SOM lattice; (b) variable bar plots by cluster for confined reaches in Clusters 4, 5, and 3; (c) bar plots for unconfined reaches in Clusters 6 and 7 (n = number of reaches per cluster; y-axis represents range-normalized values). Color shading relates to clusters in panel a. Note: for clarity of presentation, bar plots have been rendered using different vertical scales.

The remaining reach observations aggregated to Clusters 1 and 2, where clustering was less distinct. In general, Class 1 contained reaches associated with a higher-than-average incision ratio, lower-than-average entrenchment ratio and coarser bed material. Class 2 reaches, however, were much less incised (on average), and exhibited greater entrenchment ratios, lower gradients, and finer-grained bed materials (Figure 6).

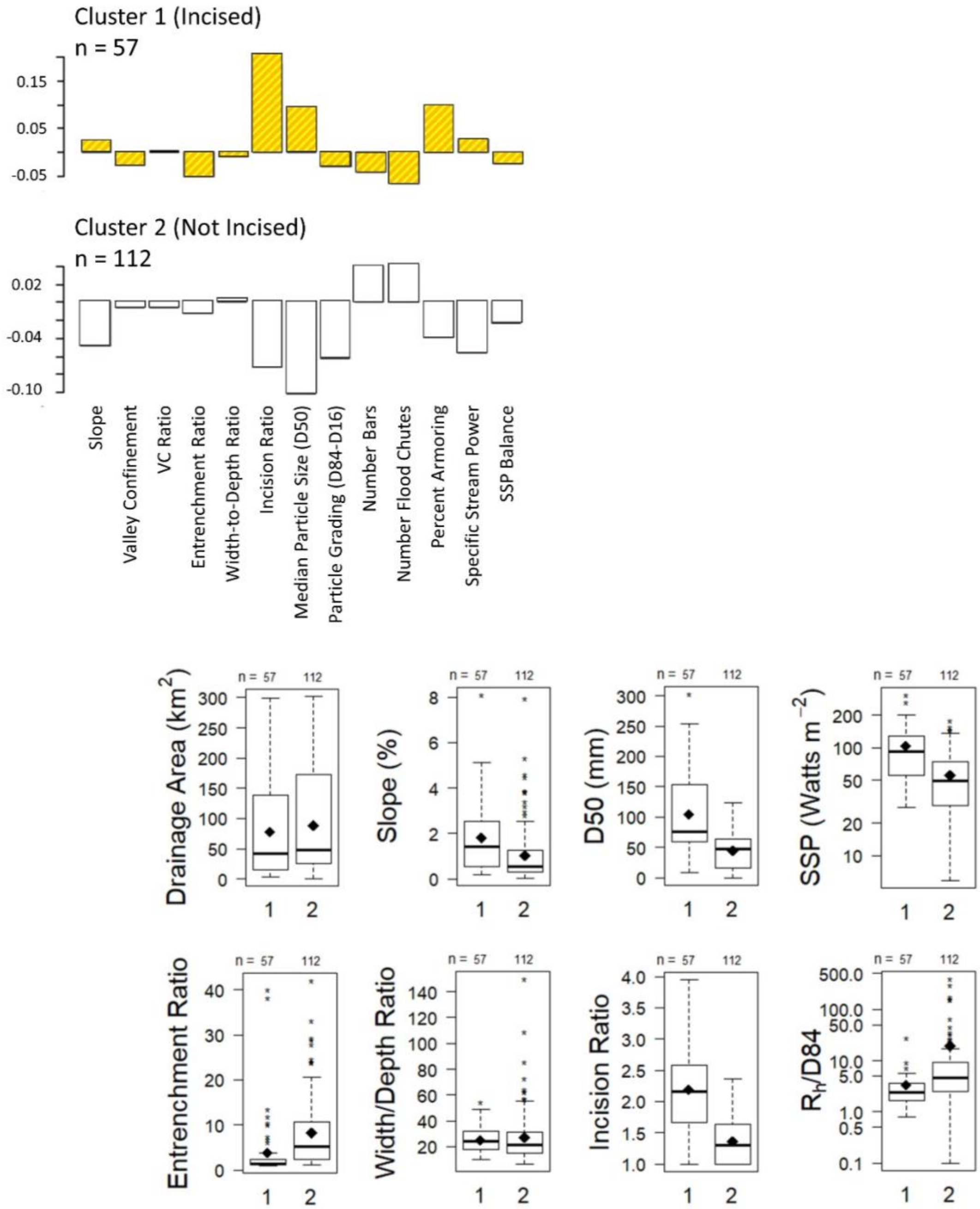


Figure 6. (a) SOM clustering outcomes for unconfined study area reaches in Clusters 1 and 2. (n = number of reaches per cluster; y-axis represents range-normalized values). Color shading relates to clusters in panel a of Figure 5. Note: for clarity of presentation, bar plots

have been rendered using different vertical scales; (b) box plots of stream geomorphic assessment variables by Clusters 1 and 2.

To evaluate the utility of the SOM clustering method for classification of sediment regimes after Kline (2010), we overlaid reach observation numbers on the lattice nodes to which they clustered, color-coded by the sediment regime classification assigned by practitioners in the VTANR DMS (Figure 7). The outcome illustrates the value of SOM methods over other multivariate statistical techniques (e.g., PCA) for visualization of patterns. Along the lattice-horizontal dimension, input vectors corresponding to the reach observations have self-organized into a configuration that is suggestive of the continuum of reach types from supply-limited (figure-left) to transport-limited (figure-right), as proposed by Montgomery & Buffington (1997). Along the lattice-vertical dimension, a gradient of increasing degree of channel or catchment stressors is suggested by the distribution of parameter values that can be visualized on the component planes (Figure 8).

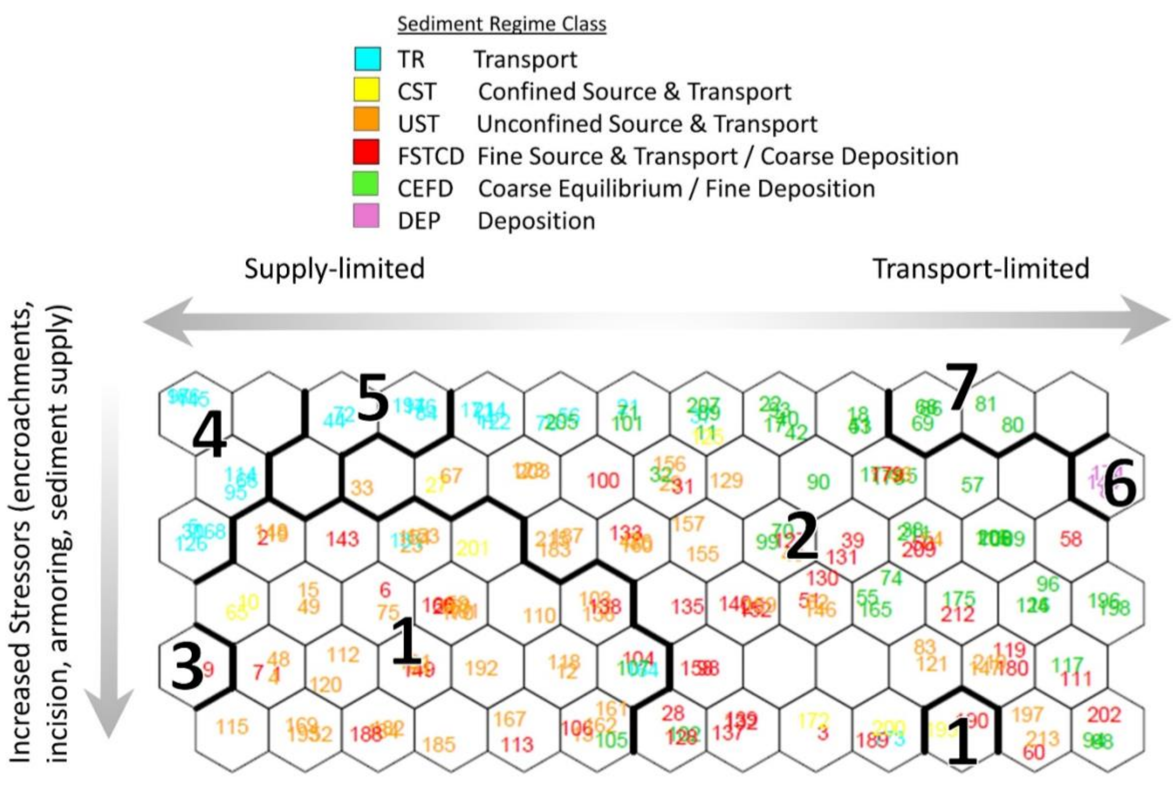


Figure 7. Reach observation numbers color-coded by expert-assigned sediment transport regime (see key above) plotted to SOM to visualize where observations clustered.

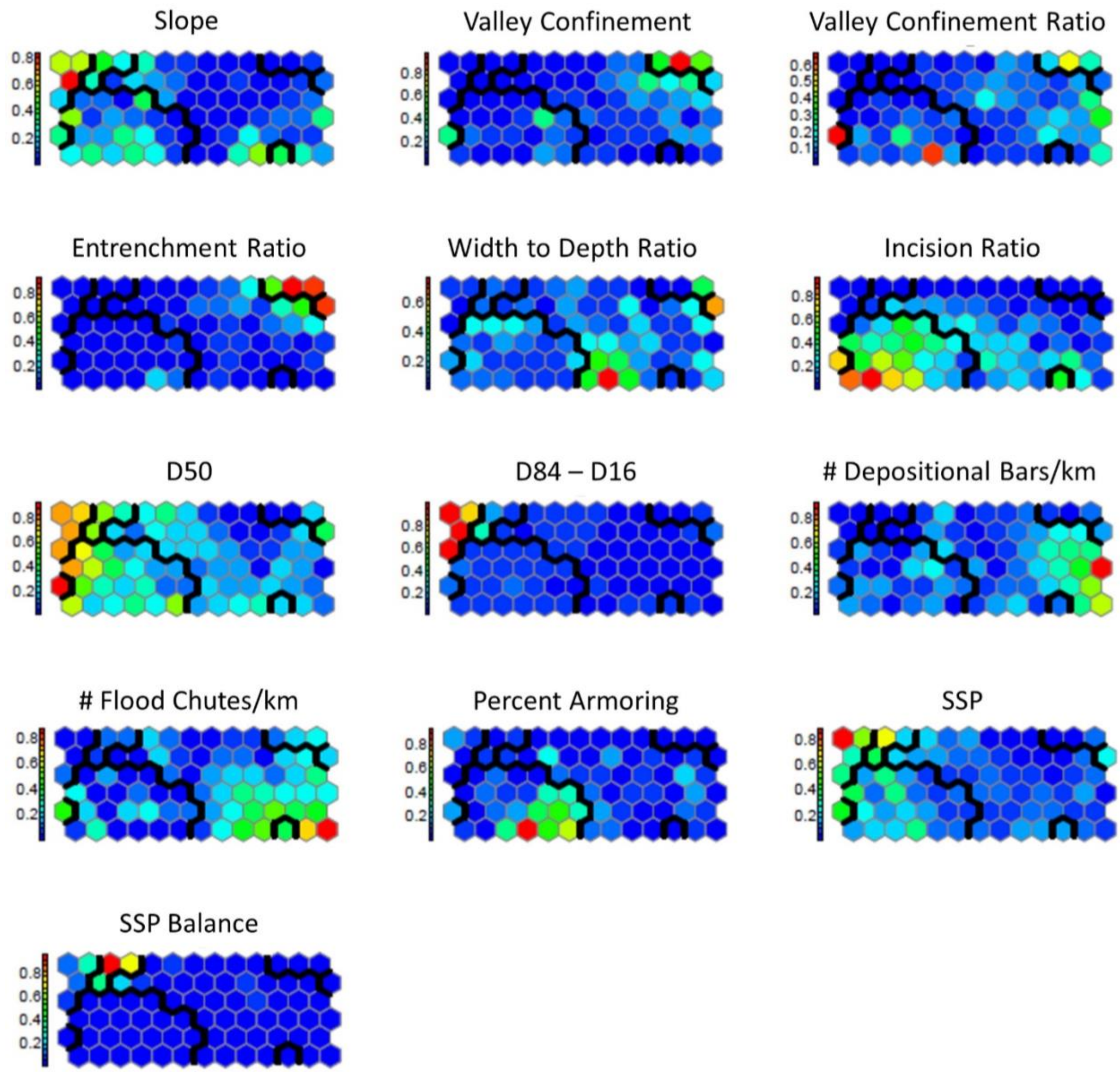


Figure 8. Component planes for each of the input variables to the SOM. Color scheme represents a “heat map” grading from low (cool blue tones) to high (warmer red tones) range-normalized values for each independent variable.

19. Discussion

A Self-Organizing Map has been used to cluster multivariate stream geomorphic data for 193 stream reaches in Vermont into sediment transport regimes. Results broadly replicated classifications of Kline (2010) offered in VTANR River Corridor Planning Guide. These classes are analogous to reach-scale fluvial process domains (Montgomery, 1999; Brardonini & Hassan, 2007; Lisenby and Fryirs, 2016), and can be superimposed on the continuum of stream types proposed for montane systems by Montgomery & Buffington (1997).

To infer process domains from large data sets of geomorphic and topographic variables, previous researchers have used parametric, multivariate statistical methods. Brardonini & Hassan (2007) applied multivariate discriminant analysis (DA) paired with principal components analysis (PCA) to channel and floodplain metrics for dimension reduction and classification of process domains, identifying a variation on the downstream continuum of stream types of Montgomery & Buffington (1997), related to legacy glacial landforms in British Columbia. Phillips and Desloges (2015) used k-means clustering, PCA, and DA to analyze geomorphic parameters and classify alluvial channels from a glacially-conditioned setting in southern Ontario. Their analysis (limited to low-gradient, single-thread, channels in unconfined settings) identified four broad channel-floodplain types (corresponding generally to C3, C4, E5, and E6 stream types of Rosgen, 1996). Importantly, their analysis suggested an additional variable (channel entrenchment, or degree of vertical disconnection from the floodplain) as a factor contributing to within-class variability.

The nonlinear, nonparametric clustering algorithm in our study more closely mimics the nonlinear and complex feature interactions at work in the natural environment. The SOM is more robust than traditional parametric methods to correlations that are inevitably present among independent variables. The reduction of multi-dimensional data to two dimensions represented by the lattice in Figure 7, has simplified data analysis, and the examination of component planes for each input variable (in Figure 8) provides insight into which variable (or combinations of variables) may be governing any particular regime. The second dimension of the lattice might suggest regime "departures" in response to increasing channel and floodplain disturbances, or stressors. For example, if we consider a low-gradient, gravel-dominated, riffle-pool reach that has been channelized and dredged, leading to channel incision and floodplain disconnection, the individual component planes for incision ratio and W/D ratio, presented in Figure 8, demonstrate a monotonic trend in the lattice-vertical dimension that is consistent with this idea. The pre-disturbance reach would plot near the top center of the lattice. Upon dredging, this same reach would shift vertically downward on the lattice and left to areas characterized by higher incision ratio and lower W/D ratio. With subsequent widening, this reach could move lattice-right to a region typified by higher width/depth ratios. As channel widening reduces stream competence leading to progressive aggradation, this reach might transition toward a more transport-limited state (i.e., "Fine Source & Transport and Coarse Deposition) – moving lattice-right and -up toward a region characterized by increasing numbers of depositional bars (and lower W/D ratio). The SOM lattice provides a way to explicitly consider and map the geomorphic process domains within the context of stream type (i.e., on the continuum from bedrock to dune-ripple).

The configuration of clusters (i.e., distribution of sediment transport regimes) on the reduced-dimension lattice has informed the conceptual model presented in Figure 9, which extends to the continuum of reach types (Montgomery & Buffington, 1997), by adding the potential influence of channel and floodplain stressors and resultant shifts in sediment transport regime.

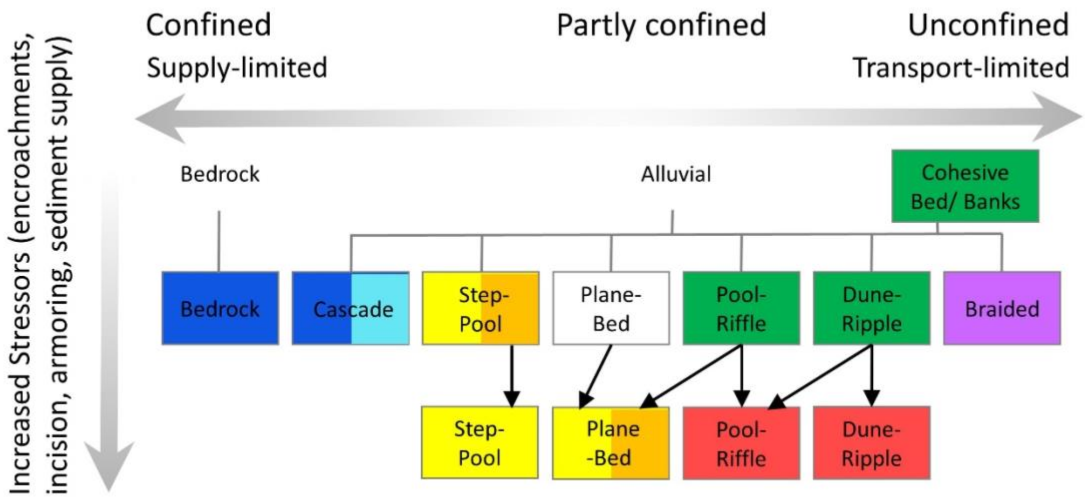


Figure 9. Conceptual model of shift in sediment transport regime and dominant bedform with increasing degree of channel and watershed disturbances, such as dredging, armoring, berming, increased peak flows, increased sediment supply. Color scheme reflects the sediment transport regime classifications presented in Figure 3.

To our knowledge, this current study is the first application of a neural network to examine geomorphic data for a range of stream types and classify reach-based sediment transport regime. Previous work of the PI Rizzo, and collaborator Mike Kline, utilized a hierarchy of ANNs to classify erosion hazard (sensitivity) in river networks using similar data (Besaw *et al*., 2009). PI Rizzo also incorporated a Naïve Bayesian classifier within an SOM architecture for the prediction of instream habitat (Fytilis & Rizzo, 2013).

Future automation of this algorithm, and linkage to the VTANR Data Management System could enable model predictions for many more Vermont river reaches. More importantly, such a framework could facilitate scenario testing – to evaluate how sediment transport regimes of a given reach (or river network) might shift in the event of future channel and floodplain manipulation (or restoration)

20. Training and Outreach

The PIs mentored one graduate student, PhD candidate Kristen Underwood, over the one-year project duration. Underwood honed skills in nonparametric and neural-network computational methods. Data and methods were incorporated in the syllabus for *Applied River Engineering* (CE295) a Civil and Environmental Engineering class developed and taught by Underwood in the Spring 2018 semester. Research results will be published in a peer-reviewed journal and presented at a regional or national conference(s) to disseminate findings to academic and agency personnel in the field of water resources. Results of this research will also be shared with VTANR and VTrans staff and regional stakeholders (e.g., VTANR brown bag series), with a focus on the possible interface of computational tools with the VTANR Stream Geomorphic

Assessment Data Management System (https://anrweb.vt.gov/DEC/SGA/ Default.aspx) and the web-based Natural Resources Atlas (http://anrmaps.vermont.gov/ websites/anra/).

21. Investigator's qualifications.

Donna Rizzo is a Professor in Civil and Environmental Engineering. She is a surface and groundwater hydrologist whose research focuses on the development of new computational tools to improve the understanding of human-induced changes on natural systems and the way we make decisions about natural resources. She has been the PI on two previous Water Center projects.

Mandar Dewoolkar is an Associate Professor in Civil and Environmental Engineering. Through his graduate and post-doctoral research work and industry experience, he has developed significant expertise in the fields of *in situ*, field, and laboratory soil testing, equipment and instrument development and computer-aided slope stability and flow analyses among other types of analytical methods. He has been the PI on three previous Water Center projects.

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DONNA M. RIZZO

(I) PROFESSIONAL PREPARATION

Institution and Location	Major	Degree & Year
University of Connecticut, Storrs	Civil Engineering	B.S., 1984
University of California, Irvine	Civil Engineering	M.S., 1990
University of Vermont (UVM), Burlington	Civil & Environmental Engineering	Ph.D., 1994

(II) APPOINTMENTS

2013-present	**Professor**, **Dorothean Chair**, Civil & Envir. Engr., Univ. of Vermont, Burlington, VT
2008-2013	**Associate Professor**, UVM Civil & Envir. Engineering, Burlington, VT
2005-present	**Adjunct Professor**, UVM Computer Science Deptartment, Burlington, VT
2002-2008	**Assistant Professor**, UVM Civil & Envir. Engineering, Burlington, VT
1995-2002	**Co-founder**, Subterranean Research, Inc., Burlington, VT.
1991-1994	**Research Assistant**, UVM, Burlington, VT. *Research*: site characterization, artificial neural networks, optimal groundwater remediation design, highly-parallel implementation of numerical methods for geohydrological applications.
1992-1995	**Instructor & PC Laboratory Instructor**, Princeton Transport Code Short Course; IBM PC Applications in Ground Water Pollution & Hydrology Short.
1992-1994	**Participating Guest**, Lawrence Livermore National Laboratory, Livermore CA.
1986-1990	**Research Assistant and Graduate Teaching Assistant**, University of California, Irvine, CA. *Research*: mathematical modeling multi-phase flow & transport in unsaturated soils.
1986-1988	**Civil Engineer**, Born Barrett & Associates, Civil Engineering & Consulting, CA.
1984-1986	**Civil Engineer**, State of Connecticut Department of Environmental Protection.

(III) PUBLICATIONS†

Gaddis, E.J.B. A. Voinov, R. Seppelt and **D.M. Rizzo**, "Spatial optimization of best management practices to attain water quality targets", *Water Resources Management*, doi: 10.1007/s11269-013-0503-0, Accepted, 2014.

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† Students Authors are Underlined

(V) Synergistic Activities

Dr. Rizzo has a strong record of accomplishment in applied research and development prior to her tenure-track faculty position:

1. Her experience with optimization methods and groundwater modeling experience using geostatistics, optimization and artificial neural networks (ANNs) led to the procurement of five Small Business Innovation Research (SBIR) grants from the Federal government (NSF, DOE and USDA) during the years she co-founded a small business.
2. She was PI for DOE funded SBIR Phase I and Phase II projects that developed tools for rapid joint inversion and imaging of multiple geophysical and geotechnical data types to characterize subsurface fluids and media (U.S. Patent 6,067,340. "Three-Dimensional Stochastic Tomography with Upscaling", Combines simulation, stochastic filtering, and "data-driven zonation" to improve parameter estimation.)

Undergraduate education research & broadening underrepresented groups in science, math & engineering:

3. Rizzo's commitment to recruit and retain culturally diverse students helped lead to the NSF (DBI) sponsored Environmental Biology Mentoring Program with Co-PI L. Stevens. The 4-yr award supports underrepresented science and engineering students in research activities.
4. PI on Barrett Foundation grant, which provides scholarships to engineering undergraduates (62 students over past 9 years) to pursue research projects under faculty mentorship.

Service to the scientific and engineering community:

5. Worked with the Federal Emergency Management Agency during 1998 and 2012 VT floods.

MANDAR M. DEWOOLKAR
Associate Professor and Program Head of Civil and Environmental Engineering
School of Engineering, College of Engineering and Mathematical Sciences, University of Vermont

(a) Professional Preparation

Institution and Location	Major	Degree & Year
University of Mumbai, India	Civil Engineering	B.E. - 1990
Indian Institute of Technology, India	Civil Engineering	M.Tech. - 1992
University of Colorado at Boulder	Civil Engineering	Ph.D. - 1996
		Postdoctoral – 1997 to 2000

(b) Appointments

07/16-current	Interim Chair, Civil & Environmental Engineering, University of Vermont
09/12-current	Program Head, Civil & Environmental Engineering, University of Vermont
09/09-current	Associate Professor, University of Vermont
09/03-08/09	Assistant Professor, University of Vermont
09/00-08/03	Geotechnical Engineer, GEI Consultants, Inc., Englewood, Colorado
	Adjunct, Colorado School of Mines, Golden, Colorado
	Visiting Lecturer, University of Colorado at Boulder
01/97– 07/00	Research Associate, University of Colorado at Boulder
08/92–12/96	Research Assistant and Teaching Assistant, University of Colorado at Boulder
08/90–07/92	Research Assistant and Teaching Assistant, Indian Institute of Technology

(c) Select Publications

Anderson, I., Rizzo, D. M., Huston, D. R., and Dewoolkar, M. M., “Stream power application for bridge damage probability mapping based on empirical evidence from Tropical Storm Irene”, ASCE *Journal of Bridge Engineering*, in print.

Anderson, I. A., Rizzo, D. M., Huston, D. R., and Dewoolkar, M. M., “Network-wide analysis of over 300 Vermont bridges damaged in Tropical Storm Irene”, *Structure and Infrastructure Engineering*, in print.

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(d) Synergistic Activities

1) Innovations in Teaching: Dr. Dewoolkar has been very active in developing innovative experiential educational modules: (i) Semester-long *service-learning* projects are introduced in his foundations course where students work on historic structures with foundations, retaining structures or slope stability problems. Service-learning projects are also conducted in his capstone senior design course. Thus far, he has mentored over 250 undergraduate students working on about 65 service-learning projects for over 30 different Vermont Towns and non-profit organizations; and (ii) Inquiry-based research projects are conducted in his geotechnical engineering and foundations courses: (a) Atterberg limits tests on the same soil using both Casagrande method and fall cone apparatus followed by statistical analysis to assess the effects of operator variability; (b) Steady state seepage through physical models of earth structures and verify hand-drawn and finite element based (computer program SEEP/W) flow nets and seepage quantities (flow rate, piezometric heads); (c) Construction and analysis of a physical model to design a pervious concrete pavement system; and (d) An instructional centrifuge is used to study undrained slope stability, active and passive lateral earth pressures and shallow foundations to validate Taylor’s stability chart, Rankine’s earth pressure theory, and Prandtl’s bearing capacity solution, respectively – all lead to undergraduate students writing research papers. He also developed formative and summative assessment strategies and reflection exercises for the above educational activities.

2) Training and Broadening Participation of Underrepresented Students: In the past 12 years, over three dozen undergraduate students have worked with Dr. Dewoolkar on various research projects, over a dozen of whom were from underrepresented groups. A total of nine of his undergraduate researchers pursued graduate school. Although majority of these students were from Civil and Environmental Engineering, some were from Historic Preservation, Biology, and Mechanical and Electrical Engineering. He has mentored three female doctoral students and three female Master’s students, five of whom were non-traditional. Nineteen out of 20 of his research graduate students are U.S. nationals.

3) Outreach: Dr. Dewoolkar Developed hands-on modules on (i) slope stability and soil reinforcement for the HELiX/EPSCoR Outreach Program for High School Students and Teachers, and (ii) pervious concrete for high school students attending Transportation Summer Institute at UVM.

Pharm-free surface waters: Identifying barriers and motivators that influence Vermonters' participation in pharmaceutical take-back programs

Basic Information

Title:	Pharm-free surface waters: Identifying barriers and motivators that influence Vermonters' participation in pharmaceutical take-back programs
Project Number:	2017VT88B
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Funding Source:	104B
Congressional District:	Vermont-at-Large
Research Category:	Social Sciences
Focus Categories:	Water Quality, Education, Law, Institutions, and Policy
Descriptors:	None
Principal Investigators:	Christine Vatovec, Alexandra Millar

Publications

1. Millarhouse, A. Z. 2017. What's in Your Body of Water? Reducing the Psychological Distance of Pharmaceutical Pollution through Metaphor in Risk Communication, Master’s Thesis, Rubenstein School of Environment and Natural Resources, The University of Vermont and State Agricultural College, Burlington, Vermont. 112pp.
2. Millarhouse, Alexandra Z., Christine Vatovec, Meredith T. Niles, Adrian Ivakhiv. 2017. What’s in Your Body of Water? Reducing the Psychological Distance of Pharmaceutical Pollution through Metaphoric Framing in Risk Communication. In Preparation.

13. **Title**: Pharm-free Surface Waters: Identifying Barriers and Motivators that Influence Vermonters' Participation in Pharmaceutical Take-Back Programs

14. **Statement of regional or state water problem**: Nationally, a growing body of literature documents the presence of pharmaceutical compounds in ground water (Banzhaf, Krein, & Scheytt, 2011), a majority of surface waters (Kolpin et al., 2002; Lara-Martín, González-Mazo, Petrovic, Barceló, & Brownawell, 2014; Lara-Martín, Renfro, Cochran, & Brownawell, 2015), and untreated (Focazio et al., 2008) and treated (Stackelberg et al., 2007) drinking water. Listed as "contaminants of emerging concern" by the Environmental Protection Agency (Environmental Protection Agency, 2008), aquatic pharmaceutical contamination poses ecotoxicological risks to the environment and human health.

Pharmaceuticals have been discovered in multiple aquatic species (Brandao, Pereira, Goncalves, & Nunes, 2014; Ramirez et al., 2009), including edible species (Antunes, Freitas, Figueira, Gonçalves, & Nunes, 2013); and have been shown to cause reproductive and behavioral effects in fish (Jobling et al., 2006), bivalves (Antunes et al., 2013), and zooplankton (Flaherty & Dodson, 2005). Increasingly found in the drinking water supply (Padhye, Yao, Kung'u, & Huang, 2014), more research is needed to understand the human health effects of pharmaceutical contamination of the water system. Some laboratory studies suggest developmental and chronic exposure to certain synthetic drug compounds may lead to susceptibility to cancer and contribute to tumor formation among humans (Birnbaum & Fenton, 2003).

In Vermont, a preliminary study of pharmaceuticals in surface waters detected 54 compounds directly entering Lake Champlain through the treated effluent of the Main Burlington Wastewater Treatment Facility (WWTF) (Vatovec, Phillips, Van Wagoner, Scott, & Furlong, 2016). Consumer behavior is a principal source of pharmaceutical occurrence in municipal wastewater (Daughton & Ruhoy, 2008). To better understand the sources of pharmaceutical contaminants in Burlington's wastewater, the study timed effluent sampling to coincide with the spring move-out period for UVM students and then surveyed the UVM student population (n=385) to determine students' prescription and over-the-counter medication purchasing, use, and disposal practices. Survey results indicate that 63% of respondents reported having leftover over-the-counter medications in the past year, and 33% had leftover prescription drugs, while only 1% of respondents disposed of their drug waste at an environmentally-preferred drug take-back program. This study was the first time data about how people are purchasing, using, and disposing of leftover medications was correlated with data characterizing the pharmaceutical content of the corresponding municipal wastewater. We leveraged these results to further identify key points of behavioral intervention for minimizing the volume of these compounds in Vermont's surface waters.

Encouraging best practices for disposal of leftover household medications is a sound first step (Glassmeyer et al., 2009) for minimizing the volume of pharmaceutical compounds entering Vermont's surface and ground waters via wastewater and landfill leachate. Consistent with the VT survey data mentioned above, national studies of U.S. consumers indicate a widespread incidence of leftover household drugs which are often reportedly stored in households (Kotchen, Kallaos, Wheeler, Wong, & Zahller, 2009; Seehusen & Edwards, 2006). Stored medications pose an additional risk of ingestion by unintended users (Daughton, 2007). In 2015, 11% of Vermont teenagers (n = 21,013) self-reported consuming a prescription pain reliever or stimulant not prescribed to them (Vermont Department of Health, 2015).

Pharmaceutical collection programs, such as the bi-annual National Drug Take-Back Day (NTBD), offer opportunities for consumers to safely dispose leftover medications to be incinerated, which may result in improved human and environmental health by reducing instances of diversion, accidental exposure, and environmental occurrences (Stoddard, 2012). The U.S. Drug Enforcement Agency collected a cumulative 5,525,021 pounds of drugs from 2010-2015 (U.S. Drug Enforcement Agency, 2015). The previously mentioned survey data indicate the possibility that a much larger volume of unwanted medications is accumulating in consumers' medicine cabinets based on the small percentage of respondents who reported participating in a NTBD. There is interest at both the Vermont Department of Health Drug Abuse Workgroup and Vermont Agency for Natural Resources Pharmaceutical and Personal Care Product Workgroup for developing other pathways for appropriate drug disposal take-back programs.

To be effective, these collection programs will need to successfully address critical barriers and motivators for increasing the participation of Vermont health consumers. In the preliminary study respondents identified the following barriers to participating in take-back programs: being unaware of program, unable to participate on the scheduled date of the event, and uncomfortable with the required police presence at the collection site (Vatovec et al., in preparation). Additionally, previous research suggests people's perceptions about aquatic pharmaceutical contamination and drug disposal may also be a barrier to adopting safe drug disposal practices. Reports show that consumers perceive commonly used medications (e.g. over-the-counter painkillers) to be less environmentally-threatening than unfamiliar drugs (e.g. antiepileptics) (Bound, Kitsou, & Voulvoulis, 2006), and flushing familiar drugs down the drain or toilet is perceived to be unlikely to have harmful environmental impacts (Dohle, Campbell, & Arvai, 2013). And yet, common pain relievers, such as acetaminophen, are one of the most frequently detected classes of pharmaceutical chemicals in the aquatic environment (Dohle et al., 2013) and are highly ecotoxic (Ortiz de García, Pinto Pinto, García-Encina, & Irusta-Mata, 2014).

By identifying attitudes and behaviors towards aquatic pharmaceutical contamination, this project provides better understanding of the barriers and motivators for increasing participation in Vermont take-back initiatives, as well as how to best increase awareness of these programs through strategic communication tools.

15. **Statement of Results and Benefits**: Results from this interdisciplinary research include an assessment of current attitudes and behaviors, as well as, how different pilot messaging influences participants' perceptions of aquatic pharmaceutical contamination and their willingness to participate in a drug collection program. This project informs communication and community outreach strategies encouraging the proper disposal of household drugs through take-back initiatives in Vermont. The Vatovec Lab will apply and expand upon results in current and future research into sources and interventions for aquatic pharmaceutical contamination. Additionally, as our results are published, they are intended to be shared with community partners to improve the effectiveness of local pharmaceutical take-back campaigns, and increase UVM student participation.

16. **Objectives**: Focusing on Vermont, our objectives were to: 1) assess knowledge of and attitudes towards aquatic pharmaceutical contamination, 2) identify current disposal behaviors, and 3) test pilot messaging promoting participation in drug collection programs.
Nature and scope: In this mixed-methods study, we used cognitive interviewing to investigate the barriers and motivators that influence participation in drug collection programs among UVM students. Our sample frame, the UVM student population, was selected for: 1) previous population data indicating a prevalence of stored leftover medications and a lack of participation in drug collection programs (Vatovec et al., 2016); 2) the existence of multiple nearby collection programs; and, 3) the adjacency of Lake Champlain. UVM students compose nearly 25% of the population in Burlington, VT, so being able to

design an effective take-back program for this target audience will be a good start to keeping future pharmaceutical waste out of Lake Champlain.

7. **Methods, procedures, and facilities**: To accomplish our objectives, we (the Graduate Research Assistant) conducted semi-structured interviews with UVM students that involved asking students a series of questions before, during, and after they look at various posters that promote collection programs as a safe drug waste disposal option.

The interview had five parts: 1) background knowledge and baseline levels of concern, 2) responses to the first of two poster advertisements for safe drug disposal, 3) responses to the second poster advertisement for safe drug disposal, 4) current prescription and over-the-counter drug purchasing and disposal practices, 5) environmental paradigm (Dunlap, Van Liere, Mertig, & Jones, 2000), and 6) demographics (the interviewer will not read aloud or ask the subject to explain their responses to the demographic questions).

During the interview, participants were given a sheet of survey-like questions to read along with the interviewer. The interviewer moved step by step through each question, reading the question aloud and asking the participant to select the answer that best fits their response and to briefly describe why they chose that answer.

The interviews were audio-recorded, and took place in one of two conference rooms in the Aiken Center on UVM campus. Audio-recordings were transcribed and coded and analyzed for pattern identification using directed content analysis (Miles & Huberman, 1994).

Analysis: Between and within group results were compared using descriptive statistical analysis and qualitative analysis. A Wilcoxon signed ranks test was used to assess whether any within group changes after the first and second visual treatments were statistically significant at $p < 0.100$. This test assumes a null hypothesis of no change in mean response between pairs. Qualitative data was coded and analyzed using inductive, directed content analysis. Coding was done manually. Analytic memos were used as an additional heuristic. The purpose of analytic memo writing was to enable the transition in analysis from the systematic process of coding to the more formal write-up of the research, leading to development of theoretical insights (Saldaña, 2015).*Following analysis, we completed the final write-up of the research, prepared a manuscript for publication, and presented findings at a graduate thesis defense on May 31, 2017.*

18. **Findings**:

Background knowledge and current purchasing, use and disposal behavior

Questions assessed whether participants had previously heard of the issue of aquatic pharmaceutical contamination and how informed they felt, as well as their current drug purchasing, use and disposal behaviors (adopted from Vatovec, 2016).

Background Knowledge: Overall, a majority of participants (65%) had previously heard of the issue of pharmaceuticals in the water (30% had never heard of the issue before and 5% were unsure). Of those who were aware of it, most cited hearing about the issue in an academic context (40%). Others heard about it from news media (10%), a doctor (5%), family members (5%), or because they had disposed of their own medication via a drain (5%). Importantly, only half of the participants in group A had previously heard of the issue (versus 80% of Group B). More than half of all respondents (55%) tended to or strongly disagreed that they felt informed about the issue; while less than half (45%) tended to or strongly agreed that they felt informed.

Current purchasing, use and disposal practices of over-the-counter (OTC) medications: In the past 12 months, nearly all (95%) respondents had purchased OTC medication (5% had not). Of those who had purchased OTC medications, 10% used all of their purchased medication(s) and had none leftover, whereas 30% were still using their medication(s) and 60% did not use all of their medication(s) and had some leftover. The majority of people (60%) said they still had their leftover medication(s) and 20% said they gave them to a friend or family member. Respondents were asked to share if and how they disposed of medication in the last 12 months. A majority (65%) said they did not throw any OTC drugs away in the past 12 months, 25% said they threw them out in the garbage and 5% said they flushed their drugs down the toilet. No one took OTC medication(s) to a National Drug Take-Back Day.

Current purchasing, use and disposal practices of prescription medications: A majority of respondents (75%) said they had purchased prescription medication(s) in the past 12 months, although fewer people bought prescription medication than OTC medication. A quarter of participants did not purchase any prescription medication in the past 12 months. Unlike those who had purchased OTC medications, most people (30%) had used their prescription medication and there was none left. Twenty five percent were still using the medication(s) and 20% did not use all of their medication(s) and had some leftover. Although most people (70%) did not dispose of prescription medications in the past 12 months, of those who said they did, 10% threw them out in the garbage, 5% threw out an empty prescription medication bottle after the medicine had been used and 5% flushed their medication down the drain. No one took prescription medication(s) to a National Drug Take-Back Day.

Drug collection programs: Most people (55%) had heard of drug collection programs in the past (45% had not heard of these programs in the past). However, only 10% had taken unneeded medications to a drug collection program (90% had not). Interestingly, when asked why they had not taken medications to a collection program, those who had heard of it said that they never had medication to throw out (35%), didn't know when or where it was held (15%), couldn't make it to the time or location where it was held (10%), did not feel comfortable taking drugs there (5%), or just forgot to utilize the resource (5%).

Contrasting with the results above, during earlier sections of the questionnaire assessing perceptions, attitudes and behaviors towards aquatic pharmaceutical contamination, at least 20% of people mentioned that they take little or no medication when considering if they felt prepared to participate in a take-back program (although 95% and 75% of participants had purchased OTC and prescription medications, respectively, in the last 12 months). Additionally, at least 65% of people said they did not know what a pharmaceutical take back initiative is and were unfamiliar with the concept, while 15% had heard of the concept but were unfamiliar with this name for it. For example, one participant asked, "what is a pharmaceutical take back initiative?" Then, after receiving an explanation, they said, "Oh, I knew that… I feel like I've just heard of people being able to bring their meds back in, I didn't know it had a name." Note: in another section of the interview, most people (55%) agreed that they had heard of drug collection programs in the past.

Initial perceptions of psychological distance, concern and willingness to act

Overall, people perceive the issue of pharmaceuticals in the water as more geographically and socially distant (happening in other places to other people) and are more concerned about distant geographic and social impacts (e.g. concern for distant people and places). People perceive the issue as certain (versus uncertain) and believe it to be temporally both distant and proximal (happening presently and in the future). They are equally concerned about the issue in the near and far future. While people agree that they are motivated and prepared to participate in take-back programs, they feel more motivated than prepared, on average.

Effect of metaphoric framing on perceptions of, concern and willingness to act on pharmaceuticals in the environment

Perceptions: After viewing the metaphor visual, group A participants perceived aquatic pharmaceutical contamination as geographically ($p = .083$) and socially ($p = .034$) significantly closer than their baseline. Group A also expressed increased certainty about scientists' knowledge of the issue and increased agreement that the effects will be temporally close, although these were not significant. Treatment group B, who saw the non-metaphor treatment first, more strongly agreed that the issue was distant across social and temporal dimensions (happening to other people in the far future), compared to their baseline. Exposure to the non-metaphor treatment had no significant impact on initial perceptions.

Concern: Representing the issue through metaphor had no direct, statistically significant effect on treatment group A's initial levels of concern, although overall concern increased. The non-metaphor treatment significantly increased treatment group B's concern for geographically distant impacts ($p = .083$), compared with their baseline responses. In general, this treatment also increased concern, although no other change was statistically significant.

Behavior: Metaphor use had no direct, statistically significant impact on group A's behavioral intentions, although people felt equally prepared and motivated to participate in a drug collection program (versus initially being more motivated than prepared). The non-metaphor visual also had no significant effect on group B's behavioral intentions. People continued to feel more motivated than prepared.

19. Discussion: Pharmaceutical contaminants pose a range of risks to ecosystems and public health that range from reproductive failure in aquatic species (Kidd et al., 2007; Nash et al., 2004; Pettersson & Berg, 2007) to the development of pathogen resistance to antibiotics (Jones, Voulvoulis, & Lester, 2004). The USGS conducted two studies at the Main Burlington WWTF on the effect of combined sewer overflow (CSO) events on the loading of chemicals including pharmaceuticals into Burlington Bay (Phillips & Chalmers, 2009; Phillips et al., 2012). Findings from those studies show that the concentration of various pharmaceutical compounds can be up to ten times greater during CSO events, showing that the wastewater treatment process is capable of effectively removing at least some of these compounds before WWTF effluent is released into surface waters.

However, aging WWTF were designed to remove solids from the wastewater stream—not chemical contaminants (Tambosi, Yamanaka, Jose, Moreira, & Schroder, 2010). As a result, pharmaceuticals are increasingly being detected in surface waters (Cunningham, Perino, D'Aco, Hartmann, & Bechter, 2010; Doerr-MacEwen & Haight, 2006; Ferguson, Bernot, Doll, & Lauer, 2013) as well as drinking water (Leal, Thompson, & Brzezinski, 2010; Padhye et al., 2014). The primary sources of pharmaceuticals in municipal wastewater are human consumption (i.e. excretion or washing off topical medications during bathing) and disposal of unwanted medications (Daughton & Ruhoy, 2008).

Therefore, a practical first step in reducing the risk of aquatic pharmaceutical contamination is to address consumer attitudes and behavior, including increasing participation in drug collection programs, the environmentally-preferred disposal practice. This project was conducted to improve understanding of points of intervention that may minimize pharmaceutical contaminant loading in Vermont's surface waters by exploring social factors influencing the movement of these compounds through our social-ecological landscape.

Our findings suggest that most of the sampled population are aware of the issue of aquatic pharmaceutical contamination, as well as the existence of drug collection programs. Additionally, consistent with Vatovec et al. (2016), most people purchased and consumed OTC and/or prescription medications in the last 12 months and chose to store (rather than dispose of) leftover medications. These findings reinforce the importance of understanding how people perceive the issue relative to themselves and their actions, since awareness and education alone may not change individual behaviors and attitudes. It also suggests the utility of developing communications for drug collection programs that effectively target university students.

Baseline perception results indicate that participants more strongly agreed that pharmaceutical pollution is a distant geographic and social issue (which does not preclude or conflict with the belief that local areas and people will also be impacted) and expressed higher levels of concern for the issue at greater geographic and social distances. The perception that the issue is more likely to impact other places and people may be due to spatial bias (environmental problems are believed to be worse at global versus local levels (Uzzell, 2000), especially by younger and happier people (Schultz et al., 2014), and/or spatial optimism (environmental conditions are better here than elsewhere) (Gifford et al., 2009; Milfont et al., 2011). These cognitive biases have implications for behavior. Believing environmental problems to be more severe at a global level can lead to decreased feelings of self-efficacy (feeling able to do something about the problem) and responsibility for the problem (Uzzell, 2000), which in turn discourages public engagement. Likewise, our baseline results indicate people felt more motivated (a value-driven, high-level construal) than prepared (a low-level construal motivated by feasibility concerns) to participate in pharmaceutical take-back initiatives, which may be connected to perceptions that aquatic pharmaceutical contamination is a distant issue.

Results indicate that metaphor use shifted perceived distance of the issue from more geographically and socially far to more proximate. Further, the majority of respondents preferred the metaphor visual to communicate about drug take-back programs. Previous research indicates positive associations between perceived proximity and concern for and willingness to act on an issue (Haden et al., 2012; Jones et al., 2016; Niles et al., 2013). As well, others have found that more proximate issues activate preparedness to act. While metaphor use did not directly affect concern or willingness to act, recent research suggests perceived distance mediates the impact of message frame manipulations, like metaphoric framing, on concern and behavioral intentions. Jones et al. (2016) found that framing messages to manipulate (reduce) perceived distance indirectly increased concern and willingness to act, but had no direct, statistically significant effect on either construct. According to Rabinovich et al. (2009), reducing perceived distance may be especially critical when specific individual actions are needed to achieve a relatively abstract goal, like participating in a drug collection program to reduce aquatic pharmaceutical contamination, which cannot be detected through the senses. Therefore, risk communication efforts to bring this issue closer may indirectly lead to greater concern and preparedness to act at an individual level.

20. **Training**: This project trained one Master's level graduate student in the Natural Resources program at the University of Vermont. Mentored and advised in social science research methods by Vatovec (PI), this student was responsible for designing and implementing each stage of the project, including project

conception. Finally, Vatovec teaches undergraduate-level courses at UVM and has incorporated information from this project into her coursework.

21. **Investigator's qualifications** (please see attached resume for further information): Christine Vatovec, PhD, is a Research Assistant Professor with appointments in both the Rubenstein School of Environment and Natural Resources and the College of Medicine at the University of Vermont. Dr. Vatovec is an environmental health social scientist with a major research focus on the environmental and public health consequences of medical care. Through her previous work, pharmaceutical contamination arose as one of the primary impacts of medical care on the environment. As a result, Dr. Vatovec has recently shifted her focus to examining the question of pharmaceutical contamination in Lake Champlain. In 2014, Dr. Vatovec recruited Dr. Patrick Phillips, USGS, as a collaborator to investigate the types and volume of pharmaceuticals entering Lake Champlain via the Main Burlington WWTF. Results from that study include one recent publication (Vatovec et al., 2016), as well as two others that are in preparation for publication.

Graduate Student's qualifications: Alexandra Millar completed her M.S. in UVM's Natural Resources program, concentrating in Environment, Society and Public Affairs in July 2017. She was a Graduate Student Fellow at the Gund Institute for Ecological Economics, and was recognized for her excellence in scholarly advancement and contributions to the school, receiving the 2016 Master's Student Award for Outstanding Research & Scholarship from the Rubenstein School, and the Outstanding Service Learning Teaching Assistant Award from UVM's Office of Community-University Partnerships & Service Learning. She maintained a 4.0 GPA across a broadly interdisciplinary array of coursework and completed a Certificate in Ecological Economics. She also served as the Vice President/Aiken Chair of the Rubenstein School Graduate Student Association (RGSA) for the 2015-16 academic year.

22. Theses, Presentations, etc.:

- Millarhouse, A. Z. (2017). *What's in Your Body of Water? Reducing the Psychological Distance of Pharmaceutical Pollution through Metaphor in Risk Communication* (Master's Thesis, The University of Vermont and State Agricultural College).
- Millar, A. Z. (2017, May). *What's in Your Body of Water? Reducing the Psychological Distance of Pharmaceutical Pollution through Metaphoric Framing in Risk Communication.* Master's Thesis Defense Seminar, Burlington, VT.
- Millarhouse, A.Z., Vatovec, C., Niles, M.T., Ivakhiv, A.. (2017). What's in Your Body of Water? Reducing the Psychological Distance of Pharmaceutical Pollution through Metaphoric Framing in Risk Communication. *In Progress.*

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Global to local assessment of cyanotoxins in fish

Basic Information

Title:	Global to local assessment of cyanotoxins in fish
Project Number:	2017VT89B
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Principal Investigators:	Jason Stockwell, Todd S Miller

Publications

1. Flores NM, Miller TR and Stockwell JD (2018) A Global Analysis of the Relationship between Concentrations of Microcystins in Water and Fish. Front. Mar. Sci. 5:30. doi: 10.3389/fmars.2018.00030
2. Flores, N. M., Miller, T. R., and Stockwell, J. D. (2018). Concentrations of cyanotoxins in fresh water and fish. Environmental Data Initiative doi: 10.6073/pasta/0c250018ee7732d984be74e2043b84b4
3. Flores, N. M., T. R. Miller & J. D. Stockwell (2017) "Cyanobacteria Toxins in Fish: A Global Analysis". Student Research Conference, UVM, Burlington, VT. [poster presentation]
4. Flores, N. M., T. R. Miller & J. D. Stockwell. (2017) "Cyanotoxins in Fish: A Global Analysis". 9th US Harmful Algal Bloom Symposium, Baltimore, MD. [poster presentation]
5. Flores, N. M., T. R. Miller, J. Kraft & J. D. Stockwell. (October 2018). "Cyanobacteria bloom impacts on fish: Insights from an ongoing study at a shallow, hypertrophic lake in Vermont (USA)". 18th International Conference on Harmful Algae, Nantes, France. [poster presentation]

1. **Title**. Global to Local Assessment of Cyanotoxins in Fish

2. **Statement of regional or State water problem**. **Include an explanation of the need for the project, who wants it, and why.**

Cyanobacteria pose a serious public health concern due to their ability to produce toxins (i.e. cyanotoxins) with effects ranging from skin reactions and tumors to liver cancer and neurological disorders (Codd 2000; Hitzfeld et al. 2000; Koreivienė et al. 2014). Microcystins are among the most prevalent cyanotoxins globally. Exposure to high doses causes acute liver failure whereas chronic exposure to low doses has been associated with liver disease, cognitive disorders, reproductive toxicity, and cancer (Agudo et al. 2006; Maidana et al. 2006; Chen et al. 2011; Zhang et al. 2011; Wang et al. 2013; Li et al. 2014). Cylindrospermopsin is another liver and kidney cyanotoxin of emerging importance as it is produce by the invasive cyanobacterium *Cylindrospermopsis raciborskii* (Ohtani et al. 1992; Sinha et al. 2012). Cyanobacteria also produce several peripheral neurotoxins. One of the most commonly detected and/or monitored is anatoxin-a which binds to muscarinic acetylcholine receptors causing over activation of these neuronal synapses, convulsions, eventual desensitization, and heart failure (Kofuii et al. 1990). Anatoxin-a(s) has similar net effects, but is structurally unrelated to anatoxin-a and instead prevents the breakdown of acetylcholine by binding to and inhibiting the action of acetylcholinesterase (Mahmood & Carmichael 1986). Several unique analogs of saxitoxin, otherwise known as paralytic shellfish toxins, have been described in *Lyngbya* cyanobacteria from Lake Erie (Carmichael et al. 1997). *C. raciborskii* with a growing prevalence in temperate areas of the U.S. is also capable of producing saxitoxins. Recent research has associated diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's and Parkinson's to certain amino acids produced by cyanobacteria (Cox et al. 2016; Otten & Paerl 2015). The cyanobacterial toxin β-N-methylamino-L-alanine (BMAA) is the leading suspect surrounding high incidences of ALS in the proximity of Lake Mascoma in Einfield, NH where the presence of the cyanotoxin was detected in fish and air samples (Banack et al. 2015; Caller et al. 2009). While these associations are still under investigation, other neurotoxic amino acids are also produced by cyanobacteria including 2,4-diaminobutyric acid (Jiang et al. 2012). Finally, cyanobacteria produce hundreds of other classes of bioactive compounds (e.g. cyanopeptolins, and anabaenopeptins) that produce varying effects in eukaryotic cells including anti-microbial, ani-cancer, and blood pressure lowering compounds. While many of these are explored for their potential medicinal benefits, the toxicology and ecological consequences of these compounds in nature and threats to human health individually or in mixtures is not well studied. Indeed, some of have recently been shown to be toxic to fish (Gademann et al. 2010; Faltermann et al. 2014). All of these compounds are potentially taken up by fish tissues and could be accumulated within certain organs and/or proteins.

In Vermont (and elsewhere), cyanoHABs are a familiar sight in shallow lakes including our two study sites, Shelburne Pond (Ferber et al. 2004; Stockwell, unpublished data) and Lake Champlain (Smeltzer et al. 2012, Isles et al. 2015). CyanoHABs have prompted beach closures (Lake Champlain Basin Program 2015), reduced property values (Rathke 2015), and are a cause of concern for ecosystem function (Gearhart et al. 2016). Over the last two decades, pet deaths have been attributed to cyanotoxin poisoning after drinking contaminated water from Lake Champlain, bringing the issue of cyanotoxins into focus. The state has developed a monitoring program for both visual detection of blooms and weekly cyanotoxin analysis of drinking water from 12 municipalities from around Lake Champlain (http://healthvermont.gov/enviro/bg_algae/weekly_status.aspx).

While avoiding direct contact with waters containing cyanoHABs reduces the risk of exposure to any toxins that might be in the water, no information exists about the potential for exposure through consumption of fish from Vermont water bodies. Studies from across the globe have found evidence of cyanotoxins in freshwater fishes (e.g., Amé et al. 2010; Amrani et al. 2014; Papadimitriou et al. 2013; Trinchet et al. 2013) and contamination of food is becoming a growing concern (Ibelings & Chorus 2007; Meneely & Elliott 2013). However, despite the volume of research on cyanotoxins in water and fish, no study has tested the hypothesis that cyanotoxins in the water and cyanotoxins in the fish are positively correlated.

We proposed to (1) conduct a data synthesis of cyanotoxins in water and fish from lakes around the world, and (2) generate baseline information on cyanotoxin presence in concurrently-collected water and fish samples in two Vermont lakes. Our project allowed us to answer, or begin to address, four main questions. Do fish cyanotoxin concentrations rise with increasing water toxin concentrations when data are examined at a global scale? Where do fish from our two Vermont study lakes fall on the global cyanotoxin scale? Do these Vermont fish follow a similar trend to global patterns? Are cyanotoxins in our Vermont study lakes at a concentration that could pose a risk for human exposure to toxins with fish consumption? The proposed research is the first two-years out of a 4-year PhD project, and we requested funds to support the second year of this project.

Our effort to assess cyanotoxins in fish is of high interest to the Vermont Department of Health, the Vermont Department of Environmental Conservation, and the Vermont Department of Fisheries and Wildlife. Furthermore, many local stakeholders support tracking toxicity in fish as a management goal in Lake Champlain (Smyth et al. 2009).

3. **Statement of results or benefits**. **Specify the type of information that is to be gained and how it will be used.**

The first part of our project compiled available data across the globe in a single database to provide a summary analysis of the relationship between cyanotoxins in freshwater systems and fish from those same waters. Assembling and analyzing data from across lake types and regions provides a broader and novel view of a potential pathway for cyanotoxins to enter humans. The second, and local, component of our project provides state agencies and public citizens a first view of cyanotoxin levels in fishes of Vermont, and when combined with the first component, an assessment of where cyanotoxin concentrations in fish from our study sites fall within the global spectrum. If we find expected positive relationships between cyanotoxins in water and fish in our global analysis, and if our analysis of water and fish samples from our Vermont sampling sites are consistent with the global analysis, the results could be used to predict potential cyanotoxin levels in fish populations across Vermont based on water samples. Our work may thus help focus management efforts to areas where frequent cyanobacteria blooms might be an additional issue for the angling public, by linking water quality to fish and public health.

Finally, because we tracked ancillary information such as types of methods used for cyanotoxin analysis and amount and type of fish tissue analyzed, we will be able to assess differences among methodologies and provide suggestions for analytical techniques, possible caveats about comparing results from different methods, and representation or under-representation of certain types of cyanotoxins in the literature and where future needs may be. The data we compiled from the literature was made publicly available for others to conduct analyses, providing a potential broader impact beyond our own research (data available on DataONE.org through the Environmental Data Initiative).

4. **Nature, scope, and objectives of the project**, **including a timeline of activities.**

Research on cyanobacteria and cyanotoxins has increased dramatically in the last two decades as a result of the increase and expansion of harmful algal blooms around the world (Carmichael 2001; Merel et al. 2013). The expectation that cyanobacteria blooms will increase with climate and land use change (Jöhnk et al. 2008, Paerl and Huisman 2008, Elliott 2010, Brookes and Carey 2011, O'Neil et al. 2012) further exacerbates the need to understand the ways in which cyanobacteria will impact natural and human systems. We used a literature-based and field-based approach to address two objectives: (1) to test the hypothesis that cyanotoxin concentrations in fish increase with cyanotoxin concentrations in water using data mined from the literature, and (2) to establish a baseline of cyanotoxin concentrations in fish tissues across a gradient of cyanobacteria conditions in two productive Vermont freshwater lakes

Our field approach includes collection of water and fish samples from Lake Champlain and Shelburne Pond to examine spatial and temporal changes, respectively, in cyanotoxin concentrations in fish and relate them to water concentrations. Targeted fish species collected from both locations included edible-sized yellow perch (*Perca flavescens*), a popular target of anglers and a popular fish for human consumption, and golden shiners (*Notemigonus crysoleucas*), a species low on the food chain that directly eats phytoplankton and zooplankton. Because each species' place in the food chain differs, we can ask additional questions, such as do toxins concentrations differ between yellow perch and golden shiners, and can this be attributed to different routes of exposure to toxins (i.e., direct exposure versus indirect exposure through the food chain)? Because yellow perch are commonly eaten by people, it is essential to know if they contain cyanotoxins that maybe be consumed by humans, and the relative risk this may pose to the citizens of Vermont, surrounding states, and travelers who may consume fish from these systems.

Timeline:

June-October 2016 [All completed]: PhD student Natalie Flores began graduate studies at UVM. Started to review literature and mine data for global analysis. Weekly water and monthly yellow perch and golden shiner samples collected from Shelburne Pond. Samples shipped to Miller Lab at UW-Milwaukee. Laboratory processing of samples started.

August 2016 [All completed]: Yellow perch samples collected from three sites across Lake Champlain (Missisquoi Bay, St. Albans Bay, and Mallets Bay) and samples shipped to Miller Lab at UW-Milwaukee.

October 2016-December 2016 [All completed]: Continue and complete laboratory processing of samples and complete data mining.

January 2017-May 2017 [All completed]: Analyzed and completed global data set analysis, completed first manuscript, which was submitted to a peer-reviewed journal. Begin data analysis of Shelburne Pond and Lake Champlain toxin data.

June-September 2017[All completed]: Collect second year of weekly water and monthly fish samples from Shelburne Pond from June-October. Ship samples to Miller Lab at UW-Milwaukee. Begin laboratory processing of second-year samples. Manuscript revision and publication of global data set. Continue data analysis of Shelburne Pond and Lake Champlain toxin data from first year.

September-December 2017[All completed]: Continue laboratory processing of samples. Begin analysis of Shelburne Pond toxin data from second year. Begin draft of manuscript on toxin analyses (second manuscript).

January-May 2018 [in progress]: Complete analysis of toxin data, complete manuscript and submit to peer-reviewed journal, present research at conference.

June-August 2018: Manuscript (second one) revision and publication. Archive data in public repository such as DataONE.

5. **Methods, procedures, and facilities**. **Provide enough information to permit evaluation of the technical adequacy of the approach to satisfy the objectives.**

Global Data Analysis

To investigate the relationship between water cyanotoxin concentration and cyanotoxin concentration in fish, data were mined from primary and gray literature that reported cyanotoxin concentrations in fish and the water from which they were taken. Online searches through Web of Science and Google Scholar were used to find relevant literature. Key words including, but not limited to "cyanobacteria", "cyanotoxin", "toxin", and "fish" were used in the search engines in various combinations with truncation or wildcard symbols. Concentrations of water toxins and toxins in fish were recorded, along with other information when available including fish species and trophic level, tissue types, lake trophic status, lake depth, and lake size. In cases where only fish toxins were presented, we also recorded these data. Only studies that reported data from the natural environment were used (i.e., not aquaculture facilities). When data of interest were only reported in graphical format, we used WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/) to extract data from figures. We used appropriate statistical analyses to test our hypotheses of relationships between cyanotoxin concentrations in water and fish. We considered linear and nonlinear regression and analysis of covariance as starting points for our analyses, however the data did not conform to any parametric assumptions (normality, homoscedasticity) and sample sizes were sometimes low for subsets of the data (i.e., data was subset by toxin type & toxin analysis method). Instead, we used nonparametric bootstraps to conduct our statistical testing. Statistical analyses of the data were conducted in RStudio version 1.0.136 (R Core Team, 2016).

Field Study

Sampling: In 2016 we collected samples to assess temporal and spatial patterns in the relationship between cyanotoxins in water and fish. Water samples were collected each week and fish samples (yellow perch and golden shiners) were collected each month from June through

October in hypertrophic Shelburne Pond to assess temporal trends (i.e., before, during and after cyanobacteria blooms). In Lake Champlain, water and fish (yellow perch) samples were collected once in late August from each of three locations that spanned an expected gradient in bloom conditions (Missiquoi Bay and St. Alban's Bay which typically experience blooms, and Malletts Bay which typically does not experience blooms and is considered oligo-mesotrophic; Smeltzer et al. 2012). Water samples were placed in 125 mL amber Nalgene bottles on ice while out in the field, then frozen and stored in a -20°C freezer until further processing. Yellow perch and golden shiners were collected from Shelburne Pond using gill nets, fyke nets, and angling. Ten yellow perch of edible size (around 200 mm) and 10 golden shiners were targeted during each sampling event. We targeted 10 yellow perch of edible size from each site in Lake Champlain using gill nets and angling. Upon capture, all fish were placed in an ice water bath in the field. Immediately upon return from the field, fish were measured for length, weighed, and dissected to determine sex and retrieve tissues, including liver, muscle, and brain. We collected a total of 48 yellow perch, 32 golden shiners, and 40 water samples from Shelburne Pond, and 29 yellow perch and 12 water samples from Lake Champlain in 2016.

Bloom conditions were relatively weak in 2016 compared to previous years (Fig. 1). Consequently, we proposed to collect a second year of samples from Shelburne Pond in June-October of 2017 to facilitate a cross-year comparison. We used only gillnet sampling to standardize collection methods in 2017. We collected 46 water samples and 103 fish from Shelburne Pond in 2017, including 53 perch and 50 shiners.

Cyanotoxin Analyses: Cyanotoxin identification and quantification for all water and fish samples were conducted by Dr. Todd Miller at the University of Milwaukee Zilber School of Public Health. The Miller Laboratory contains state-of-the-art facilities to conduct toxin and other analyses, including an AB Sciex 4000 qTrap tandem mass spectrometer with an attached Shimadzu Prominence HPLC. The system is designed for LC/MS/MS analyses and allows concurrent qualitative and quantitative analyses. Various compound libraries are available for fast method development and screening of samples for unknown compounds. In addition to the LC/MS/MS system, the laboratory contains a nitrogen gas generator, ventilation hood and nitrogen gas solvent evaporation unit, an HP5890 gas chromatograph with flame ionization and electron capture detectors, a Teledyne total organic carbon analyzer and a Dionex ion chromatograph. Cyanotoxins measured in water and fish tissues included the hepatotoxins and neurotoxins: Nodularin; Microcystin-RR; Microcystin-YR; Microcystin-LR; Microcystin-LA; Desmethyl-LR; Microginin-690; Cyanopeptolin-1020; Cyanopeptolin-1041; Cyanopeptolin-1007; Anabaenapeptin-F; Anabaenapeptin-A; Anabaenapeptin-B; 2-methyl-3-methoxy-4-phenylbutyric acid; Cylindrospermopsin; Homoanatoxin-a; Anatoxin-a; Tetrodotoxin; dcGTX-2; dcGTX-3; dcGTX-2,3; GTX-2b; GTX-3b; dcNEO; dcSTX; STX; NEO-b; Beta-N-methylamino-L-alanine; Beta-amino-N-methyl-alanine; 2,4-diaminobutyric acid; N-(2-aminoethyl) glycine; and 2,6-diaminopimelic acid.

Statistical Analyses: We will use analysis of covariance to determine if there are differences in cyanotoxin concentrations between trophic levels (yellow perch vs golden shiners) or among the different tissues (brain, liver, and muscle) while using toxin concentration in water as the covariate. If necessary, data will be transformed to meet assumptions of ANCOVA.

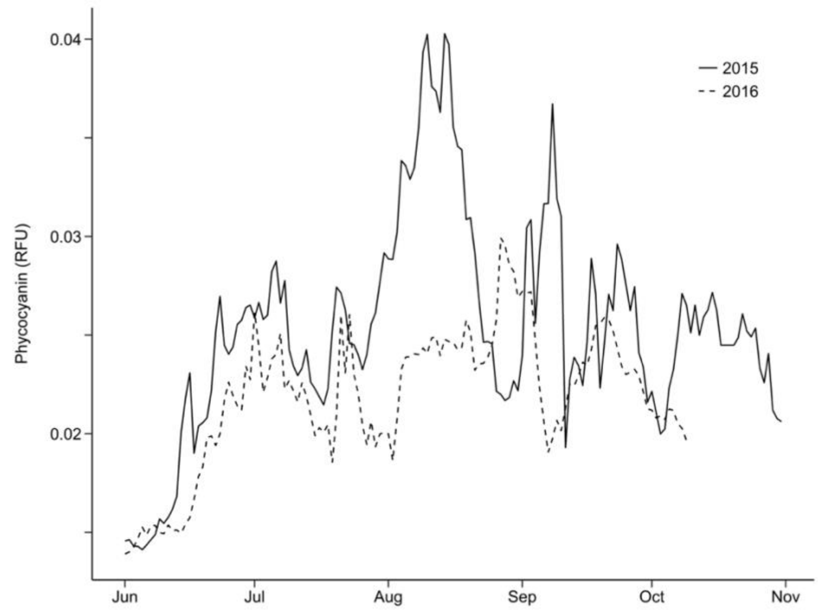

Figure 1. *Relative frequency units (RFU) of the cyanobacteria pigment phycocyanin recorded continuously from a sensor buoy in Shelburne Pond in 2015 and 2016.*

6. **Findings**

Global Data Analysis

We found fifty-eight studies (from 99 bodies of water, including lakes, ponds, and reservoirs) that met our data criteria. Most studies (90%) were conducted on the liver toxins microcystins. Some studies also tested for other toxins, including liver toxins (cylindrospermopsin) and neurotoxins (BMAA, DABA, anatoxin-a, and saxitoxin). Research questions were only addressed for microcystins (MCs) because data on the other toxin types were too sparse for statistical analyses. However, we provide a qualitative summary of all toxins and their concentrations in wild, freshwater fish tissues from the assembled global data (see Table 2 in Flores et al., 2018). Up to ten different methods have been used to test fish for specific cyanotoxins. Consequently, the results of our analysis depended on the method used to test for toxins. For some methods, water bodies with higher levels of MCs in the water also had higher levels in fish (positive relationship). For other methods, no clear relationship existed between MC levels in the fish and MCs in the water. Omnivorous fish accumulated the highest concentrations of MCs regardless of the method used to test for the toxins. Patterns of accumulation between different organs could not be generalized across methods because different results were obtained depending on the analysis method. The full dataset is published in the Environmental Data Initiative data repository (https://portal.edirepository.org/nis/mapbrowse?packageid=edi.160.2).

Field Study

We completed analyses of all water samples (both years) and all fish samples from 2017. We are currently re-analyzing the 2016 fish samples because we have since improved our tissue extraction methods. Statistical analyses will be conducted as indicated in the methods (section 17, above) when all laboratory analyses are completed.

We detected multiple cyanobacteria peptides in Shelburne Pond water samples throughout the entire sampling period in each of 2016 and 2017, including microcystins (Figure 2),

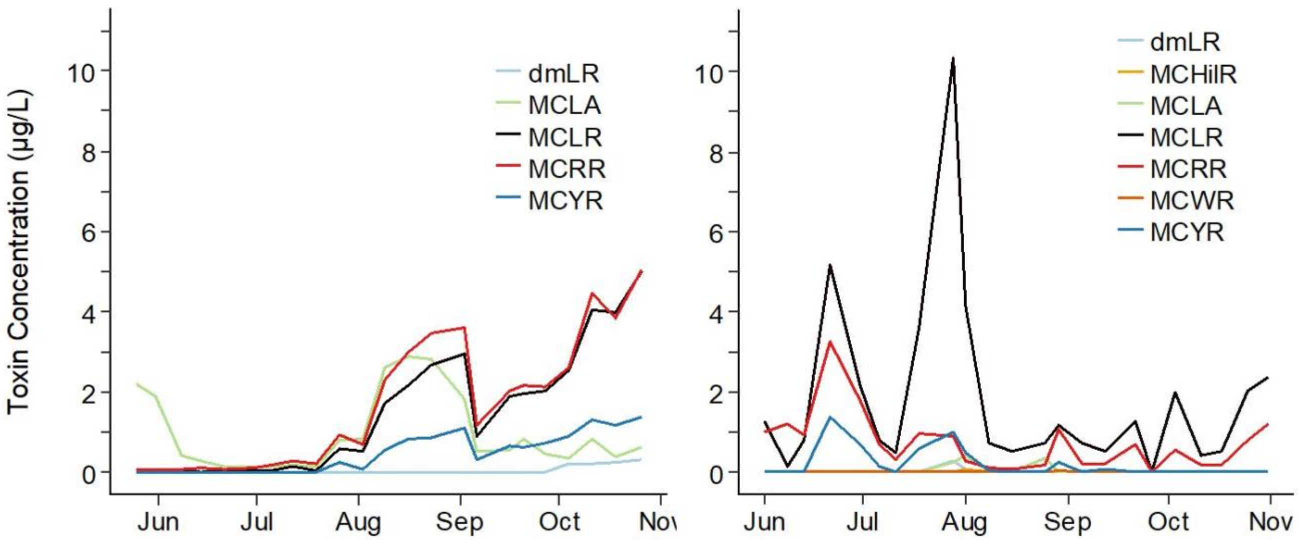

Figure 2. *Microcystin congeners present in Shelburne Pond in 2016 (left) and 2017 (right).*

anabaenopeptins (Figure 3), and cyanopeptolins (Figure 4). Cyanopeptolins were only detected in 2017. Concentrations of the detected cyanotoxins were generally higher in 2017 compared to 2016 with a few exceptions. We also detected many peptides simultaneously in the sampled fish tissues from 2017 (Table 1). Cyanotoxins were detected most often in the liver, followed by the muscle, with highest concentrations in the liver. Toxins were detected more frequently in yellow perch than golden shiners.

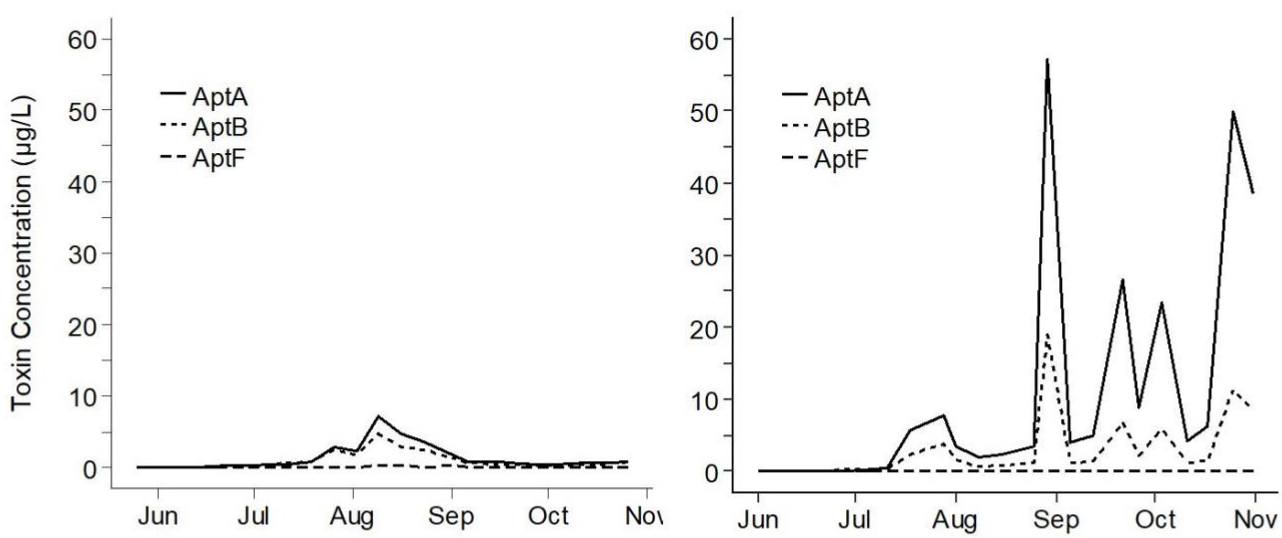

Figure 3. *Anabaenopeptins in Shelburne Pond in 2016 (left) and 2017 (right).*

Table 1. *Detected concentrations of cyanotoxins in Shelburne Pond golden shiners and yellow perch in 2017.*

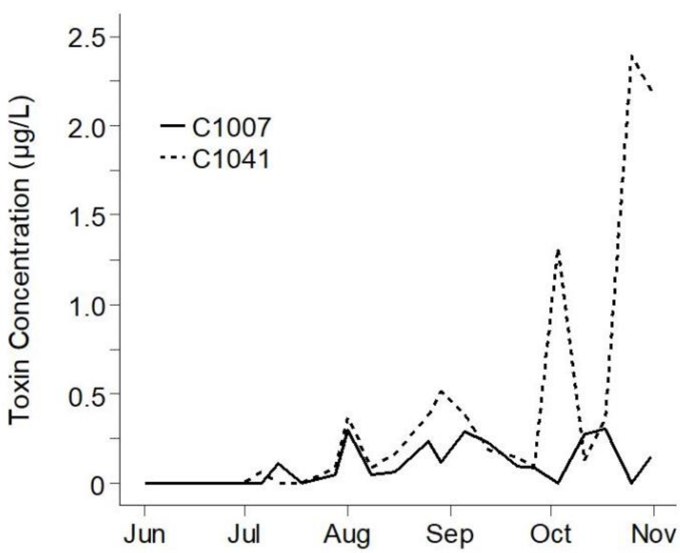

Figure 4. *Cyanopeptolins in Shelburne Pond (detected in 2017 only).*

Date	**Tissue**	**Toxin concentration (ng/g dry weight)***								
		AptB	*C1007*	*C1041*	*dmLR*	*MCHtyR*	*MCLA*	*MCLR*	*MCRR*	*MCYR*
						Golden Shiners				
7/6/2017	brain								2.258	
9/12/2017	brain								4.708	
7/6/2017	liver				5.306					
8/8/2017	liver	3.69								9.004
9/12/2017	liver	0.676								
10/17/2017	liver	1.465								

10/17/2017	liver	0.629					0.697		
9/12/2017	muscle		2.848	5.809	0.48			0.42	
10/17/2017	muscle	0.292							
					Yellow Perch				
9/12/2017	brain								1.772
10/17/2017	brain			35.117					
10/17/2017	brain								0.767
6/8/2017	liver					10.181		24.578	
6/8/2017	liver					8.651		18.916	
7/6/2017	liver					11.826		26.32	
8/8/2017	liver					1.98	1.929		
8/11/2017	liver						0.989	6.894	
9/25/2017	liver					15.669		20.172	
10/17/2017	liver					17.225			
10/17/2017	liver						2.008		
7/6/2017	muscle							0.287	0.177
9/12/2017	muscle		1.956	4.94	0.291			1.176	
9/12/2017	muscle		2.499	3.577	0.244			0.761	
9/12/2017	muscle		1.902	4.531				0.716	
9/12/2017	muscle		2.9	5.491	0.206			0.751	
9/25/2017	muscle	0.204							
10/17/2017	muscle				0.269			0.999	
10/17/2017	muscle		2.423						

* Toxin abbreviations: *AptB*, Anabaenapeptin-B; *C1007*, Cyanopeptolin-1007; *dmLR*, Desmethyl-LR; *MCHtyR*, Microcystin-HtyR; *MCLA*, Microcystin-LA; *MCLR*, Microcystin-LR; *MCRR*, Microcystin-RR; *MCYR*, Microcystin-YR.

Low concentrations of microcystins were detected in Lake Champlain water samples on 24 August 2016 (Figure 5). No toxins were detected in Malletts Bay water samples on 25 August 2016. Fish were only sampled from Lake Champlain in 2016 and will be re-analyzed along with the Shelburne Pond fish from 2016.

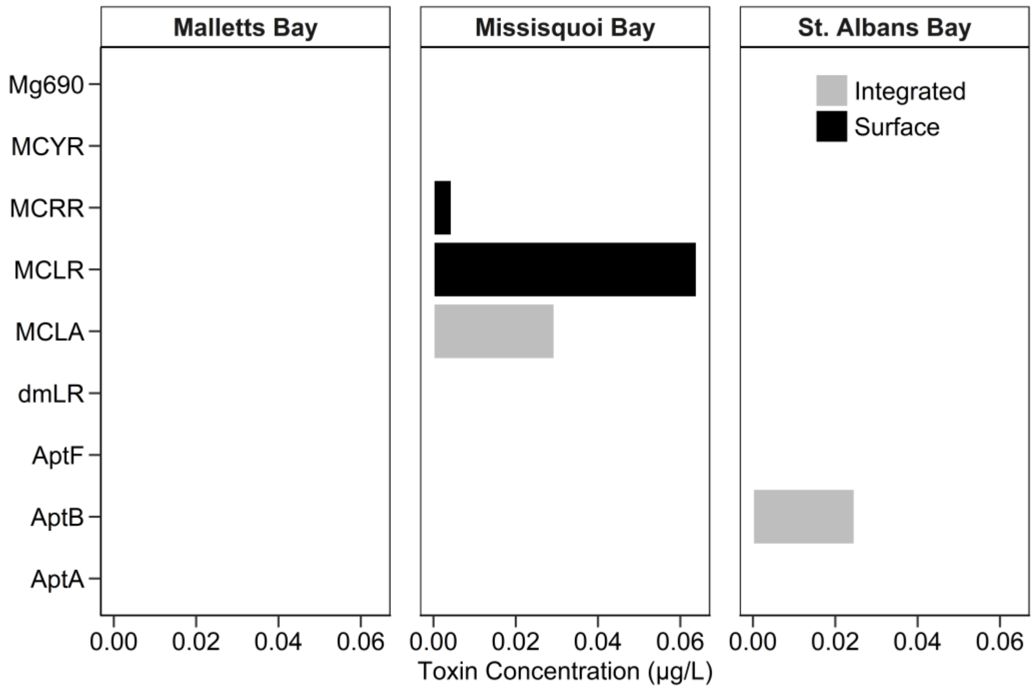

Figure 5. *Cyanotoxins in Lake Champlain water samples, 24 August 2016 (Missisquoi and St. Albans Bay) and 25 August 2016 (Malletts Bay).*

7. **Discussion**

Many studies have tested for the presence of cyanotoxins in fish through laboratory experiments or field observations (Liang et al. 2015; Magalhães et al. 2003; Mohamed et al. 2003; Soares et al. 2004; Zhang et al. 2013). A few studies have conducted data syntheses on cyanobacteria toxins in aquatic organisms, including fish. However, many of these have been qualitative in nature, focused on comparing biodilution factors to determine toxin bioaccumulation in different aquatic biota, or focused explicitly on the cyanotoxin microcystin (Ibelings & Chorus 2007; Ibelings & Havens 2008; Ferrão-Filho & Kozlowsky-Suzuki 2011; Kozlowsky-Suzuki et al. 2012, Mulvenna et al. 2012; Pavagadhi & Balasubramanian 2013). In fact, much of the previous literature has been biased toward microcystins (Ibelings & Havens, 2008; Flores et al., 2018), and other toxins should be studied in more detail because cyanobacteria can produce hundreds of secondary metabolites and potentially toxic compounds.

The data synthesis component of our project was the first to combine previous field research from individual sites across the globe into a single analysis to quantitatively assess the *relationship between cyanotoxin concentrations found in the water and concentrations found in fish from the same lakes*. The relationship was presumed to be positive but had never been tested across a gradient of lakes. Although much can be learned from single lake studies, combining data across lakes can provide novel insights into our understanding of the relationship between cyanotoxins in water and fish. In our data synthesis of cyanotoxins in wild, freshwater fish, we found that most of the studies were focused on microcystins. As a result, we were only able to conduct statistical analyses on microcystins. Even within microcystins, numerous methods have been used to determine concentrations in fish. Differences between analytical methods used to obtain toxin concentrations present difficulties in detecting broad patterns of accumulation of microcystins in fish. An increased effort to standardize and improve analytical methods to test for microcystins in fish, and expansion of such tests to include cyanotoxins other than MCs, is needed. The recommended method currently available for analysis of fish tissues is LC-MS/MS due to its sensitivity and specificity for quantitative analyses (Flores et al., 2018).

Consumption of fish is a possible vector for human exposure to cyanotoxins, yet it is a poorly researched area (Hardy et al. 2015; Magalhaes et al. 2001). Assessment of cyanotoxins in fish is a first step in this process, but to our knowledge, Vermont fishes had not been previously surveyed for cyanotoxin levels. Further, our proposed work focuses on a full suite of cyanotoxins, thereby expanding our view beyond microcystins (Ibelings & Havens 2008). We know of one parallel, ongoing study that is also assessing cyanotoxin levels in fish in Lake Champlain. That study, led by Mark Swinton (Rensselaer Polytechnic Institute, RPI) and supported by Greg Boyer (SUNY-ESF), also sampled fish from several areas of Lake Champlain in 2016 to measure cyanotoxins in fish. We have talked with Swinton and Boyer about our projects, and both groups plan to share results with each other to provide a more comprehensive picture of cyanotoxin levels in Vermont fishes. Our assessment of cyanotoxin levels in water and fish samples from Vermont will allow us to place our local conditions in the context of global cyanotoxin exposures. Using the recommended LC/MS/MS analytical methods to test for a suite of cyanotoxins, we found multiple cyanobacteria toxins in Shelburne Pond water samples that were frequently above the World Health Organization's guideline value (1 µg/L) for exposure to microcystin in drinking water, in addition to more stringent state guidelines (0.16 µg/L for microcystin; Vermont Department of Health). Guideline values are based on the congener microcystin-LR, due to limited data for other congeners, and can be used as a surrogate value for other microcystins. Though people do not drink water from Shelburne Pond, pets and wild animals that drink the lake water may be at risk for exposure. More importantly for people, concentrations of microcystins in Shelburne Pond also exceeded the U.S. Environmental Protection Agency's preliminary recreational guideline for microcystins (4 µg/L) and Vermont's Department of Health guideline for beach closure (6 µg/L microcystin-LR equivalents) on at least one occasion in 2017. In comparison, Lake Champlain cyanotoxin levels were very low and likely not an immediate concern for health, although our sampling did take place prior to the major blooms in 2016 in Lake Champlain.

8. **Summary of plans for year 2**

We plan to finish the extraction and analysis of cyanotoxins from the 2016 fish samples by July 2018. Upon completion, we will conduct statistical analyses to determine relationships between cyanotoxins in water and Vermont fish, and how Vermont levels of cyanotoxins compare to those in water and fish from the global dataset. Field study results will be published in a peer-reviewed journal and presented at an international conference (the 18th International Conference on Harmful Algae in Nantes, France, 21-26 October 2018).

9. **Training potential**. Estimate the number of graduate and undergraduate students, by degree level, who are expected to receive training in the project.

This project broadened participation in science. The funding supported one PhD student, Natalie Flores, who is a member of an underrepresented group in STEM disciplines.

10. **Investigator’s qualifications**. Include resume(s) of the principal investigator(s). No resume shall exceed two pages or list more than 15 pertinent publications.

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Professional Preparation

B.S. 1991 (Biology, Mathematics) Northland College
Ph.D. 1996 (Zoology) University of Toronto
Postdoctoral Fellow 1996-1997 (Fishery Biology) Colorado State University
Postdoctoral Fellow 1997-1998 (Fisheries and Wildlife) Michigan State University
Fellow 2008 National Conservation Leadership Institute

Appointments

2018-present	Full Professor of Aquatic Ecology, Rubenstein School of Environment and Natural Resources, University of Vermont
2011-present	Associate Professor of Aquatic Ecology, Rubenstein School of Environment and Natural Resources, University of Vermont
2011-present	Director, Rubenstein Ecosystem Science Laboratory, University of Vermont
2007-2011	Research Scientist, Gulf of Maine Research Institute, Portland, Maine
2003-2007	Field Station Supervisor/Research Fishery Biologist, Great Lakes Science Center, U.S. Geological Survey, Ann Arbor, Michigan
2001-2003	Senior Statistical Analyst, The Jackson Laboratory, Bar Harbor, Maine
1998-2000	Aquatic Biologist III, Massachusetts Division of Marine Fisheries, Gloucester, Massachusetts

Five Relevant Products (*denotes undergraduate students, # denotes graduate student)

*****Bockwoldt, K.A., E.R. Nodine, T.B. Mihuc, A. Shambaugh, and J.D. **Stockwell**. *In review*. Reduced phytoplankton and zooplankton diversity associated with increased cyanobacteria in Lake Champlain, USA. *Journal of Contemporary Water Research and Education.*

#Isles, P.D.F., Y. Xu, J.D. **Stockwell**, and A.W. Schroth. *In review*. Climate-driven changes in energy and mass inputs systematically alter nutrient concentration and stoichiometry in deep and shallow regions of Lake Champlain. *Biogeochemistry*.

#Gearhart, T.G., K. ***Ritchie, E. ***Nathan, J.D. **Stockwell**, and J. Kraft. 2017. Alteration of essential fatty acids in secondary consumers across a gradient of cyanobacteria. *Hydrobiologia* 784:155-170.

#Euclide, P.T., and J.D. **Stockwell**. 2015. Effect of gut content on δ^{15}N, δ^{13}C and C:N of experimentally-fed *Mysis diluviana*. *Journal of Great Lakes Research* 41:926-929.

Hayes, D., M.L. Jones, N. Lester, C. Chu, S. Doka, J. Netto, J.D. **Stockwell**, B. Thompson, C.K. Minns, B. Shuter, and N. Collins. 2009. Linking fish population dynamics to habitat conditions: insights from the application of a process-oriented approach to several Great Lakes species. *Reviews in Fish Biology and Fisheries* 19:295-312.

Five Additional Products

Hampton, S.E., A.W.E. Galloway, S.M. Powers, T. Ozersky, K.H. Woo, R.D. Batt, S.G. Labou, C.M. O'Reilly, S. Sharma, N.R. Lottig, E.H. Stanley, R.L. North, J.D. **Stockwell**, R. Adrian, G.A. Weyhenmeyer, L. Arvola, H.M. Baulch, I. Bertani, L.L. Bowman, Jr., C.C. Carey, J.

Catalan, W. Colom-Montero, L.M. Domine, M. Felip, I. Granados, C. Gries, H.-P. Grossart, J. Haberman, M. Haldna, B. Hayden, S.N. Higgins, J.C. Jolley, K.K. Kahilainen, E. Kaup, M.J. Kehoe, S. MacIntyre, A.W. Mackay, H.L. Mariash, R.M. McKay, B. Nixdorf, P. Nõges, T. Nõges, M. Palmer, D.C. Pierson, D.M. Post, M.J. Pruett, M. Rautio, J.S. Read, S.L. Roberts, J. Rücker, S. Sadro, E.A. Silow, D.E. Smith, R.W. Sterner, G.E.A. Swann, M.A. Timofeyev, M. Toro, M.R. Twiss, R.J. Vogt, S.B. Watson, E.J. Whiteford, and M.A. Xenopoulos. 2017. Ecology under lake ice. *Ecology Letters* 20:98-111.

Stockwell, J.D., D.L. Yule, T.R. Hrabik, M.E. Sierszen, and E.J. Isaac. 2014. Habitat coupling in a large lake system: delivery of an energy subsidy by an offshore planktivore to the nearshore zone of Lake Superior. *Freshwater Biology* 59:1197-1212.

Sierszen, M.E., T.R. Hrabik, J.D. **Stockwell**, A.M. Cotter, J.C. Hoffman, and D.L. Yule. 2014. Depth gradients in food web processes in large lakes: Lake Superior as an exemplar ecosystem. *Freshwater Biology*. DOI: 10.1111/fwb.12415

Stockwell, J.D., T.R. Hrabik, O.P. Jensen, D.L. Yule, and M. Balge. 2010. Empirical evaluation of predator-driven diel vertical migration in Lake Superior. *Canadian Journal of Fisheries and Aquatic Sciences* 67:473-485.

Jones, M.L., B.J. Shuter, Y. Zhao, and J.D. **Stockwell**. 2006. Forecasting effects of climate change on Great Lakes fisheries: models that link habitat supply to population dynamics can help. *Canadian Journal of Fisheries and Aquatic Sciences* 63:457-468.

Selected Synergistic Activities

- Associate Editor, Journal of Great Lakes Research, 2016-present
- Invited Presentation, Lake Champlain Basin Program Toxics Working Group, “Lake Champlain Food Web”, Autumn 2016
- Facilitator: (1) Faculty Development Seminar on Undergraduate STEM Research Mentoring at the University of Vermont, 2015; and (2) Lake Champlain REU Mentor Training, 2015 and 2016.
- Invited Member, Site Visit Committee, Natural Sciences and Engineering Research Council (NSERC) of Canada, Strategic Partnerships Grant Network, University of Windsor, April 2016
- Project Co-Leader, “Storm-Blitz: Impact of Storms on Phytoplankton Composition”, Theoretical Working Group of GLEON (Global Lakes Ecological Observatory Network)
- Organizer and Host, 2015 Annual Meeting of the International Association of Great Lakes Research, University of Vermont
- NSF Panel Review Member, BIO REU Program, 2014-2016

Graduate Student and Post-Doc Advising

MSc Students: Mitchell Jones (2012-2014), Peter Euclide (2012-2014), Victoria Pinheiro (co-advised with E. Marsden, 2014-2015), Hannah Lachance (2017-present)

PhD Students: Peter Isles (co-advised with A. Schroth, 2012-2016), Trevor Gearhart (2013-present), Brian O’Malley (2014-present), Allison Hrycik (2016-present), Taylor Stewart (2016-present), Natalie Flores (2016-present), Ben Block (co-advised with E. Marsden, 2017-present)

Post-Docs: Emily Nodine (2014-2015), Rosalie Bruel (2018-present), Jon Doubek (2018-present)

Todd Rex Miller, Ph.D

A. **Professional Preparation**

1999-2004 Ph.D., Marine Estuarine Environmental Sciences, Marine Molecular Biology and Biotechnology, University of Maryland, College Park, MD

1994-1998 B.S., Biology, St. Norbert College.

B. **Positions Held**

2016-present Associate Professor, Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI.

2011-2015 Assistant Professor, Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI.

2011-2014 NIEHS Children's Environmental Health Sciences Core Center Member.

2009-2010 Research Associate, Department of Bacteriology and Department of Civil and Environmental Engineering, University of Wisconsin- Madison, Madison, WI. Mentor: Katherine McMahon, Ph.D.

2007-2009 Research Associate, Limnology and Marine Science, University of Wisconsin-Madison, Madison, WI. Mentor: Katherine McMahon, Ph.D.

2004-2007 Postdoctoral Scholar, Environmental Health Engineering, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, Mentor: Rolf Halden, Ph.D., P.E.

1999-2004 Graduate Research Assistant, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD. Mentor: Bob Belas, Ph.D.

C. **Special Honors and awards**

2014 University of Wisconsin - Milwaukee Research Excellence Award

2013 Nominated University of Wisconsin - Milwaukee Undergraduate Research Mentor of the year

D. **Publications Relevant to the Proposal**

1. Xiao, X., He, J., Huang, H., Miller, T.R., Christakos, G., Reichwaldt, E.S. et al. (2016) A novel single-parameter approach to forecast algal blooms. *Water Res* Submitted.

2. Beversdorf, L., Rude, K., Weirich, C., Bartlett, S., Seaman, M., Biese, P. et al. (2016) Microcystin and cyanobacterial peptide concentrations in eutrophic Lake Winnebago and associated drinking water treatment plant raw water. *Water Res* Submitted.

3. Miller, T.R., Beversdorf, L., Weirich, C.A., and Bartlett, S. (2016) Comprehensive review of cyanobacterial toxins and their potential human health effects in the Great Lakes region. International Joint Commission, Health Professionals Advisory Board.

4. Weirich, C.A., and Miller, T.R. (2014) Freshwater harmful algal blooms: toxins and children's health. *Curr Probl Pediatr Adolesc Health Care* **44**: 2-24.

5. Beversdorf, L.J., Chaston, S.D., Miller, T.R., and McMahon, K.D. (2015) Microcystin mcyA and mcyE gene abundances are not appropriate indicators of microcystin concentrations in lakes. *PLoS One* **10**: e0125353.

6. Beversdorf, L.J., Miller, T.R., and McMahon, K.D. (2015) Long-term monitoring reveals carbon-nitrogen metabolism key to microcystin production in eutrophic lakes. *Front Microbiol* http://dx.doi.org/10.3389/fmicb.2015.00456

7. Miller, T.R., Beversdorf, L., Chaston, S.D., and McMahon, K.D. (2013) Spatiotemporal molecular analysis of cyanobacteria blooms reveals Microcystis--Aphanizomenon interactions. *PLoS One* **8**: e74933.

F. Budget Summary (See Attachment E). The application system will generate this form automatically by compiling information from the budget breakdown forms for all of the projects

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23. Products that resulted from the first year of funding:

Funding provided by the Vermont Water Resources and Lake Studies Center resulted in the publication of 1 research article, published in the scientific journal *Frontiers in Marine Science*; 1 published dataset (corresponding to the scientific article); and 3 graduate student presentations (2 poster, 1 oral presentation). Natalie Flores plans to present results of the field study at Shelburne Pond during the 18th International Conference on Harmful Algae in Nantes, France (21-26 October 2018). Please see below for references to the above-mentioned products.

Publications:

Flores NM, Miller TR and Stockwell JD (**2018**) A Global Analysis of the Relationship between Concentrations of Microcystins in Water and Fish. *Front. Mar. Sci.* 5:30. doi: 10.3389/fmars.2018.00030

Flores, N. M., Miller, T. R., and Stockwell, J. D. (**2018**). Concentrations of cyanotoxins in fresh water and fish. *Environmental Data Initiative* doi: 10.6073/pasta/0c250018ee7732d984be74e2043b84b4

Presentations:

Flores, N. M. & J. D. Stockwell (**2016**) "A Global to Local Analysis of Cyanobacteria Toxins in Fish". *Ecology, Evolution, and Environmental Biology Seminar*, UVM, Burlington, VT. [**oral presentation**]

Flores, N. M., T. R. Miller & J. D. Stockwell (**2017**) "Cyanobacteria Toxins in Fish: A Global Analysis". *Student Research Conference*, UVM, Burlington, VT. [**poster presentation**]

Flores, N. M., T. R. Miller & J. D. Stockwell. (**2017**) "Cyanotoxins in Fish: A Global Analysis". *9th US Harmful Algal Bloom Symposium*, Baltimore, MD. [**poster presentation**]

Flores, N. M., T. R. Miller, J. Kraft & J. D. Stockwell. (**October 2018**). "Cyanobacteria bloom impacts on fish: Insights from an ongoing study at a shallow, hypertrophic lake in Vermont (USA)". *18th International Conference on Harmful Algae*, Nantes, France. [**poster presentation**]

Information Transfer Program Introduction

The Vermont Water Resources and Lake Studies Center facilitates information transfer in a variety of ways. The Director, Dr. Breck Bowden, and Program Coordinator, Elissa Schuett, participate in committees and meetings as representatives of the Vermont Water Center. Dr. Bowden is a member of the Technical Advisory Committee and Steering Committee for the Lake Champlain Basin Program, sharing work being funded by the Vermont Water Center with others in the region. He also is actively involved in the “Common Circle” steering committee and the “Solutions” team for the Network for Clean Water, a program being led by the ECHO, Leahy Center for Lake Champlain to address water quality issues in the region. Ms. Schuett leads communications for both the Vermont Water Center and Lake Champlain Sea Grant and is a member of both the Sea Grant Communicators Network and the University of Vermont Communicators Network. These networks offer numerous training and professional development opportunities, which are applicable to the Water Center communications and information transfer. Involvement with these other networks also broadens the reach of the Water Center.

The Water Center maintains several websites, including the Vermont Water Resources and Lake Studies site that highlights emerging research funded by the Center or relevant to water resources management in Vermont. A regional network website was developed for the New England Regional Water Resources and Research Centers. The website is updated with news relevant to regional water resources issues, RFP announcements, and links to each of the Water Resources Research Institutes and the Water Science Centers in the New England region (Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, Vermont).

Support by the Water Center of the e-digest publication, ecoNEWS VT (http://www.econewsvt.org/), continued in 2017, highlighting ecological research from across Vermont, including research funded by the Vermont Water Center. Research digests are derived from peer-reviewed reports and articles and distilled into short articles for a non-expert audience. All digests are archived on the website as a resource for managers, lawmakers, and researchers. Quarterly emails are sent to approximately 300 subscribers with featured articles and information. Three email issues with 14 new stories were produced in 2017, including several articles about Water Center funded projects. The publication is a collaboration with several other organizations, including Lake Champlain Sea Grant, Northeastern States Research Cooperative, and Vermont Monitoring Cooperative.

In addition to the e-digest issues, all articles are archived online and tagged for cross-reference of similar topics. A total of 56 articles are now archived on the site. A section of the website is used to capture ecological research being conducted outside Vermont, but relevant to issues found in Vermont. Events are also maintained on the website to inform visitors about seminars, public meetings, and workshops. Social media is also used to alert followers to recent digests and relevant ecological research news. A graduate student was supported part-time by the Water Center to assist Ms. Schuett with writing feature stories and management of the outreach efforts of ecoNEWS VT.

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	3	0	0	0	3
Masters	1	0	0	0	1
Ph.D.	3	0	0	0	3
Post-Doc.	0	0	0	0	0
Total	7	0	0	0	7

Notable Awards and Achievements

Publications from Prior Years

1. 2011VT57B ("Determining phosphorus release potential from eroding streambank sediments in the Lake Champlain Basin of Vermont") - Dissertations - Ishee, E.I. 2011. Characterizing phosphorus in eroding streambank soils in Chittenden County, Vermont. M.S. Thesis, Department of Plant & Soil Science, University of Vermont, 90 pp.
2. 2011VT57B ("Determining phosphorus release potential from eroding streambank sediments in the Lake Champlain Basin of Vermont") - Articles in Refereed Scientific Journals - Young, E. O., D. S. Ross, B. J. Cade-Menun, and C. W. Liu. 2013. Phosphorus Speciation in Riparian Soils: A Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy and Enzyme Hydrolysis Study. Soil Sci. Soc. Am. J. 77:1636-1647. doi:10.2136/sssaj2012.0313
3. 2011VT57B ("Determining phosphorus release potential from eroding streambank sediments in the Lake Champlain Basin of Vermont") - Articles in Refereed Scientific Journals - Young, E. O., D.S. Ross, C. Alves, and T. Villars. 2012. Soil and landscape influences on native riparian phosphorus availability in three Lake Champlain Basin stream corridors. Journal of Soil and Water Conservation. 67:1 (1-7).
4. 2011VT57B ("Determining phosphorus release potential from eroding streambank sediments in the Lake Champlain Basin of Vermont") - Articles in Refereed Scientific Journals - Ishee, E. R., D. S. Ross, K. M. Garvey, R. R. Bourgault, and C. R. Ford. 2015. Phosphorus Characterization and Contribution from Eroding Streambank Soils of Vermont's Lake Champlain Basin. J. Environ. Qual. 44:1745-1753. doi:10.2134/jeq2015.02.0108