Introduction

Maine is fortunate in being a water-rich state with extensive surface water and groundwater resources. These water resources are essential to the regional economy in terms of energy generation, water supply, agriculture, tourism, industry, and ecosystem services. The state is not without water resources concerns due to flooding, sea-level change, urbanization, stormwater, endangered aquatic species, harsh winters, and natural contaminants such as arsenic in drinking water. The Maine Water Resources Research Institute is the primary independent source of water resources research in the state. Situated in the Senator George J. Mitchell Center for Sustainability Solutions, there is a focus on resource sustainability, we facilitate the advancement of water science in the state by supporting research, graduate studies, and outreach. It is our mission to facilitate the process to identify, understand, and solve water resources problems that are important to the citizens of Maine.

During the FY15 period, the Maine Institute supported three (3) research projects, with prominent student involvement:

(1) Plankton N cycling and detection of cyanobacterial blooms across a gradient of lake trophic states; (2) Toward a more efficient UV disinfection system: The development of a TiO2-based photocatalyst to enhance the degradation of taste and odor compounds in drinking water; and, (3) Water budget, groundwater exchange and hydrologic variability of central Maine’s seasonal forest pools.

The Institute supported additional Information Transfer activities such as working with municipalities to manage road salt to protect water quality, the Maine Sustainability and Water Conference and groundwater education in rural communities (GET WET!) activities. These projects directly provided support to two graduate students and involved several undergraduate students in a variety of roles.

The federally authorized Maine Water Resources Research Institute provides fundamental and essential functions, none of which would not exist without explicit Congressional re-authorization and appropriations. The federal money that supports the Institute is highly leveraged with other funds provided by other granting agencies, stakeholders, universities, and researchers. In order to address key problems in the best way possible, research project proposals are evaluated by peer-review and approved by our Research Advisory Board composed of members from the U.S.G.S. Water Science Center, State Environmental Agencies, academia, and industry.

The Maine Institute Co-Directors, John Peckenham and David Hart, also serve as the Associate Director and Director, respectively, of the Senator George J. Mitchell Center for Sustainability Solutions. The Mitchell Center provides the administrative home for the Water Resources Research Institute. The broader mission of the Mitchell Center enhances our efforts to have the Maine Institute increase the breadth and accessibility of water research in Maine. The Mitchell Center is the recipient of a five-year EPSCoR grant from the National Science Foundation to develop the Sustainability Solutions Initiative. This grant is fostering even greater multi-institutional interdisciplinary research, including several projects related to water resources.

The 21th annual Maine Water and Sustainability Conference was held in March 2015 and continues to be the leading regional event for the water community. Research supported through the Maine Institute is prominently featured at the conference. The recent conference attracted more than 300 registrants. The number of people and organizations who support and contribute to this conference reflect the importance of water to the people of the State of Maine. Through the hard work of Institute staff, the Conference Steering Committee, and other key supporters, we have been able to address the important water issues in Maine and to bring together diverse interest groups.
The Water Resources Research Institute's affiliation with the Mitchell Center gives us the ability to support both large and small projects that address important local needs. It also provides us leverage to develop and attract funding from other agencies. In FY15, the Maine Institute had projects that brought in other funds and contributions from state agencies (e.g. Department of Environmental Protection), federal agencies (e.g. Fish and Wildlife, Environmental Protection Agency, National Oceanic and Atmospheric Agency), and foundations. None of these projects would be possible without the support of the federal Water Resources Research Institutes program and the U.S. Geological Survey.
Research Program Introduction

The Maine Water Resources Research Institute supports research, information transfer projects, and seed grants using Section 104b funds. Grants funded under Section 104b each deals with important aspects of Maine's highly-valued water resources. Projects are awarded on a competitive basis using a two-stage selection process. The Institute issues a call for pre-proposals in the spring. The pre-proposals are reviewed by the Executive Committee (Co-Directors and invited topical experts) and full proposals are solicited for 150% of available funds. Full proposals are sent out for external review to experts selected by the Co-Directors. The Research Advisory Committee members (stakeholders) read the proposals and reviews to provide the Institute Directors with a ranked selection of proposals to fund. Much effort is made to solicit suggestions for themes, to diversify the types of projects funded, and to include researchers from the small colleges and universities in the state. Preference is given to support new faculty and projects developed by students. Investigators are encouraged to collaborate with state and federal agencies and to seek additional contributions for their projects.
Controls of phosphorus cycling in Lake Auburn, Maine, USA: Spatial and temporal interactions among sediment, water column, and climate change

Basic Information

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<td>Aria Amirbahman, Linda Bacon, Stephen A. Norton</td>
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Publications


Title: Controls of phosphorus cycling in Lake Auburn, Maine, USA: Spatial and temporal interactions among sediment, water column, and climate change

Project Number: 2014ME297B
Project Type: Research
Focus Category: Geochemical Processes, Hydrogeochemistry, Sediments
Descriptors: Phosphorus, Eutrophication, Geochemistry, Lakes, Water Quality
Start Date: 4/1/2014
End Date: 3/31/2015

Report to the Water Resources Research Institute
April 10, 2015

Submitted by Aria Amirbahman, Department of Civil and Environmental Engineering, University of Maine

In December 2013, a grant was awarded through the WRRI to assess the geochemical cause of algal blooms in Lake Auburn, a public water supply having a sensitive population of coldwater fish threatened by resultant high turbidities. It was envisaged that the results could inform decisions regarding future treatment strategies of the lake and provide an approach suitable for use in other Maine lakes and water supplies. The award amount was considerably less than what was required to conduct a thorough study, so we approached the Maine Outdoor Heritage Fund (MOHF) and the Maine DEP for additional funding so that the project could move forward. At this time we have made considerable progress toward our objectives. We have a graduate student in a MS program who has been conducting most of the research and have learned much about the geochemistry of Lake Auburn.

Abstract of the project’s results. We have determined that Lake Auburn sediment has very low concentrations of aluminum (Al) relative to iron (Fe) and phosphorus (P) with Al:Fe ratios ranging from 0.3 to 1.7 and Al:P ratios ranging from 2.0 to 14.5. Several studies, some of which include Maine lakes, have shown lakes sediments with molar Al:Fe ratios < 3 and Al:P ratios < 25 to be susceptible to P release under anoxic conditions. We sampled sediment from 11 locations in Lake Auburn. Our analyses show that Lake Auburn sediment is well below both the threshold of 3 for Al:Fe and 25 for Al:P (see figures next page), indicating that Lake Auburn may be vulnerable to internal P loading during summers with widespread hypolimnetic anoxia, as occurred in 2011 and 2012.

A survey of sediments from upstream lakes in the watershed (Mud Pond and Little Wilson Pond) showed that these lakes have more favorable Al:Fe and Al:P ratios with values >3 and >25, respectively. High concentration of Fe in Lake Auburn sediment may be linked to the presence of the Basin and Townsend Brook wetlands. Water samples taken at several locations stretching from Mud Pond to Lake Auburn and from upstream Townsend Brook to Lake Auburn have shown an increase concentration of total Fe in both wetland systems. It is possible that the presence of these wetlands are causing an increased flux of Fe to Lake Auburn, which becomes incorporated into the sediments, making the lake at risk to internal P loading. However, it is also
possible that the presence of these wetlands decreases nutrient inputs (N and P) to the lake and, as such, it is important that the role these wetlands play in lake productivity is further evaluated.

*Follow-up work.* We are currently analyzing two 60 cm sediment cores that were extracted from Lake Auburn in March 2015. These cores are being dated using radioactive isotopes ($^{210}\text{Pb}$) and chemically analyzed to determine if there have been changes in sediment chemistry over the last several hundred years. We hope to determine any differences in sedimentation to Lake Auburn as land use within the watershed changed from agricultural to forested and if the emplacement of the dam, which created the Basin wetlands, has had a significant impact on sediment Al:Fe in the lake.

*Presentations related to the project.* The results of this study have been presented at the Oklahoma State Student Water Conference (March 2015, Stillwater, OK), the Maine Water and Sustainability Conference (March 2015, Augusta, ME), and the New England Water Works Association annual meeting (April 2015, Worcester, MA). The results from this study will also serve as the basis for the MS thesis in Civil and Environmental Engineering for Ms. Heather Doolittle at the University of Maine. She expects to graduate in December 2015.

*Color photographs or figures related to the project.* The following figures were included in the presentations made by Ms. Doolittle over the last few months. Contour maps of Lake Auburn surficial sediments show the Al:Fe ratios (left) and Al:P ratios (right). Both maps highlight the low concentration of aluminum relative to iron and phosphorus in the sediment. Photographs below the maps were taken while obtaining sediments from Lake Auburn. The first photograph illustrates use of a gravity coring device to obtain surface sediments (summer 2014), and the second photograph shows a deep core obtained in March 2015 which was collected by drilling through the ice using a Davis-Doyle coring device. The sediments at the bottom of the deep core were likely deposited around 500 years ago. As mentioned above, we are presently working to characterize the age and chemical/physical makeup of these sediments.
Connecting climate and land use to Sebago Lake drainage network processes

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Publications

Final Report to: The Maine Water Resources Research Grants Program, FY 14

Submitted by: Dr. Sean M.C. Smith, School of Earth and Climate Sciences, Sen. George J. Mitchell Center, University of Maine, Orono, Maine

Date: May 2, 2016

Title: Connecting Climate and Land Use to Sebago Lake Drainage Network Processes

Project Number: 5406025

Focus categories: G&G, HYDROL, MOD, NPP, SED, SW, WQL, WQN, WS

Keywords: Channels, Climate, Geomorphology, Impoundments, Land Use, Streams, Urban Drainage, Watershed Management

Project Duration: March 2014 – Aug. 31, 2015 (with an extension to December 31, 2015)

Funds: $34,004

Project Investigator: Dr. Sean M.C. Smith, School of Earth and Climate Sciences, Sen. George J. Mitchell Center, University of Maine, Orono, Maine

Students Supported Under the Award: Brett Gerard, School of Earth and Climate Sciences

Congressional District: Second
A. RESEARCH PROJECT

A.1 Summary

The geomorphology of Northern New England is influenced by a postglacial history of erosion combined with direct and indirect landscape modifications by humans following European colonization. Better information on the relative influence of glacial processes and anthropogenic activities on modern streams is necessary for watershed management and restoration strategies. Little information is available on headwater stream channel dimensions and dynamics in varied settings of Maine despite their composition of the majority of drainage networks lengths. Here we provide a set of observations of headwater stream channel dimensions, hydrology, and hydraulics relevant to Central and Coastal Maine to help fill this information gap.

Our investigation examined fluvial systems in central and coastal Maine with a focus on the Sebago Lake watershed in the Lakes Region of southern Maine and the Webhannet River and Cromwell Brook watersheds on the coast. Field measurements were combined with existing data from federal and state agency sources to characterize stream channels in the region and advance the ability to predict their dimensions and responses to climate and land use changes. Our approach is centered on an analysis of physiographic conditions affecting surface runoff, sediment supply, and channel dimensions.

Landscape analysis focused on the Sebago Lake watershed as an area representative of Central Maine locations. We identified physiographic settings within the region at the scale of watershed sub-basins based on a collection of attributes affecting surface runoff and thereby influencing stream channel conditions. Approximately half of the variation across the landscape was statistically differentiated based on the presence of well drained, till-dominated surficial materials versus poorly drained soils. Hydrologic analysis demonstrated predictable associations between hydrologic conditions that can be quantified in terms of surface water storage and local physiographic conditions. The Central Maine locations we examined have somewhat homogenous similar hydrologic characteristics with the exception of locations that are poorly drained. The Central Maine observations were different in comparison to coastal areas dominated by bedrock with limited surface water storage capacity.

We quantified stability of headwater streams in Central Maine based on the incipient motion of channel bottom sediment. Entrainment predictions based on hydraulic conditions and the grain size distributions representative of our study sites correlated with the 3.2 year discharge rate, on average. The result indicates that the headwater channels are stable during sub-annual flows. An explanation for this observation of threshold channel conditions can be tied to the watershed sediment supply that is influenced by the glacial history of the landscape.

Published downstream hydraulic geometry relations relating bankfull width, depth and velocity to discharge in the region held for the stream locations we measured that had contributing drainage areas within the range of USGS gage station sites in Central and Coastal Maine. However, geometry curves produced from our measurements had lower slopes based on the dimensions of the relatively small streams in our dataset. The geometry predictions for small headwater channels thereby depart from the relations for larger streams in the region. At-a-station hydraulic geometry relations indicate a gradual transition in channel shape with increasing drainage area. This conforms to the decreases in stream corridor confinement with distance downstream from the drainage divides that can be observed on topographic maps.
A.2 Problem and Research Objectives

The rates and magnitudes of watershed hydrologic processes in Northern New England are influenced by the post-glacial terrain and landscape modifications made by humans. Knowledge of the physical stream characteristics and the extent to which factors linked to climate and humans influence them is important for the development of watershed restoration and management strategies in the region. Better measurements in more places are necessary to quantify the processes that influence watershed surface flows and the physical connections between the upland hillslopes and downstream rivers. In particular, there is a growing demand for better information on the configuration and stability of small headwater channels that dominate the total length of stream networks in Maine. This report summarizes a set of measurements and analyses focused on headwater stream channel conditions in Coastal and Central Maine to address related knowledge gaps.

Channel conditions respond to the balance between slope, water discharge, and sediment load described by Lane (1955) (Figure 1). The modern topography and supply of surface runoff and sediment in Maine are strongly influenced by the presence and retreat of the Laurentide Ice Sheet ~15ka (Borns Jr et al. 2004). Till dominated surficial geology, thin soils, and high density of instream ponds resulted from the glacial processes. The conditions produced stream networks with a small sediment supply and transport (Snyder et al. 2009) and extensive surface water storage in Central and Coastal Maine. During deglaciation much of Coastal Maine became submerged and overlain with glacio-marine deposits. The extent of submergence is demarked by the line of marine transgression. The surficial deposits that dominate extensive areas to the east of the line are relatively fine grained and poorly drained.

Extensive European colonization of the region in the early 1600’s (Maine Historical Society) also influenced contemporary watershed conditions. The colonization introduced large scale industrial activities (agriculture, forestry, mills, etc.) that directly and indirectly altered streams and river valleys. Although many of the perturbations from extensive forest clearing are less evident in many places today, small run-of-the-river dams, flash dams, and other modifications to stream channel networks persist.

This project was framed with the goal of quantifying the hydrologic and dimensional characteristics of streams over a range of watershed scales, identifying generalizable patterns, and explaining factors affecting modern physical conditions. We address the components of Lane’s balance and evaluate the spatial-temporal dynamics of this relation across the glacially modified landscape of Central and Coastal Maine. The approach to meeting this goal was based on four objectives: 1) Identification of dominant watershed physiographic settings influencing stream channel conditions; 2) Analysis of stream hydrograph characteristics in varied watershed settings; 3) Quantification of the competence and capacity of stream channels in varied watershed settings; and 4) Evaluation of in-channel conditions in a range of watershed settings.

A.3 Methodology

Site Selection Study Area Description

We collected field data from watersheds associated with Sebago Lake in Central Maine, the Webhannet River on the southern coast, and Cromwell Brook in Mid-Coast Maine. The primary
study location is the Sebago Lake watershed which is an area of concern because the lake is an important recreation destination and drinking water source for a large portion of Maine’s population. The surrounding region is predominantly rural and forested; however, there are projected land cover (LULC) changes that can directly or indirectly impact streams in the region (U.S. EPA 2009). The coastal areas surrounding the Webhannet River and Cromwell Brook are also a recreation destination and source of income from both tourism dollars and commercial fishing. This area is more extensively developed, often impacted by non-point source pollution, and under more substantial development pressure.

Stream channels in our study watershed vary in size, bottom sediment composition, and history of disturbances from humans. Study reach locations were selected to span a range of watershed drainage areas and physiographic settings, providing varied channel slope and riparian corridor conditions including presence of man-make infrastructure such as dams and culverts. We selected 37 stream reaches for geomorphic reconnaissance (27 in the Sebago watershed, 8 in the Webhannet watershed, and 2 in the Cromwell watershed). These data were coupled with information from previous investigations by the USGS (Dudley 2004) and for Instream Flow Incremental Methodology (IFIM) (Kleinschmidt 1999a; Kleinschmidt 1999b) evaluations to provide a more robust dataset across the study region (Figure 1).

Relief of the terrain in the 1,144 km² Sebago Lake watershed is generally modest with an average elevation standard deviation of ~30 m. The surficial geology is dominated by glacial till with a scattered mix of wetland, glacio-fluvial, and glacio-marine deposits. The latter of these are dominant along the southern end of the watershed, east of the marine transgression limit. Land cover throughout the watershed is predominantly rural with pockets of higher development along the lake perimeter.

The Webhannet River watershed covers 27 km² of mostly low relief terrain (elevation range of 71 m and a standard deviation of 9 m). The area is moderately developed (~11%), much of which is concentrated along the coast, and has a long history of human interventions. The surficial geology of the watershed is predominantly composed of marine clays and sands (Smith 1999a; Smith 1999b) corresponding to its location east of the line of marine transgression.

The Cromwell Brook watershed is located in Mid-Coast Maine on Mount Desert Island just outside of Acadia National Park. The watershed is ~18 km² and has moderate to high relief (elevation range of 465 m and a standard deviation of 114 m). The location is dominated by the presence of shallow granites and has thin or absent soils, and it is moderately developed, (19%, mostly outside of Acadia National Park). Much like the Webhannet watershed and other parts of coastal Maine, this development is concentrated along the coast.

**Watershed Characterization**

We used a Principal Component Analysis (PCA) and a K-means Cluster Analysis to identify dominant watershed physiographic settings influencing stream channel conditions within the Sebago Lake Watershed, what we term Geomorphic Response Units (GRUs). A Principal Component Analysis (PCA) finds linear combinations of variables that capture maximum variation in a dataset, or landscape (Harris 2001). This can be represented mathematically as:

\[ PC_1 = b_{1,1}X_1 + b_{1,2}X_2 + \ldots + b_{1,m}X_m = Xb_1 \]

\[ PC_2 = b_{2,1}X_1 + b_{2,2}X_2 + \ldots + b_{2,m}X_m = Xb_2 \]
where PC is a principal component; X is a variable; and b is a loading or weight applied to a variable. An iterative process assigns weights to each variable trying to maximize the total variation while requiring that the squares of the coefficients involved in any PC sum to one. Cluster analysis is another iterative multivariate technique that attempts to find structure in data by minimizing within cluster sums of squares based on Euclidean distances from K user specified points (Sokal and Rohlf 1995; Harris 2001) (Figure 3).

Landscape units were defined in the Sebago Lake Watershed by a 2,500 m resolution grid, corresponding to the average drainage area of 2nd order basins in the region defined by the National Hydrography Dataset (NHD). For each unit we quantified the distribution of six variables that govern watershed surface drainage processes in the landscape (i.e., relief ratio, surface geology, bedrock geology, depth to bedrock, land cover, and soil hydrologic conditions) using spatial data from the Maine Office of GIS (Maine Office of GIS 2014). The value of these variable were used in both multivariate approaches.

**Hydrologic Characterization**

Ongoing research in the Sebago Lake watershed provided almost five years of discharge data from six study reaches (Reeve et al. 2013). These hydrographs were analyzed along with a USGS gauge station in a coastal watershed, Otter Creek on Mount Desert Island (USGS 01022840), and another USGS station in a North-Central Maine watershed, East Bear Brook (USGS 0102294). Analysis focused on the “sensitivity function”, g(Q), as described by Kirchner (2009). This parameter describes the sensitivity of discharge in a stream to changes in storage within a landscape, where a large g(Q) indicates less storage and a smaller g(Q) indicates more storage. An example of this is presented in Figure 4. In this example watershed A (red) has less storage than watershed B (black), indicated by a steeper recession limb assuming the same amount of rain falls onto each watershed.

The sensitivity parameter can be solved for starting with a simple water balance:

\[
\frac{dS_k}{dt} = P - E - Q
\]

where \(S_k\) is storage; t is time; P is precipitation; E is evapotranspiration; and Q is discharge. Using the linear reservoir theory, discharge can be defined as a function of storage.

\[
Q = g(Q) \cdot S_k
\]

Through differentiation and substitution we can define discharge over time as:

\[
\frac{dQ}{dt} = \frac{dQ}{dS_k} \cdot \frac{dS_k}{dt} = \frac{dQ}{dS_k} (P - E - Q)
\]

This can be rearranged to show that the sensitivity function can be found using only the discharge hydrograph when precipitation and evapotranspiration are small.
Sediment Dynamics

The ability of flows in the study region to move the observed sediment distribution, stream competence, was estimated by evaluating flow recurrence intervals and the estimated shear stress generated by these flows relative to the critical shear stress required for the initiation of movement. For steady, uniform flow conditions the total boundary shear stress is:

$$\tau_0 = \rho_f ghS$$

where $$\rho_f$$ is the density of the fluid; $$g$$ is the acceleration due to gravity; $$h$$ is the flow depth; $$S$$ is the slope of the water surface. We can partition this stress to find only that portion which is acting on the sediment trough use of the Strickler relation:

$$n_L = 0.013D^{1/6}$$

where $$n_L$$ is a roughness coefficient related to sediment and $$D$$ is the grain size diameter of interest in meters. Solving for $$\tau_0$$ in Manning’s equation:

$$v = \frac{1}{n} R^{2/3} S^{1/2}$$

where $$v$$ is flow velocity; $$n$$ is a reach averaged roughness coefficient; and $$R$$ is the hydraulic radius; we can substitute $$n_L$$ for $$n$$ and find the shear stress acting on the sediment ($$\tau_g$$) (Wilcock et al. 2009):

$$\tau_g = \rho g (0.013)^{1/2} (SD)^{1/4} U^{3/2}$$

The relation between $$\tau_g$$ and the critical shear stress required for the initiation of motion of a grain of diameter $$i$$ ($$\tau_{ci}$$) represents the average conditions for bed mobility at the reach segment scale. $$\tau_{ci}$$ is described by:

$$\tau_{ci} = \tau^*_c (\rho_s - \rho_f) g D_i$$

where $$\rho_s$$ sediment density; $$D_i$$ is a grain of diameter $$i$$; and $$\tau^*_c$$ is the spatially and temporally averaged dimensionless critical shear stress (Shields parameter) which is often reported as $$\sim 0.045$$ from visually determined laboratory studies (Buffington and Montgomery 1997; Diplas et al. 2008). If the recurrence interval flow for our locations generates $$\tau_g$$ in excess of $$\tau_{ci}$$, the reach averaged conditions for movement of channel bed sediment have been met.

Stream capacity, the rate at which sediment is transported given an adequate sediment supply, was also estimated for the region relative to the current recurrence interval of flows and projections. We choose to estimate transport rates using the surface based Wilcock and Crowe (2003) transport model. The expression of this transport model is:

$$W^*_i = \frac{\tau_g}{\tau_{rsg}} \left( \frac{D_{sg}}{D_i} \right)^{\chi}$$
where $W'_t$ is the dimensionless, fractional transport rate; $\tau_{rs_g}$ is the reference shear stress for the mean grain size; $D_{sg}$ is the mean grain size for the gravel portion of the bed; $D_i$ is the grain size of the size fraction of interest; and $\chi$ varies relative to grain size $D_i/D_{sg}$. The use of this surface-based model decreases the logistical difficulties of sub-surface sampling, and its explicit treatment of sand is important because of the sandy nature of streams in Central Maine.

Field data collection for these analyses included 100-pebble count surveys to characterize the sedimentary environment of the channel bottom (Wolman 1954). Most of the surveyed locations did not have available flow time series data, so drainage area normalization of nearby monitoring station data was used to estimated flow time series (Hirsch 1982). This data was used to estimate flow recurrence intervals using methods described by Gupta et al. (1989).

**Hydraulic Geometry**

Hydraulic geometry expresses the fundamental relations between discharge, flow velocity, and channel dimensions (Leopold and Maddock 1953). This relation can be expressed for a single station as discharge changes over time, at-a-station geometry, or for multiple stations as discharge changes relative to drainage area, downstream geometry. These relations can be expressed as the following functions:

\[
\begin{align*}
    w &= aQ^b \\
    d &= cQ^f \\
    v &= kQ^m
\end{align*}
\]

where $Q$ is streamflow and interchangeable with drainage area (DA); $w$ is channel width; $d$ is channel depth; $v$ is flow velocity; $a$, $c$, and $k$ are derived coefficients; and $b$, $f$, and $m$ are derived exponents. Because discharge is a function of cross section area (depth x width) and velocity, the exponents of these relations sum to one as does the product of the coefficients.

Following the work of Dudley (2004), we investigated these relations for Coastal and Central Maine with particular interest in smaller stream systems than previously examined. We completed topographic surveys in 37 stream reaches throughout the Sebago Lake, Webhannet River, and Cromwell Brook Watershed using a total station (Sokia CX 105). Drainage area calculations for each location were calculated using ArcGIS (ESRI) and elevation data from the Maine Office of GIS (Maine Office of GIS 2014). Downstream relations were evaluated relative to bankfull conditions, identified in the field using features such as the top of the bank, the backs of point bars, benches, changes in sediments, or changes in vegetation (Williams 1978). At-a-station relations were defined using a meta-data analysis incorporating our survey sites, nine USGS gauge stations, and four IFIM study sites. Because of incomplete flow data for many of these locations, we did not address velocity and evaluated a modified at-a-station relation.

\[
\frac{w}{d} = a(DA)^b
\]
A.4 Principal Findings and Significance

Findings

PCA and Cluster Analysis based on landscape variables governing runoff production and routing to determine geomorphic response units indicates that ~50% of the variance between landscape units can be described by the difference between well drained, forested, till dominated units (GRU 1) and less well drained units with more wetland coverage (GRU 2). We can see the spatial distribution of these units in Figure 5. Well drained, till dominant units appear to generally be associated with higher elevations, so we reference them as “upland”, and more poorly drained, wetland rich units are generally present at lower elevations, so we refer to them as “lowland.” This parsing of the landscape allows for a general idea of the transferability of more fine scale geomorphic and hydrologic investigations. The pattern is anticipated to be present at similar watershed scales across central Maine. Ongoing work seeks to expand the investigation to the entire central and coastal Maine regions.

Hydrologic analysis based on hydrograph recession slopes indicates similarities in storage among Central Maine basins dominated by glacial till and “upland” GRU (Figure 6). Central Maine basins with more poorly drained soils and a higher proportion of the “lowland” GRU have distinct responses which indicate more storage in the watershed. Comparison with a coastal watershed hydrograph indicates substantially less storage in locations dominated by bedrock exposures and glacio-marine deposits. These patterns that appear to be consistently correlated to watershed characteristics indicate that reliable prediction the hydrologic signature of rainfall-runoff is possible using generalizable watershed information.

Recurrence interval for entrainment estimates indicate that sediment transport is initiated by flows with a recurrence interval between 3 and 4 years, on average (Figure 7). The result provides an indication of the stability of headwater channels relative to sub-annual flow conditions. This outcome is influenced by the supply of glacially deposited materials with a grain size distribution exceeding the competence of the corresponding surface flows at the sampled locations. We observed a large amount of variation between sites due to the presence of different glacially formed deposits (e.g. dispersed eskers, and glacio-fluvial or glacio-marine deposits).

Sediment rating curves for two similar sized second order streams representative of the Sebago Lake region are shown in Figure 8. Data from these sites and field observations illustrate some of the variability between fluvial systems in Central Maine. For example, observations indicate that Hill Brook mechanically operates as a “threshold channel” controlled by gravel and cobble materials derived from local bank sources (Wilcock et al., 2009). Moss growth observed on point bars is supportive evidence of the relatively low transport rates derived by calculation. Pykes Brook is quite different with a much larger fraction of the bottom composed of sand sized materials. The channel appears to behave as an alluvial system dependent on the balance of sediment input and output from the study reach. Both of the sites have been instrumented with scour chains and tracer gravels for further evaluations of channel bed dynamics linked to sediment transport and water flow conditions.

At-a-station geometry calculations across the study region indicate that a gradual progressive transition from confined v-shaped to rectangular shaped channels with distance downstream from the drainage divides (Figure 9). There is greater variability in channel width and depth dimensions towards the upper ends of drainage networks. Increasing relevance of local variations
in disturbance from human activities is assumed to have a substantial influence on the headwater stream conditions. Even more variability would be expected if steeper channels on hillslopes with relative high relief were included in the dataset.

The bankfull channel dimensions measured at our study sites produce downstream geometry relations that differ slightly from the relations generated using measurements of the larger channels and watersheds at USGS gage stations in Central and Coastal Maine (Dudley et al. 2004). There is overlap of the upper range of the drainage areas to our study sites with the lower end of the watershed sizes flowing to USGS gage stations. The previously published relations provide reasonably accurate predictions of the geometry of the largest channels in our dataset. However, they would result in progressive more substantial under-prediction of channel width and depth in smaller headwater channels, particularly for depth (Figure 10). The result is attributed to progressive erosion of the headwater threshold channels, many of which do not recover from erosion events through subsequent infill of sediment from sources upslope.

Scatter in the dataset makes identification of a threshold drainage area at which the hydraulic geometry relations in small versus large streams diverge inappropriate to pursue. However, observations also indicate that the transition from threshold to alluvial channel conditions can be a long distance from the drainage divide in many Central and Coastal Maine drainage networks because of the size distribution and magnitude of the watershed sediment supply. This contrasts with observations of alluvial valley deposits associated with streams draining landscapes below the southern extent of glaciation in the Northeast.

**Significance**

In National Academy of Science’s summary of overarching themes of sustainability science, Bob Kates identifies geophysical research focusing on the earth as a system with interconnections among the earth’s climate, biogeochemical cycles and human activities as a distinct line of research relevant to sustainability (Kates, 2003). Watershed sustainability requires place based research that provides a foundational understanding of how social actions and the environment interact spatially and temporally in a system (Kates et al. 2001; Loucks 2000). This research provides a basis for watershed management practices in Central and Coastal Maine that support to the sustainability of water resources by quantifying interactions between hydrology, sediment, and channel attributes that influence water quality and aquatic habitat conditions. The observations we have assembled expand the capacity to develop tools for the prediction of stream responses to varied watershed conditions influenced by both climate and land use alterations.

We have targeted metrics for quantification of stream conditions based on the input of stakeholders focused on maintenance or restoration of aquatic habitat conditions conducive to good water quality conditions, fish passage, and aquatic habitat supportive of viable fish communities. Our dialogue continues with multiple organizations involved with stream and watershed management in the region including the U.S. Fish and Wildlife Services, United States Geological Survey, National Park Service, Lakes Environmental Association, Maine Inland Fisheries and Wildlife, Sebago Trout Unlimited, Saco Salmon Restoration Alliance, and the Portland Water District. The structure of our meetings have ranged from formal to impromptu with the purposes of establishing interactive communication and understanding watershed management interests in the region.
The information assembled through this research provides a foundation for more detailed research on channel bed dynamics and surface water-groundwater exchange based on feedback from stakeholders. Responses from fisheries-focused groups highlighted interest in stream sediment dynamics because of the implications to spawning of game species. Other groups that influenced our research approach are attentive to water quality conditions and have interests in resource optimization to preserve watershed land areas and limit nonpoint sources of nutrient pollution.

Research addressing the relations between hydrology, sediment, and channel conditions in Coastal and Central Maine is limited. This research provides groundwork for future projects aimed at expanding understanding of connections between climate, land use, surface runoff, fluvial processes, and nonpoint source pollution. Future research targets include the hydrologic effects of varied small dam management scenarios, the implications of modern-urban drainage networks to surface runoff routing, and the linkages between stream channel dynamics and surface water quality conditions. Partnerships that have been established and matured over this research project will provide a useful network to pursue related topics with a network of collaborators and stakeholder engagement.
A.5 Figures

Figure 1: The Lane/Borland stable channel stability relation.

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Figure 2: Location map indicating primary data collection points and stations used in meta-analysis.
Figure 3: Example of cluster analysis in two dimensions where K equals 3 user specified clusters. Figure 2.A through Figure 2.D are sequential representations of iterations.
Figure 4: Adaptation of Gupta (2008) indicating the components of a simple storm hydrograph for a watershed with less storage (red) and more storage (black).
Figure 5: Cluster analysis results. Orange represents “upland” clustered settings and green represents “lowland” clustered settings.
Figure 6: Recession plots of the "sensitivity function" for selected sites within the study area.
Figure 7: Recurrence interval plots with critical discharge for two monitoring stations.
Figure 8: Sediment rating curves for two monitoring stations.
Figure 9: A.) At-A-Station hydraulic geometry plotted against drainage area; B.) Graphic indicating the general shift in channel shape from smaller to large drainage areas.
Figure 10: Downstream hydraulic geometry relations from this study in blue, plotted against previous results from the region (Dudley 2004) in black.
A.6 References


B. INFORMATION TRANSFER PROJECT

N/A

B. OTHER REQUIRED DOCUMENTATION

C.1 Student Support

The graduate student supported by this project, Brett Gerard, is a fourth year PhD candidate in the School of Earth and Climate Sciences at the University of Maine. Work on this project provided the framework for his dissertation research, and the research addressed by this grant composes a chapter of his dissertation. This work provided the student with opportunities to improve skills in field data collection (stream flow measurements and geomorphological surveys), data analysis (processing, cleaning, exploratory analyses, and statistical evaluation), and numerical modeling (parametrization, calibration, and evaluation). Engagement with various individuals and groups related to the project framed stakeholder perspectives. This interaction improved the students understanding of the outside interest and applicability of this research, framing the ability to and direction with which to pursue the implementation of knowledge to action.

C.2 Notable Awards and Achievements

2016-2017 Micheal J. Eckardt Dissertation Fellowship: This fellowship was awarded to Brett Gerard for outstanding academic performance (related to research activities described by this project).

2016 Maine Sustainability and Water Conference Graduate Poster Competition (Honorable Mention): This award was given for a poster presentation of this research by Brett Gerard.

2015 University of Maine Graduate Student Exposition Poster Competition (2nd Place): This award was given for a poster presentation of this research by Brett Gerard.

2014 Senator George Mitchell Center Undergraduate Mentorship Award: This award acknowledge the mentoring activities of an undergraduate student assisting on this project by the PI and graduate student over the summer of 2013.

C.3 Presentations

Invited Presentations

Conference Presentations


**C.4 Proposal Submissions**

Senator George J. Mitchell Center for Sustainability Solutions Graduate Student and Postdoctoral Fellow Supplemental Funding. Submitted by Brett Gerard. Awarded funding in 2013 ($1000), 2015 ($500), and 2015 ($1000). *Total funding award of $2,500.*

University of Maine Graduate Student Government Grant. Submitted by Brett Gerard. Awarded funding in Spring 2014 (Requested: $850; Received: $850), Fall 2014 (Requested: $850; Received: $637.50), Spring 2015 (Requested: $850; Received: $637.50), Fall 2015 (Requested: $850; Received: $850), and Spring 2016 (Requested: $850; Received: $637.50). *Total funding award of $3,612.50.*


**C.5 Publications**

*Articles in Refereed Scientific Journals*


*Dissertations*

Plankton N cycling and detection of cyanobacterial blooms across a gradient of lake trophic state

Basic Information

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Publications

“Plankton N cycling and detection of cyanobacterial blooms in two Maine lakes”

Part A: Research Project

Summary

Our project examined dynamics of *Gloeotrichia echinulata* blooms in low nutrient Maine lakes. To better understand the causes and consequences of *G. echinulata* blooms, we sampled an oligotrophic lake (Long Pond) and a mesotrophic lake (Great Pond) weekly through the summer of 2015. At each lake, we sampled three shallow, littoral sites and two pelagic sites to better understand spatial variation in bloom events. We sampled a suite of water chemistry and physiochemical variables, as well as ammonium (NH$_4^+$) uptake and regeneration to examine how the nutrient dynamics of each lake may favor bloom events. We examined the consequences of *G. echinulata* blooms by examining genetic diversity of plankton samples and measuring the cyanotoxin microcystin-LR. Finally, we pioneered a DNA-based detection and enumeration assay for *Gloeotrichia echinulata* based on quantitative polymerase chain reaction (qPCR), and we compared the results of this assay with traditional counting techniques.

To date, our results show that oligotrophic Long Pond experiences larger *G. echinulata* blooms than mesotrophic Great Pond, and that these blooms extend from littoral to pelagic sites rather than clustering in littoral zones only. Interestingly, both lakes experienced peak *G. echinulata* blooms in early-mid August, suggesting regional cues for blooms are important. The peak blooms events also correspond with periods of stable stratification and warm temperatures in each lake. Patterns of chlorophyll-a concentrations suggest that peak *G. echinulata* blooms may be an important contributor to overall lake production during bloom events. We have successfully developed a qPCR technique to measure *G. echinulata*, and we are currently analyzing approximately 695,000 16S sequences for the planktonic bacterial community, including *G. echinulata*. We also continue to analyze samples for microcystin-LR and NH$_4^+$ uptake and regeneration processes. Upon assembly of the complete dataset, we will have an assessment of how water chemistry, nitrogen cycling, and physiochemical lake conditions interact to enhance conditions for bloom events, and we will have an understanding of how *G. echinulata* blooms influence bacterial community diversity and toxicity of the blooms.

A team of six undergraduate students have been directly involved in this research, three of whom are presenting this work at regional or international scientific conferences. We have fostered collaborative relationships between researchers at Colby College, Bigelow Laboratory for Ocean Sciences, and Bates College centered on questions of *G. echinulata* blooms. Additionally, we have strengthened collaborative ties with conservation organizations, lake associations and local citizens concerned about the impacts of cyanobacteria blooms on the Belgrade Lakes. As a result of our work, the citizen science efforts of monitoring *G. echinulata* blooms have re-surged with the assistance of the Belgrade Regional Conservation Alliance.
Problem and Research Objectives

Maine lakes are facing increasing pressures from anthropogenic activities, and water quality of many Maine lakes is in decline (McCullough et al. 2013). Cyanobacterial blooms have been increasing both globally (Brooks and Carey 2011; Paerl and Paul 2012) and in the state of Maine, with over 40 lakes listed by the Maine Department of Environmental Protection as lakes that commonly experience blooms. In particular, *Gloeotrichia echinulata* blooms in low nutrient lakes are increasingly common in Maine and more broadly across New England (Carey et al. 2012). Cyanobacterial blooms are more commonly linked to eutrophic lakes, but *G. echinulata* typically occurs in lower nutrient systems. The *G. echinulata* blooms in the northeastern US are capable of producing the hepatotoxin microcystin-LR (Carey et al. 2007). Maine DEP has measured microcystin toxin in the water of some Maine lakes and detected concentrations that were above the guideline for safe drinking water. These toxins become a concern for human health in lakes that are used as sources of drinking water and the economic costs of water treatment must be considered. It is anticipated that an increasing number of Maine lakes that serve as drinking water sources will face cyanobacteria blooms in future due to predicted climate change.

Like many other species of cyanobacteria, *G. echinulata* can fix di-nitrogen gas ($\text{N}_2$) for use in biomass production (Stewart et al. 1967). Traditionally, phosphorus (P) limitation was thought to prevent cyanobacterial blooms in low nutrient lakes, but *G. echinulata* has been shown to overcome this limitation by using P from the sediment. Although research has shown how *G. echinulata* presence may be influenced by water column N:P ratios (Noges et al. 2008) or sediment P concentrations (Carey et al. 2008; Carey et al. 2009), no previous research has directly measured N uptake, regeneration and N fixation during *G. echinulata* blooms in northeastern US lakes. N fixation is an energetically-expensive process. If *G. echinulata* have luxuriant stores of sediment P, measurement of their use of dissolved inorganic N (ammonium, $\text{NH}_4^+$) from the water column and the timing of when they resort to N fixation under different N:P scenarios will be important to improve our understanding of the role of nutrient availability in bloom formation.

Additionally, recent experimental research suggests *G. echinulata* can increase the biomass and diversity of other phytoplankton taxa, potentially by leaking N and P (Carey and Rengefors 2010; Carey et al. 2014b). Parallel measurements of microbial community structure will help to assess the impact of *G. echinulata* blooms on the diversity, structure, and ecological functions of planktonic microbial communities. The Belgrade lakes region of Maine provides aquatic ecosystems with a range of trophic states (from low-nutrient to high-nutrient ecosystems) and a range of both N and P limitation (Bruesewitz, unpublished data). These gradients of trophic status and nutrient limitation provide natural variation that will be useful for developing a better understanding of cyanobacterial bloom dynamics. As local drinking water resources become impacted, future scenarios may result in significantly more costly ways of meeting demands for freshwater (Brooks et al. 2014). Therefore, research resources directed at improved understanding of the physiochemical and biological factors that may result in cyanobacterial blooms, particularly those that produce toxins, are an important investment in securing our water resources.
Our collaborative research examined two critical pieces of information: (1) *G. echinulata* N use, including uptake of dissolved inorganic N, and (2) microbial genetics, including the characterization of microbial taxa comprising algal blooms and the amounts of toxin production. This suite of measurements allows us to examine potential physiochemical and biological controls on *G. echinulata* blooms. The physiochemical parameters we examine focus on N concentrations and N:P ratios in each lake through time, but also include high-frequency monitoring of light, temperature profiles, and dissolved oxygen at Great Pond. The biological controls focus on characterization of changes to the microbial community composition through time in each lake (see below for detail). We worked directly with a group of 6 undergraduate students who gained experience with a unique, diverse training in ecosystem ecology and molecular microbial ecology. The undergraduate students continue to be directly involved in field work, lab work, data analysis and communication of the research.

Our objectives were to:

- Measure the microbial taxonomic diversity for bacteria and microbial eukarya in Great Pond and Long Pond through the ice-free season.
- Develop a DNA-based detection and enumeration assay for *Gloeotrichia echinulata*.
- Determine how the microbial populations alter their use of NH$_4^+$ in the water column temporally through the ice-free season, determine how the uptake and regeneration of NH$_4^+$ change across lakes, and during *Gloeotrichia echinulata* blooms.
- Measure water column N fixation rates and determine how rates of N fixation change during cyanobacterial blooms.
- Measure microcystin concentrations in oligotrophic and mesotrophic lakes prior to a visible bloom of *Gloeotrichia echinulata*.
- Link the presence of microcystin to physiochemical variables, including high-frequency data collected at the buoy in Great Pond, and to *Gloeotrichia echinulata* abundance.

**Methodology**

We sampled Long Pond and Great Pond of the Belgrade Lakes region as a representative trophic gradient from oligotrophic to mesotrophic conditions to measure pelagic microbial community dynamics, toxin concentrations, and N-cycling through the ice-free season with higher frequency sampling during the summer and cyanobacteria bloom events. Long Pond and Great Pond regularly experience *G. echinulata* blooms.

Within each lake, we monitored five sampling sites weekly that were established in summer 2014 for monitoring of *G. echinulata* abundances (Figure 1). At each site, we took plankton tows for sampling *G. echinulata* abundance. We also measured a profile of physiochemical parameters, and collected samples for nutrients (Total N, Total P, NH$_4^+$ and NO$_3^-$) and extracted chlorophyll-α as well as pheophytin, an indicator of cyanobacteria. We collected additional water samples for molecular and N-cycling research as described below. The five study sites were monitored weekly from June-
August during the summer bloom season, and then move to bi-weekly sampling until no
G. echinulata were observed at any site in mid-September 2015.

Under co-PI Countway’s direction Rebecca Chmiel and Brian Kim assisted
in making a suite of molecular measurements. We estimated the
abundance of G. echinulata populations using a newly-developed quantitative PCR
assay (BioRad CFX96 real-time PCR system) for all sites on during the summer
bloom period. Changes in the microbial community structure for bacterial and
protistan assemblages will be assessed by deeply sequencing (e.g., ~20,000
sequences per sample) PCR amplicons of 16S and 18S rRNA genes on Bigelow
Laboratory’s high-throughput next-generation DNA sequencer (Illumina
MiSeq). Sequencing for 16S/18S
diversity was performed on 16 selected
samples during the expected cyanobacterial
bloom season. Preference for DNA
sequencing was given to samples from
sites in which G. echinulata blooms are
detected to determine how the blooms will impact overall microbial diversity and
taxonomic structure. Concentrations of microcystin toxins (including MC-LR, MC-RR,
MC-YR, MC-LA, MC-LW, MC-LF) and nodularin hepatotoxins will be analyzed by
Bigelow Analytical Services on a ‘Triple Quadrupole’ LC-MS (Agilent 6460). High-
precision LC-MS analysis of microcystins will be performed on approximately 30
samples from probable G. echinulata bloom locations.

Additionally, we measured several metrics of N-cycling in each lake at each
sampling event. We collected water with each sampling for $^{15}$N tracer studies. Tracer
studies were performed in an environmental chamber over a period of 24-hours.
Undergraduate students will assist in making these measurements using facilities at
Colby College. We will measure water column $^{15}$NH$_4$ uptake and regeneration using $^{15}$N
tracers and a newly developed LC-MS-TOF technique to measure 14:15N ratios. These
methods use conventional colorimetric methods for NH$_4^+$ to produce colored indophenol
(Patton and Crouch 1977; Moorcroft et al. 2001). Total concentrations are obtained from
the visible absorbance of the dye complexes. Isotope ratios are obtained from the relative
mass abundance of the $^{15}$N to $^{14}$N complexes using Electrospray- Time Of Flight Mass
Spectrometry.

We had planned to measure water column N fixation rates bi-weekly using N$_2$:Ar
ratios of water samples measured on a Membrane Inlet Mass Spectrometry (McCarthy et
al. 2013). However, after attending the Society for Freshwater Sciences meeting in
Milwaukee, WI in May 2015, we were made aware of several methodological problems
with this method for measuring N fixation rates. The largest concern was that it appears
virtually impossible to stop bubble formation in the sample tubes as a result of primary production during daylight hours of incubation, which greatly impede the ability of the MIMS to measure real changes in N$_2$:Ar over time. After consultation with a number of colleagues, we elected to use an acetylene technique to measure N fixation in large glass syringes. Unfortunately, we had great difficulty in getting the necessary equipment for doing these experiments, and we did not receive all the needed equipment until August 2015. Therefore, we will be measuring N fixation this summer, along with the N uptake experiments and ongoing monitoring of G. echinulata in Great Pond and Long Pond.

Once all variables are measured, we will use network analysis (Steele et al. 2011) to link the biotic and physiochemical variables to reveal significant correlations that may suggest direct interactions and/or processes that structure the microbial communities in each season and lake trophic state. We will take particular advantage of the high-frequency monitoring data collected on Great Pond for this analysis, including metrics of gross primary production and lake stratification. These metrics have been calculated for the 2015 season (see results below).

**Principal Findings and Significance:**

Given that our sampling and experiments continued into Fall 2015, our analyses for this project are ongoing. To date, we have completed the density counts of G. echinulata at all sites across all dates, as well as our water chemistry and chlorophyll analyses. We have successfully developed a qPCR process for *G. echinulata* and now have an extensive dataset of 16S sequences from Great and Long Ponds to measure bacterial community diversity. We completed 50 NH$_4^+$ uptake experiments for each lake, and these samples are currently being run on the TOF mass spectrometer after refinement of our analytical procedure. Toxin analysis is also ongoing. Below, we present a summary of our results to date.

This research addresses key water quality issues for Maine lakes. Much of our previous knowledge on the dynamics of *G. echinulata* blooms in Maine lakes was focused on lower-frequency sampling. Through our weekly sampling of 5 sites in both Long Pond and Great Pond, we have significantly increased our understanding of the spatial and temporal variation of these blooms across the oligotrophic to mesotrophic trophic state of lakes. Once our toxicity analyses are complete, we will be able to increase our understanding of how these spatial and temporal patterns of blooms relate to the toxicity of the blooms. We received strong interest by the local community regarding their interest in the issue of algal blooms and the potential for these blooms to be toxic. PI Bruesewitz presented to the Maine Lake Trust, a group of leaders of the various Belgrade Lake Associations during summer 2015 on the preliminary results of this research. We worked with the Belgrade Regional Conservation Alliance (BRCA; [www.belgradelakes.org](http://www.belgradelakes.org)) to link our quantitative *G. echinulata* data to the ongoing citizen science monitoring of *G. echinulata* blooms in both lakes. Student researchers have begun to explore connections between the citizen science data record of blooms, which categorize blooms based on citizen observations, to our density counts. The citizen science efforts to monitor blooms are continuing under the guidance of the BRCA, and our sampling of *G. echinulata* densities will continue as well. Thus, this project has done
much to strengthen our relationship with the many lake associations active in the Belgrades and with the ongoing efforts of the BRCA.

This grant has done much to further research collaborations between the Bruesewitz and King labs at Colby College and the Countway lab at Bigelow Laboratory. Once we are able to complete the analyses for this project, we plan to submit a proposal to the National Science Foundation in January 2017 to continue this research. Within the past several months, PI Bruesewitz has contributed to 2 grant submissions that are related to cyanobacterial blooms and their toxicity (see below).

Our results to date refine our understanding of the spatial and temporal dynamics of *G. echinulata* blooms in Long Pond, an oligotrophic lake, and Great Pond, a mesotrophic lake (Figures 2). The peak blooms of *G. echinulata* in Great Pond occurred in early to mid-August, and there were no colonies observed in the water by the end of September (Figure 2A). Great Pond blooms were smaller in comparison to Long Pond blooms. Long Pond blooms also peaked in early to mid-August, similar to Great Pond (Figure 2B). However, the magnitude of the blooms were higher, up to ~50 colonies/L.

When sites are categorized into deep, pelagic sites or shallow, littoral sites, we see that the deep sites follow similar patterns of the littoral sites. This suggests that the *G. echinulata* blooms are ubiquitous across the lake, not necessarily concentrated in shallow bays where sediment recruitment may be high.

The molecular component of this project had three primary goals which included:

1) **Sequencing the 16S rRNA gene and Intergenic Transcribed Spacer (ITS) region of the *Gloeotrichia* from the Belgrade Lakes**

2) **Developing a real-time quantitative polymerase chain reaction (qPCR) assay and DNA standards for *Gloeotrichia* based on the sequences that were generated above**

3) **Conducting next generation DNA sequencing** to evaluate the lake bacterial community at times of low, medium, and high *Gloeotrichia* abundance (based on qPCR data).
An ancillary goal was to attempt cultivation of *Gloeotrichia* to enable year-round study of this organism. Unfortunately, cultivation was not successful due in part to the complex life-cycle of *Gloeotrichia*, which includes a period of over-winter dormancy in close association with lake-bottom sediments. At best, we were able to keep colonies of *Gloeotrichia* in relatively good shape for about one month, after which the colonies would begin to disintegrate. A manuscript detailing the overall results of our study is currently in preparation. The highlights of our molecular-based investigations are summarized below.

**DNA sequencing of *Gloeotrichia* isolates**

*Gloeotrichia* colonies were collected from Long and Great Ponds during the summer of 2015 to genotype the Maine variety of *Gloeotrichia* (Fig. 3). One additional sample from Lake Auburn was provided to the project by Dr. Holly Ewing of Bates College. Our initial genotyping efforts focused on the 16S rRNA gene, which remains the ‘gold standard’ for microbial identification, and represents the gene with the greatest taxonomic representation in public databases. We were successful in generating 16S rRNA gene sequences for *Gloeotrichia* from all three lakes using PCR primers (CYA106F and 1492R) that were modified for this project to promote the amplification of cyanobacterial DNA. In addition, we generated Intergenic Transcribed Spacer (ITS) region sequences for *Gloeotrichia* from Long and Great Ponds using the PCR primers 16S-1247F and 23S-241R. There are very few *Gloeotrichia* 16S DNA sequences in public DNA databases (e.g., GenBank, Silva, Greengenes) and no *Gloeotrichia* sequences that we could locate representing the ITS region. The sequences in our study represent the first *Gloeotrichia* sequences that will be deposited into public databases from any site in the northeastern United States for both 16S rRNA genes and the ITS region. Our *Gloeotrichia* 16S rRNA gene sequences were most similar to 16S sequences from *Gloeotrichia echinulata* that were isolated from a lake in Finland (Sihvonen et al. 2007), demonstrating 99.6% similarity in nucleotide sequence over 1366 positions (Figure 8). *Gloeotrichia* 16S sequences from Long and Great Ponds (n = 7 sequences) were all exactly the same length (1366 nucleotides) and nearly identical, varying by just a few nucleotides among all sequences in the collection. Putative *Gloeotrichia* ITS sequences were identified by the nearly perfect alignment of the first ~200 nucleotides at the 5’ end of the ITS region to the 3’ end of the corresponding *Gloeotrichia* 16S gene sequences from our lake samples. It was necessary to perform this ‘internal
identification’ of the *Gloeotrichia* ITS sequences since there were no ITS sequences reported for *Gloeotrichia* in the scientific literature, nor any sequences deposited in GenBank by other researchers. The cyanobacterial sequences in GenBank that were most closely related to our *Gloeotrichia* ITS sequences are only 85% similar and represent other distantly-related cyanobacterial genera. Our *Gloeotrichia* ITS sequences will be the first of their kind reported in the literature and deposited in GenBank. The hypervariable nature of the ITS sequence proved useful for our goal of developing a quantitative PCR assay is highly-specific for *Gloeotrichia*.

**Development of *Gloeotrichia* qPCR**

Although colonies of *Gloeotrichia* are large and unmistakable in water samples, it is unclear if enumeration of *Gloeotrichia* based on counting the colonies that aggregate near the surface of lakes provides an accurate picture of *Gloeotrichia* bloom dynamics. Additionally, the ‘colony-based’ counting approach does not include *Gloeotrichia* cells that are not a part of recognizable colonies or cells that have settled into the lake sediments during periods of dormancy. Given these potential limitations, we developed the first quantitative PCR assay to enumerate *Gloeotrichia* in both lake water and sediment samples. The assay was designed using the new DNA sequences that we generated for the *Gloeotrichia* ITS region along with other ITS reference sequences from a variety of cyanobacteria. Specifically, the ‘TaqMan’ qPCR assay that we designed included two primers and a dual-quenched fluorescently-labeled probe that were synthesized by IDT, Inc. (Coralville, IA). Compared to other ‘flavors’ of qPCR that rely on DNA stains (e.g., Sybr Green) in the reaction mixture to provide the fluorescent signal, the TaqMan approach provides a greater level of specificity with both primers and a DNA probe, and a generally higher signal to noise ratio.

**Figure 4.** *Gloeotrichia* abundance data derived from qPCR analysis for Long Pond station #1 and Great Pond station #1. Similarity of trends suggest the microbial connectivity of the two lake ecosystems. These two stations were selected for high-throughput 16S rRNA gene sequencing to investigate the diversity of the entire bacterial community before and during *Gloeotrichia* blooms.
The qPCR approach relies on estimating copy number of the target gene based on calibration with DNA standards that are either cell-based, or cloned PCR products. Given that *Gloeotrichia* cannot currently be maintained in culture, and the fact that it exists in tightly clustered colonies (making discrete cell counting impossible), standards were developed from cloned PCR products from the *Gloeotrichia* ITS region. We assumed that each *Gloeotrichia* cell has two copies of the ITS region within its genome given the reported copy numbers of the rRNA operon from other better-sequenced cyanobacteria. Total *Gloeotrichia* cell numbers were therefore estimated by dividing qPCR-derived ITS copy number estimates by two. A total of 50 lake samples were analyzed by *Gloeotrichia* qPCR, representing ten stations (five per lake) that were sampled on five dates during the summer of 2015. Full DNA extractions were performed on the samples, creating a valuable resource for further molecular investigations in the future. For example, qPCR for any conceivable lake microbe could be conducted on these same DNA samples – which will be archived at Bigelow Laboratory indefinitely. The TaqMan assay revealed *Gloeotrichia* in 49 of the 50 samples, with a peak abundance of 808,667 (±56,847) cells per liter on August 10, 2015 along the eastern shore of Great Pond. The key advantage of qPCR is its extreme sensitivity – with capabilities of detecting as little as a few 10s of cells in a liter of water. In general, *Gloeotrichia* abundance varied from station to station on a given date, but within a particular site, there were clear seasonal trends in *Gloeotrichia* abundance (Fig. 4). The two sampling locations from Long and Great ponds that are physically closest to one another (separated by Belgrade Stream) were highly similar with respect to the trends in *Gloeotrichia* abundance data, which is suggestive of the connectivity of the two lakes.

**Next generation DNA sequencing of the lake bacterial community**

The broader bacterial community was analyzed for a subset of the stations that were sampled for *Gloeotrichia* abundance by qPCR using the ‘next generation’ (Next-Gen) high-throughput DNA sequencing approach. Twenty Figure 5. Relative abundance of bacterial taxa from August 10, 2015 in Great Pond at station #1. A total of ~50,000 sequences were generated to characterize the lake microbial community for this date and location. Although *Gloeotrichia* was detected at ~150,000 cells per liter via qPCR, cyanobacteria were a relatively small fraction of the bacterial taxa that were detected.

Samples (four stations on five dates) were sequenced via Illumina MiSeq DNA sequencing of the variable V4 region of the 16S rRNA gene. A total of ~695,000 sequences were generated from the 20 DNA samples. Of these, approximately 1,000 were a perfect match to the V4 region of our cloned *Gloeotrichia* 16S sequences. To our knowledge, the sequences from this project represent the largest collection of *Gloeotrichia* sequences ever assembled. The data are still in the process of being analyzed. In particular, we will be comparing the taxonomic structure and diversity of the bacterial community from each of the 20 discrete samples to every other sample in the study. This should reveal trends in the diversity of the lake bacterial assemblage in relation to the abundance of *Gloeotrichia*. Previous data have suggested that the presence of *Gloeotrichia* has a positive effect on the growth (and presumably the abundance) of
some phytoplankton species (Carey & Rengefors 2010). It has not been determined yet whether *Gloeotrichia* has a similar effect on the diversity and abundance of the other members of the bacterial community. In general, the bacterial community revealed by MiSeq DNA sequencing was highly diverse, representing a broad range of bacterial phylotypes even when *Gloeotrichia* was at its highest abundance (Fig. 5).

**Outreach – handheld, smartphone-enabled qPCR**

Through a collaboration with a Philadelphia-based startup company, we have been able to demonstrate our *Gloeotrichia* qPCR assay to the general public and interested stakeholders using a handheld qPCR device. It is envisioned that qPCR technology will soon be within reach of citizen scientists and that assays like cyanobacterial testing will be of interest to groups outside of the traditional research community. We have already receive positive feedback from several stakeholder groups on the development and accessibility of this new technology. We will continue to promote our research via demonstrations of the qPCR methodology, which is enabled by smartphone technology through an integrated iPhone.

**Preliminary data linking Gloeotrichia blooms to physiochemical parameters**

Chlorophyll-a concentrations, an indicator of phytoplankton activity, appear to peak for both lakes towards the end of July (Figure 6), with pheophytin (suggestive of cyanobacteria including *G. echinulata*) peaking in late July to early August (Figure 7). Peak stability and the warmest surface water temperatures (Figure 8) occurred in Great Pond in early-mid August, corresponding to peak *G. echinulata* densities. These in-lake cues of a warm, stable water column may contribute to conditions that allow for *G. echinulata* blooms. Measurements of primary production show a pattern of high GPP in the early summer, and consistent rates of GPP through July and August, with no clear signals that coincide with *G. echinulata* blooms. This may be because *G. echinulata* bodies are large in comparison to other plankton, and they are dispersed through the water column. Therefore, detection of their contribution to primary production may depend on distance of a *G. echinulata* bloom from the deployed oxygen sensor.
Figure 6: Extracted chlorophyll concentrations in Great Pond (black circles) and Long Pond (gray circles) during summer 2015. Data are averages of 5 sampling sites with standard error.

Figure 7: Pheophytin concentrations in Great Pond (black circles) and Long Pond (gray circles) over the summer of 2015. Data are averages of 5 sampling sites with standard error.
Figure 8: Total phosphorus (TP) in Great Pond (black circles) and Long Pond (gray circles). Weekly data are represented as the average of all 5 sampling sites in each lake with standard error bars.

Figure 1: A heat map of summer 2015 in Great Pond, showing the onset of seasonal stratification and fall mixing. Data are derived from high-frequency buoy data.
Figure 2: Gross Primary Production (GPP) and respiration (R) and net ecosystem production (NEP) of Great Pond based on high-frequency measurements at the buoy and one of our sampling points. GPP is shown in white circles, R in black and NEP in gray circles.
Below are a series of photos from our project.

Figure 3: Undergraduate students Brian Kim and Rebecca Chmiel collecting water samples from Great Pond.

Figure 4: Undergraduate student Brian Kim sampling Great Pond.
Figure 5: Rebecca Chmiel working on G. echinulata at Bigelow Laboratory.

Figure 6: Colby College undergraduate students Rebecca Chmiel and Brian Kim presenting their research at the Maine Sustainability and Water Conference, March 2016 with Dr. Peter Countway.
Part C: Other documentation

Student support

Our research project supported a team of undergraduate researchers, through summer research assistantships as well as during the academic year. Many of our students have presented this research at regional or international scientific conferences. In general, each student received experience in the field and the laboratory, as well as opportunities to work with the PIs and other scientists at Bigelow Laboratory, Colby College, and Bates College. A number of our students have aspirations towards graduate work in limnology or marine science.

Rebecca Chmiel ('17) is an Environmental Studies and Chemistry major at Colby College. Rebecca worked primarily at Bigelow Laboratory with Dr. Pete Countway and Dr. Stephen Archer during the summer of 2015. She will be presenting the results of this research at the Association for Limnology and Oceanography (ASLO) meeting in Santa Fe, NM in June 2016, and she presented her research and the Maine Sustainability and Water Conference in Augusta ME in March 2016 as well. Rebecca has become very involved in limnology research, and she continues to work in the Bruesewitz lab. This summer, she will work with Dr. King on the Belgrade Lakes Water Quality Initiative, and in the fall she will begin an honors thesis project on microcystin toxicity in G. echinulata. Rebecca has attended 2 annual meetings of the Northeast GLEON (Global Lakes Ecological Observatory Network), and is interested in pursuing graduate work in limnology. Through this project, she was exposed to aspects of experimental design, fieldwork, lab work, genetic analyses, and data analysis. Her engagement across the fields of biogeochemistry and microbial genetics were a unique cross-section of research in freshwater ecosystems. Rebecca was able to interact with stakeholders at the Maine Lakes Resource Center as well as at the Maine Sustainability and Water Conference. These interactions allowed her to see the importance of work on cyanobacteria blooms to the local community.

Brian Kim ('18) is an Environmental Studies and Biology major at Colby College. Brian worked full time in my lab on this project during Summer 2015 doing field sampling of the lakes, the N experiments in the lab, and other variables such as water chemistry and chlorophyll-a analyses. Brian continued this work during the academic year, and will be presenting on this project at the Society for Freshwater Science meeting in Sacramento CA in May 2016. Brian was exposed to many aspects of limnological research as a result of this work, and he is interested in pursuing graduate work in either limnology or marine sciences. Brian is still deciding which research path interests him the most, but as a result of this work he was introduced to a large network of limnologists, and he would be very well poised to being a graduate program in limnology. As a part of weekly fieldwork, Brian regularly interacted with local community members as well as staff at the Maine Lakes Resource Center. One of our field sites was at a community member’s dock, and Brian and another student Harriet developed a relationship with them. I believe that this helped reinforce the importance of our work to the local community on a regular basis.
Harriet Rothschild (’16) is an Environmental Studies major at Colby College. Harriet worked full time in my lab on this project during Summer 2015 doing field sampling of the lakes, the N experiments in the lab, and other variables such as water chemistry and chlorophyll-a analyses. Harriet continued the work in the fall as part of her senior honors thesis, and she is nearing completion of her thesis. Like Brian, Harriet regularly interacted with community members and staff of the Maine Lakes Resource Center throughout the project. After graduation, Harriet is moving towards the medical sciences, however I believe the experiences she gained doing this research will carry with her into her career.

Serena Haver (’16) is an Environmental Studies major at Colby College. Serena is interested in public health, and as a separate project is completing an honors thesis on pharmaceutical compounds in the Belgrade Lakes. Serena worked in the lab on this project as a G. echinulata counter during the Fall 2016 semester. Working towards the formidable goal of counting hundreds of G. echinulata samples was a major project, and the experience helped Serena navigate many common challenges of laboratory work. She and her lab partner Clea were able to work towards small goals through the academic year to achieve the overall goal of counting all the samples for the project. Due to the nature of her lab-based work, Serena did not have an opportunity to interact with stakeholders as a part of this project.

Clea Harrelson (’16) is an Environmental Studies major at Colby College. Clea worked in the lab on this project as a G. echinulata counter during the Fall 2016 semester. Working towards the formidable goal of counting hundreds of G. echinulata samples was a major project, and the experience helped Clea navigate many common challenges of laboratory work. She and her lab partner Clea were able to work towards small goals through the academic year to achieve the overall goal of counting all the samples for the project. Due to the nature of her lab-based work, Clea did not have an opportunity to interact with stakeholders as a part of this project.

Zachery Mondschein (’17) is an Environmental Studies major at Colby College. Zachery worked with Dr. King on refining the procedure for the $^{15}$N isotope analysis on the time-of-flight mass spectrometer. His work has been from Fall 2015 into January 2016. Zachery’s research provided him with experience in instrumental chemistry, the process of refining a laboratory procedure, and functioning of mass spectrometry. He also saw how these fundamental chemistry concepts had ties to limnology and ecology. Due to the nature of his lab-based work, Zachery did not have an opportunity to interact with stakeholders as a part of this project.

**Awards and achievements**

n/a

**Presentations**

Colby Undergraduate Summer Research Retreat (CUSRR)

Date: July 23-24, 2015

Presenter: Rebecca Chmiel
Oral Presentation

Title: *Gloeotrichia echinulata* presence and toxicity in the Belgrade Lakes

Abstract:

The state of Maine relies on the health and quality of its lakes, which make up a significant portion of the state’s economy and public drinking water systems. However, the water quality of many of Maine’s lakes is decreasing as cyanobacterial blooms are increasing, most notably in relatively clear, low-nutrient systems like the Belgrade Lakes. *Gloeotrichia echinulata* is one such cyanobacteria species that is commonly found in lakes throughout the northeast United States, yet little is known about the timing, duration, and toxicity of its blooms, or the environmental factors that favor blooms. The increasing presence of cyanobacterial blooms threatens local property values, the recreation potential of lakes, and their use as sources of drinking water due to the associated toxins and algal scums that can be produced during a bloom. It is imperative that Maine communities like those around the Belgrade Lakes determine the concentration and distribution of toxins in their lakes and the link between cyanobacteria and toxin presence. This summer, I am working in collaboration with Bigelow Laboratory to create a novel DNA-based assay for harmful cyanobacterial species such as *Gloeotrichia* that would allow us to quantify and map these potentially toxic blooms. This assay will use real-time quantitative PCR system to detect the 16S bacterial rRNA gene using primers that are specific to *Gloeotrichia* found in the Belgrade Lakes. I am also analyzing the relationship between *Gloeotrichia* abundance and microcystin toxin concentration to determine if and/or when *Gloeotrichia* is an important driver of microcystins in the lake system. Toxin analysis will use liquid chromatography in conjunction with triple quadrupole mass spectrometry (QqQ-LC/MS) to determine the presence and concentration of selected toxins in the Belgrade Lakes. We expect that our research will improve our understanding of the nature and severity of cyanobacteria blooms in northeastern lakes.

Colby Undergraduate Summer Research Retreat (CUSRR)

Date: July 23-24, 2015
Presenter: Harriet Rothschild and Brian Kim

Title: Nitrogen cycling in *Gloeotrichia echinulata* blooms in the Belgrade Lakes

Abstract:

*Gloeotrichia echinulata* are a cyanobacteria that are common in many clear-water lakes in Maine. *Gloeotrichia* appear in lakes during mid summer and last until fall. In the field, we are measuring the density of the *Gloeotrichia* colonies throughout the summer. In the lab, we are monitoring their Nitrogen uptake rates to understand how they influence the lake’s Nitrogen cycle. Our partners at Bigelow Laboratories in Boothbay Harbor are extracting *Gloeotrichia* DNA for further analysis along with researching any potential toxins. This research on *Gloeotrichia* is crucial to the Belgrade Lakes and can be applied to the entire state of Maine. The *Gloeotrichia* in the Belgrades have been blooming earlier in the summer and tend to concentrate in shallow portions of the lake. Our goal is to learn more about the how the *Gloeotrichia* affect the Nitrogen cycle and the implications on the health of the Belgrade Lakes. Limited research has been conducted on *Gloeotrichia* in Maine and it is unclear if and when the blooms will disappear. In the meantime, our research accompanies other organizations’ efforts to understand how the colonies redistribute nutrients throughout the lake. On a day-to-day basis, our sampling raises awareness in the community on lake health. In the future we hope to contribute information on the intricacies of *Gloeotrichia* nitrogen cycling to the Limnology community. Our research will help to preserve the Belgrade Lakes for future generations to enjoy.

Bigelow REU Symposium

Date: August 13, 2015
Presenter: Rebecca Chmiel

Oral Presentation

Title: *Gloeotrichia* in Maine Lakes: An analysis of genetic diversity and DNA-based detection.

Abstract:

The state of Maine relies on the health and quality of its lakes, which make up a significant portion of the state’s economy and public drinking water systems. However, the water
quality of many of Maine’s lakes is decreasing as cyanobacterial blooms are increasing, most notably in relatively clear, low-nutrient systems like the Belgrade Lakes (Maine, USA). Gloeotrichia echinulata is one such cyanobacteria species that is commonly found in lakes throughout New England, yet little is known about the genetic diversity, timing, duration, and toxicity of its blooms. Using genetic cloning techniques, I sequenced the 16S rRNA gene of Gloeotrichia from the Belgrade Lakes and compared the diversity of these sequences to Gloeotrichia and related taxa from other locations. The 16S sequences were used for the creation of a quantitative PCR assay for Gloeotrichia and other harmful cyanobacteria that will allow us to quantify, map, and provide ‘early warning’ of these potentially toxic organisms in our freshwater resources.

Colby Environmental Science Capstone
Date: December 3, 2015
Presenter: Harriet Rothschild
Oral Presentation
Title: *Gloeotrichia echinulata*: Monitoring, Effects, and Management in the Belgrade Lakes
Abstract:
Cyanobacterial blooms in oligotrophic lakes are a result of excess nutrients and should be carefully studied. Further research is necessary to understand luxury P uptake from the sediment during recruitment because it could mitigate the positive effects of alum treatment. *G. echinulata* can serve as a warning sign for lake eutrophication and specific densities have been shown mark tipping points between eutrophication states.

We are researching *G. echinulata*, a cyanobacteria species that has been increasingly forming blooms in the Northeastern United States. Our study sites are Long Pond and Great Pond in the Belgrade Lakes region which is a popular location for summer tourism and year-round residents. Research into *G. echinulata* is important to the Belgrade community because of potential effects to water quality, public health, and recreation. We are specifically looking at the patterns of *G. echinulata* bloom density throughout the summer and how it may affect the phosphorous cycle, the nitrogen cycle, and the plankton community in general. Our research question how does *G. echinulata* influence nutrient dynamics, phytoplankton communities, and human health within the Belgrade Lakes? Specifically, what are the characteristics of bloom densities throughout the summer? What is the relationship between *G. echinulata* colonies and phosphorous loading in the pelagic zone? Is there a relationship between *G. echinulata* density and shifts in the general plankton community? How can citizen science be used as a tool for future monitoring of *G. echinulata* in the Belgrade Lakes? We hypothesize that the density of *G. echinulata* shifts throughout the summer with peak densities occurring in July. We also suspect that wind direction and lake shape have a large influence on the distribution of *G. echinulata*. We hypothesize that *G. echinulata* transport P from the sediment to the epilimnion during recruitment and therefore have a large affect on the P cycle. Because *G. echinulata* is able to fix N from biologically unavailable dinitrogen gas, we hypothesize that it can increase the overall N concentration. Due to the possible increase in nutrients, we hypothesize that the general plankton community will increase. Lastly, we hypothesize that citizen science observations can be used as an effective tool for long-term monitoring of *G. echinulata*.

Colby at Bigelow Semester Presentation
Date: December 17, 2016
Presenter: Brian Kim
Oral Presentation – No Abstract

Maine Sustainability and Water Conference
Date: March 29, 2016
Presenter: Rebecca Chmiel
Poster
Title: Cyanobacteria blooms in low-nutrient Maine Lakes: Development of a qPCR Assay for *Gloeotrichia echinulata* using the ITS region
Abstract:
Maine’s lakes contribute to a significant portion of the state’s tourism economy and its public drinking water supply. Across the nation, the water quality of lakes is declining as toxic cyanobacterial blooms become more widespread. The cyanobacterium Gloeotrichia echinulata is increasingly found in lakes throughout New England, including those in our study region (Belgrade Lakes, Maine, USA). Very little is known about the timing, duration, and toxicity of G. echinulata blooms in Maine lakes and even less is known about its genetic diversity. We performed DNA sequencing on isolates of G. echinulata from our study sites for comparative phylogenetic analysis and for development of a quantitative PCR assay that was used for rapid determination of G. echinulata abundance. Time-series samples collected during the summer of 2015 were assayed via qPCR, indicating the presence of G. echinulata cells in almost every lake sample, with peak abundances approaching 200,000 cells per liter. The new qPCR assay will allow us to provide ‘early warning’ of these potentially toxic organisms in our freshwater resources prior to the onset of visually-noticeable blooms.

*** Same abstract used for Rebecca Chmiel CLAS and ALSO presentations

Maine Sustainability and Water Conference
Date: March 29, 2016
Presenter: Harriet Rothschild and Brian Kim
Poster
Abstract:
The use of 15-nitrogen (N) stable isotope tracers is a valuable tool for understanding N cycling in aquatic ecosystems, and the experimental techniques using $^{15}$N tracers range from mesocosms to stream. Traditionally, analytical measurements of $^{14}$N:$^{15}$N ratios involve incubations to concentrate N onto filters for analysis by isotope-ratio mass spectrometry. This process is time-consuming and often requires large sample volumes, a challenge for microcosm experiments. Here, we present a technique for measuring $^{14}$N:$^{15}$N in ammonium using ESI-TOF mass spectrometry. Small volumes of samples (<10 mL) are complexed with phenol using the Bertheiot reaction, producing the traditional indophenol blue complex (mass 198 AMU). Excess reagents are removed and the indophenol is concentrated using an automated C18 solid phase extraction. The extracts are run through a spectrometer to measure total concentration and passed to the ESI-TOF to obtain the $^{14}$N:$^{15}$N ratio. This technique, by allowing determination of ammonium concentrations and isotopic ratios at the ppb level, can be used to better characterize nitrogen cycling in lake and estuarine systems.

NE GLEON 2016
Date: April 16-17, 2016
Presenters: Brian Kim and Rebecca Chmiel
Oral Presentation – No abstract

Colby Liberal Arts Symposium
Date: April 28, 2016
Presenter: Rebecca Chmiel
Poster

Colby Liberal Arts Symposium
April 28, 2016
Presenter: Harriet Rothschild
Presentation
Title: The effect of Gloeotrichia echinulata blooms in the Belgrade Lakes
Abstract:
Gloeotrichia echinulata is a cyanobacteria species that has been increasingly forming blooms in the Northeastern United States. Long Pond and Great Pond in the Belgrade Lakes region are popular locations for summer tourism and year-round residents. Research into G. echinulata is important to the Belgrade community because of potential effects to water quality, public health, and recreation. Studying G. echinulata bloom density throughout the summer and how it may affect the phosphorous cycle, the nitrogen cycle, and the plankton
community in general will help scientists inform policy makers on water quality initiatives. Further research is necessary to understand luxury P uptake from the sediment during recruitment because it could mitigate the positive effects alum treatment. G. echinulata can serve as a warning sign for lake eutrophication and specific densities have been shown mark tipping points between eutrophication states.

The use of 15-nitrogen (N) stable isotope tracers is a valuable tool for understanding N cycling in aquatic ecosystems. Traditionally, analytical measurements of 14N:15N ratios involves a time consuming process of incubations to concentrate N onto filters for analysis by isotope-ratio mass spectrometry. The process also requires large sample volumes, which is a challenge for microcosm experiments. Here, we present a technique for measuring 14N:15N in ammonium using ESI-TOF mass spectrometry, to better characterize nitrogen cycling in lake and estuarine systems.

**Society of Freshwater Sciences 2016 Summer Meeting**
May 21-26
Presenter: Brian Kim
Poster
**Title:** The abundance, distribution, and toxin production of *Gloeotrichia echinulata* blooms in Maine Lakes and its Impact on Microbial Diversity and Human Health
**Abstract:**
Cyanobacterial blooms in freshwater ecosystems are increasing in frequency and magnitude across North America. Many of these blooms consist of species that are known or suspected toxin producers. *Gloeotrichia echinulata* is one example that has been confirmed to produce microcystin-LR. Although cyanobacterial blooms typically occur in eutrophic lakes during periods of elevated temperature, *G. echinulata* has been thriving and consistently found in clear, low nutrient, oligotrophic lakes. The Belgrade Lakes, a chain of seven lakes ranging from oligo-mesotrophic to eutrophic, is one region in Maine that has recently developed continuous annual blooms of *G. echinulata*. Unfortunately, very little is known about the duration, distribution, toxicity, and impact on local ecosystems of these blooms. The ever-increasing use of freshwater resources in Maine is placing the public on a collision course with a potentially toxic cyanobacterium. Therefore, using genetic cloning techniques, we have sequenced the hypervariable ITS region of *G. echinulata* to develop a species-specific assay. The ‘TaqMan’ probe qPCR assay may allow us to quantify and map blooms in order to predict and provide warning for future potentially toxic blooms.

**ASLO 2016 Summer Meeting**
June 5-10, 2016
Presenter: Rebecca Chmiel
Poster

*Gloeotrichia echinulata* in the Belgrade Lakes. Presented by Denise Bruesewitz in July 2015 to the Maine Lakes Trust in Belgrade Village at the Maine Lakes Resource Center (Presentation to community stakeholders, including representatives of 6 lake associations).

**Proposal Submissions**
1. “A comprehensive assessment of modern microcystin analyses in a realistic environmental setting” Submitted by Stephen Archer (Bigelow Laboratory) and Denise Bruesewitz (Colby College) to The Water Research Foundation Emerging Opportunities Program for $149,989 on 2 March 2016. Status: Submitted.
2. “Preliminary Proposal: Using ecosystem metabolism to capture cyanobacteria-mediated changes in low-nutrient lakes in transition” Submitted by Kathy Cottingham (Dartmouth University) and co-PIs Denise Bruesewitz (Colby College), Cayelan Carey (Virginia Tech) and David Richardson (SUNY New Paltz) to the National Science Foundation. Preproposals do not require a budget, submitted on 25 January 2016. Status: Pre-proposal Submitted.

Publications
This work is not yet published, but we will submit two manuscripts based on this work; one focused on the genetics and one on the nitrogen cycling research. Both will include undergraduate student co-authors.
References


Toward a more efficient UV disinfection system: The development of a TiO2-based photocatalyst to enhance the degradation of taste and odor compounds in drinking water

Basic Information

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Publications

A. RESEARCH PROJECT

Toward a more efficient UV disinfection system: The development of photocatalysts to enhance the degradation of taste- and odor-causing compounds in drinking water

Sudheera Yaparatne and Aria Amirbahman

Summary

Today UV disinfection is becoming a growing trend in most of the public water systems because it is a safe alternative for primary disinfection and is free of harmful byproducts associated with chemical disinfection. Pathogenic bacteria and protozoa are readily inactivated by direct UV. However, virus inactivation and removal of taste and odor compounds are not effective under UV irradiation. In this study, efficiency of UV disinfection is increased by using immobilized semiconductor photocatalyst (TiO$_2$) to degrade one of the major taste and odor compound, MIB (2-methyl isoborneol). Degussa P-25 powder modified TiO$_2$ (50g/L) was immobilized (via dip coating) on soda lime microscopic glass slides using TiO$_2$-SiO$_2$ sol gel mixture as the binder and calcined at 500 °C. These glass slides were characterized using X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). Photocatalytic degradation of MIB was investigated by irradiating aqueous MIB under UV-A light (350 nm) in the presence of P25-TiO$_2$ immobilized glass slides. Determination of MIB in water samples was performed by headspace solid phase microextraction (HS-SPME)/gas chromatography–mass spectrometry (GC–MS). According to the obtained results, powder-modified photocatalyst films that contained 3% molar concentration of Si showed an effective (around 80%) degradation of MIB within an hour.

Problem and Research Objectives

Access to safe drinking water is very important as a health concern and is a basic human right. Supply of adequate, safe and accessible water has gained a fair amount of interest by most of the authorities at local, national, and global levels not only because it is a health and development issue, but also it is an economic issue. According to the US EPA, there are five major types of contaminants in drinking water: microorganisms, disinfection byproducts, inorganic chemicals, organic chemicals, and radionuclides. In 2006, the EPA introduced the long-term 2 enhanced surface water treatment rule (LT2) to further reduce contamination by pathogens in drinking water. UV disinfection is one of the options that has gained more interest in public water systems. Though direct UV photolysis is effective in inactivating pathogenic microorganisms such as protozoa, it is not very effective with respect to inactivation of viruses and removal of taste and odor causing compounds. The changing climate, especially increasing average temperatures have led to water quality deterioration in many temperate regions. Longer periods of higher temperatures result in longer periods during which some lakes remain anoxic. Anoxia can lead to the release of phosphorus from hypolimnetic sediment in water bodies, which causes undesired algal blooms. Many lakes in temperate regions, including Maine, have experienced increased turbidity and algal blooms, including harmful algae.
Taste and odor compounds are mainly produced by cyanobacterial blooms as secondary metabolites. Removal of these taste and odor compounds is difficult because their odor threshold is very low, in the range of ng L\(^{-1}\).

Previous studies have shown that most of the taste and odor causing compounds are resistant to conventional water treatment techniques, such as coagulation, sedimentation and filtration, especially at very low concentrations. Disinfectants and oxidants such as Cl\(_2\), ClO\(_2\), and KMnO\(_4\) have proven to be ineffective in degrading these taste and odor compounds due to the resistance of tertiary alcohols towards mild oxidation\(^1\). Only few conventional water treatment methods have been successful in removing these compounds at such low concentrations. Among these treatment methods granular activated carbon, powder activated carbon, alum coagulation and sand filtration are used in some of the water treatment facilities\(^2, 3\). In the presence of dissolved organic matter (DOM), reduced adsorption of these contaminants was observed and additional treatments were needed to clean the saturated activated carbon for reuse\(^4\). Common taste and odor compounds found in surface waters are geosmin (GSM) and 2-methyl isoborneol (MIB). GSM (odor threshold; 4 ng L\(^{-1}\)) is a bicyclic tertiary alcohol produced by certain species of *Oscillatoria Anabaena* and actinomycetes. MIB (odor threshold; 15 ng L\(^{-1}\)) is a terpenoid also produced by the cyanobacterial species of *Oscillatoria* and *Phormidium* and Actinomycetes\(^5-9\). Cyanobacteria and actinomycetes growth are promoted due to the eutrophication caused by the disposal of agricultural, municipal and industrial wastes into water bodies\(^10, 11\).

Advanced oxidation processes (AOPs) have the advantage of fast reaction rate and non-selective strong oxidation over multiple contaminants and have become desirable techniques for degrading taste and odor compounds. Semiconductor photocatalyst TiO\(_2\) in the presence of UV radiation is one of the advanced oxidation processes that efficiently degrades MIB via producing hydroxyl (\(\bullet\)OH) radicals. Use of a photocatalyst suspension in a UV water treatment reactor is limited due to difficulties in separation of the suspended photocatalyst particles. Therefore, it is important to have an immobilized photocatalytic system to enhance the UV degradation of these persistent organic compounds in water.

The objective of this study is to develop an effective TiO\(_2\) photocatalyst immobilized on different substrates such as glass and stainless steel to augment the existing UV systems for degradation of taste and odor compounds. Mesoporous P25 modified TiO\(_2\)-SiO\(_2\) were immobilized on different substrates, and their efficiencies were assessed using a probe compound (i.e., terephthalate) to determine the steady-state hydroxyl radical concentration. Degradation of MIB was analyzed by headspace solid phase microextraction (HS-SPME)/gas chromatography–mass spectrometry (GC–MS) method. Development of mesoporous TiO\(_2\) catalysts following sol-gel method with a controlled hydrolysis and condensation reactions resulted in photocatalysts with enhanced structural and catalytic properties. P25-TiO\(_2\)-SiO\(_2\) nano-composites increase the hydrophilicity, thermal stability and photocatalytic activity of the thin films.
Methodology

Preparation of powder modified TiO$_2$-SiO$_2$ immobilized catalyst:

Titanium tetraisopropoxide and (TTIP) and tetraethylorthosilicate (TEOS) were used as precursors for titania and silica, respectively. Degussa P-25 TiO$_2$ powder was used to prepare sol–gel derived powder-immobilized coatings. First polyoxyethylenesorbitan monooleate (Tween 80) was homogeneously dissolved in 2-propanol (iPrOH). Then Acetic acid (AcOH) was dissolved into the solution, and TTIP was added under stirring. The molar ratios of the reactants were Tween 80: iPrOH:AcOH:TTIP = 1:45:6:1$^{12}$. The final transparent sol was stirred for 30 min before mixing it with the SiO$_2$ sol. Ethanol and HCl were added into TEOS and stirred for 30 min. Molar Ratio of the Si sol mixture was TEOS:EtOH:HCl = 1: 8: 0.05. TTIP and TEOS mixtures were mixed together to obtain different Si molar concentrations (50% and 15%) and were stirred for 60 min. Degussa P-25 TiO$_2$ powder (50 g/L) was added to the TiO$_2$-SiO$_2$ solution and stirred for 6 hr. After adding P-25, the two sol gel mixtures had 20% and 3% molar concentration of Si, respectively.

Plain microscope glass slides were cleaned with piranha solution ($\text{H}_2\text{SO}_4$:H$_2$O$_2$ =7:3 v/v) for 1 hr at 70°C, washed with deionized water and dried at 125°C in an oven. Cleaned glass slides were dip coated (figure 1) with P-25 powder modified TiO$_2$-SiO$_2$ sol gel mixture (up to 4 coatings). The dip-coating device equipped with a speed controller to maintain a withdrawal rate of 120 mm/min was used to dip in and withdraw the substrate from the catalyst solution. Catalyst coatings were dried and annealed at 125°C for 24 hr in a furnace. Catalyst-coated glass slides were calcined at a ramp rate of 3°C/min up to 500 °C, dwelled at this temperature for 1 hr and cooled down naturally.

In this report, we follow the following naming scheme for the catalysts: P: P-25 Degussa; S: SiO2; T: TiO$_2$ sol gel; 4: number of coatings, 50: P25 loading in g/L.

![Figure 1](image-url)
Photocatalytic degradation of 2-methylisoborneol (MIB)

500 ng/L of MIB in nano pure water was used for the photocatalytic degradation experiments. Glass slides were placed in a quartz beaker containing MIB. The beakers were covered with aluminum foil and the solutions in them were allowed to equilibrate for 20 min. MIB solution was placed in a photochemical chamber—figure 2 (Rayonet Model RPR-100) and illuminated with sixteen RPR-3500 Å lamps, each emitting light in UV-A range (~350 nm) for 60 min. During the irradiation, 10 mL aliquots of MIB solution were taken out at six different time intervals (0, 5, 10, 15, 30 and 60 min) and analyzed for MIB concentration.

Analytical determination of MIB

The determination of MIB in the water samples was performed by the headspace solid phase microextraction (HS-SPME) gas chromatography–mass spectrometry (GC–MS) method. Samples (10 mL) were placed in screw-capped, straight-sided headspace vials with PTFE-lined silicone septa. Sodium chloride (3.0 g) and a magnetic stirrer were added to the sealed vials and placed in a 70 °C oven for 20 min. A GC temperature gradient program from 50 °C (held for 1 min) to 250 °C (held for 6 min), using a temperature ramp (12 °C min⁻¹) under constant flow (1 mL min⁻¹), was used. Extraction of analytes by HS-SPME was achieved using a Supelco fiber coated with Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), Stableflex, 50/30 μm. Detection was performed in selected ion monitoring (SIM) and 95 (MIB).

Catalyst Characterization

X-ray diffraction (XRD) patterns of catalyst film were obtained on a PANanalytical X'pert MRD X-ray diffraction system using Cu-Kα radiation at a scan rate of 0.3° s⁻¹. X-ray photoelectron spectroscopy (XPS) was obtained on a dual anode VG Microtech X-ray source and a SPECS HSA2000 analyzer.
Principal Findings and Significance

Photocatalytic degradation of MIB

Figure 3 shows the photodegradation of MIB under 350 nm UV light in the presence and absence of the catalyst films. During a period of 1 h, MIB concentration decreased by ~30% with UV light only. This might be due to photolytic degradation, adsorption and evaporation of MIB. The presence of catalyst films results in an increase in MIB degradation rate. Among the two catalysts with different Si molar concentrations, catalyst films with 20% [Si] showed a ~50%, while films with 3% [Si] showed a ~80% degradation of MIB. A decrease in the Si content in the catalyst mixture results in an increase in the activity of the catalyst (figure 4).

Figure 3. Photocatalytic degradation of MIB (500 ng/L) under UV A (350 nm) - (♦) UV only photolysis, (●) Catalyst films with 3% [Si], (■) Catalyst films with 20% [Si], (▲) Dark experiment (without UV)

Figure 4. Photocatalytic degradation of MIB (500 ng/L) under UV A (350 nm) at different Si concentrations (▲) PST4-50 5% [Si], (●) PST4-50 10% [Si], (■) PST4-50 15% [Si]
Increased SiO$_2$ content in the sol-gel mixture results in a decreased photocatalytic activity (figure 4). This can be attributed to a lower P-25 surface coverage due to a higher SiO$_2$ content. Also higher amounts of SiO$_2$ in the sol-gel mixture results in the formation of Si-O networks that prevent the formation of anatase crystallites$^{13}$. P-25 Degussa TiO$_2$ has an excellent photocatalytic activity with respect to many pollutants in water. A major challenge in using TiO$_2$ suspension in an UV treatment facility is that the nano-size TiO$_2$ particles need to be separated after UV irradiation. Immobilization of P-25 onto a substrate eliminates the tedious and expensive separation of TiO$_2$ from water after UV treatment. As a control experiment, P-25 particles were dispersed in isopropanol without a TiO$_2$-SiO$_2$ sol-gel mixture, dip-coated onto glass slides and calcined. MIB degradation kinetics of the catalyst without TiO$_2$-SiO$_2$ sol gel mixture and PST4-50-3% [Si] catalyst are shown in figure 5. The MIB degradation kinetics show that the two catalyst films have similar activities. According to the cross-tape adhesion test (results not shown here), catalyst films synthesized without TiO$_2$-SiO$_2$ sol-gel mixture show poor adhesion compared to 3% [Si]-PST4-50 catalyst.

In order to identify the MIB peak in the mass spectral ion chromatogram, known concentrations of MIB were measured using the GC/MS. A peak at 8.29 min was observed (figure 6) and corresponding mass spectra was matched with the reference mass spectrum for MIB (figure 7).
Figure 7. (a): Mass spectrum for aqueous MIB solution ($10^4$ ng/L). (b): MIB reference mass spectra obtained from the NIST mass spectral library.
Catalyst Characterization

XRD data were obtained for three types of substrates; i.e., glass, catalyst film with TiO$_2$-SiO$_2$ sol gel coating (ST) and PST4-50. The XRD spectra (figure 8) show that the catalyst has more anatase when P-25 is incorporated into it. Rutile crystal phase is present only in the catalyst film with P-25 degussa TiO$_2$. Further XRD characterization is needed for the other catalysts that contain different SiO$_2$ concentrations.

![Figure 8. XRD spectra for PST4-50-[Si]-20%, ST4-[Si]-20%, Glass substrates.](image)

XPS analysis of the PST4-50-[Si]-20% catalyst showed that 19.7% of the catalyst surface is covered with Ti and 10.8% with Si (figure 9). Therefore, by decreasing the SiO$_2$ content in the sol-gel mixture, we should be able to increase the Ti availability on the surface which in turn increases the photocatalytic degradation of MIB.
Conclusions

The results obtained in this study show that the P-25 powder modified catalyst film has the ability to photodegrade MIB more efficiently than the UV only treatment. P-25 immobilized on to the glass substrate without TiO$_2$-SiO$_2$ did not have a strong and stable adherence. TiO$_2$-SiO$_2$ sol-gel mixtures assist in binding P-25 to the substrate. Powder modified catalyst film containing [Si] at 3% was able to degrade ~ 80% of MIB within an hour. Higher SiO$_2$ content in the catalyst films causes improved adhesion of P-25 to the glass substrate, but decreases its photocatalytic activity with respect to MIB. Efficiency of the UV treatment can be significantly enhanced by using these P-25 powder modified TiO$_2$ catalyst films. The technology developed here involving an immobilized photocatalyst enhances the UV activity without the need for the removal of particulate catalysts using separation/filtration schemes.
References


C. OTHER REQUIRED DOCUMENTATION

PRESENTATIONS

Poster –“The development of immobilized photocatalysts to enhance the degradation of persistent organic compounds in drinking water”

New England Water Works Association
2015 Spring Conference & Exhibition
April 1-2, 2015 at DCU Center, Worcester, Massachusetts

Poster-“The development of immobilized photocatalysts to enhance the degradation of persistent organic compounds in drinking water”

American Chemical Society
2015 Northeast Regional Meeting
June 10 - 13, 2015 at Ithaca College in Ithaca, New York

PUBLICATIONS

Manuscript in preparation:
Yaparatne, Sudheera; Amirbahman, Aria; Tripp, Carl, The development and characterization of immobilized P-25 modified TiO₂ catalysts for degradation of taste and odor compounds in drinking water.
Water budget, groundwater exchange and hydrologic variability of central Maine

Basic Information

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<td>Principal Investigators</td>
<td>Andrew Reeve, Aram Calhoun, Krista Arminty Capps</td>
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Publication

Final Project Report:

Water budget, Groundwater exchange and hydrologic variability of central Maine’s seasonal forest pools.

Summary

Data to assess groundwater interaction with vernal pools and the amount of snow within each vernal pool basin has been collected for six vernal pools in central Maine. Completed field activities include the installation of monitoring wells and stilling wells in each pool, deploying data logging pressure transducers in each pool to continuously record water level data, deploying temperature loggers as vertical arrays in sediments lining each pool, and collecting snow cores from each pool drainage basin. A one-dimensional heat transport model has been refined for this project and is being used to estimate the vertical flow rates from temperature data. Heat transport models best fit the measured temperature data when slow upward specific discharge values were assigned to the model. Surface water and groundwater levels have been measured through the Fall 2015 and show: (1) the pools refilled in early October and (2) vertical hydraulic gradients are upward, indicating groundwater discharge to the pools. Snow core data has been converted into water equivalent data and are similar to published Maine Geological Survey snow surveys, with snow pack through the winter only reaching two to three centimeters water equivalent depth in most of our pool basins. Time lapse photography has been collected at three pool in an attempt to monitor water levels and snow pack over time; however, cameras sporadically stopped resulting in data gaps.

Changes in Personnel

Both Dr. Krista Capps and Laura Podzikowski left the University of Maine before this project was funded. Dr. Capps is now working at the University of Georgia in a tenure track faculty position and Laura Podzikowski is now working fulltime for an Analytic Chemistry Laboratory. Kelli Straka started at the University of Maine in August 2015 to pursue a M.S. degree with a research focus on the hydrology of vernal pools and has been working on this project since arriving at the University of Maine.

Problem and Research Objective

Vernal pools are important breeding habitat for amphibians and other organisms adapted to ephemeral hydrologic regimes (Calhoun and deMaynadier, 2008). Organisms that use vernal pools during their life cycle export nutrient and energy from the vernal pool to the surrounding watersheds. Many of these organisms, especially amphibians, have declined globally and a variety of state-listed species rely on vernal pools.

The water balance of these pools regulates their hydroperiod and influences chemical fluxes to and from these systems. Quantifying the different components of a pools water balance should be a basic component of their management and is needed to predict their response to external impacts (e.g. climate change, altered land use). Many vernal pools are treated as hydraulically isolated systems (Leibowitz, 2003) and assume that only surface flows impact pool water levels (Boone et al., 2006).

We hypothesize that: [(1)] 1. vernal pools are connected to local and regional hydrologic systems through subsurface flow, and 2. the exchange between groundwater flow and vernal pools varies based on hydrogeologic setting, is a significant component of the water budget, and influences their ecological function. Additionally, we are collecting baseline data on the hydrologic components of vernal pools (storage, evapotranspiration, precipitation) to create water budgets to assess the relative importance of these hydrologic processes. The degree of hydraulic interconnection to surrounding aquatic systems has implications on their regulatory status and their response to nearby land use, and groundwater flow paths may provide the hydraulic linkage between vernal pools and other aquatic systems.
Methods

To test our hypotheses and create water budgets for vernal pools, six pools have been selected that are part of ongoing ecological work by a graduate student pursuing a Ph.D. in Wildlife Ecology. Pools cluster in two spatial groups to with the goal of: 1. comparing the hydrologic relationships between pools, 2. assessing two distinct hydrogeologic settings in Maine (underlain by low permeability silt-clay vs. located near an esker), and 3. comparing pools in areas experiencing different land use (suburban vs. managed forest). Three pools are located along Mount Hope Avenue in Bangor, Maine; two are located along Spectacle Pond Road in Osborn, Maine; and one located in the Penobscot Experimental Forest (Eddington, Maine).

Hydraulic Head Measurements

Monitoring wells were installed in each vernal pool by:

• creating a hole with a 2.5 cm diameter ‘Dutch’ gouge auger,
• driving PVC pipe (2.5 cm diameter flush threaded PVC with 30 cm long machine slotted screen) into the sediment core hole using a post driver,
• surging the well to remove sediment from the screen and then pumping out debris,
• clamping a short (about 30 cm long) perforated PVC pipe to the outside of each well to create a stilling well,
• installing Solinst Levelogger water pressure and temperature data logging sensors in each well,
• placing a 1.2 cm diameter PVC pipe within each monitoring well to reduce the storage in each well (increase sensitivity to water level changes).

Solinst Barologgers were installed at the Penobscot Experimental forest and at a vernal pool in Bangor to collect atmospheric pressure data used to compensate the water pressures measured by leveloggers and adjust these pressures to water levels.

Water levels in the wells are manually measured using a ‘blow tube’ constructed from a thin piece of plastic (tygon) tubing and a tape measure. The tube is lowered into the well while blowing through it until the water is encountered, requiring more force to blow through the tube and creating a audible bubbling sound. Manual water level measurements are used to adjust water heights measured with the data logging pressure sensors to a common datum.

Temperature Measurements

Temperature data is being collected across the upper 30 cm of the pool bed and used to estimate the vertical groundwater flow rate through the pool bed (Rau et al., 2014; Swanson and Cardenas, 2011; Stallman, 1965). Four Thermochron ibutton data logging temperature sensors (ibutton) were placed in slotted thermoplastic rods at 8 cm intervals, covered with electrical tap, and pushed into the sediments in each vernal pool, so that the upper-most ibutton was positioned at the interface between the pool bed (sediment) and water. Arrays of loggers recorded data for about 14 days and were replaced every two to three weeks. Loggers were frozen in place over the winter and no temperature data was collected from January 2016 to March 2016. We anticipate continuing to collect temperature data in these vernal pools through October 2016.

Data logger data has been analyzed using analytic solutions (Swanson and Cardenas, 2011) and a finite-difference model previously prepared using the Python scripting language and further refined for this project. The numerical model is based on the algorithm presented by Vandenbohede and Lebbe (2010) and is modified to include upwinding and QUICK (Leonard, 1979; Versteeg and Malalasekera, 1995) routines for advective heat transport. Specific discharge and porosity used in heat transport simulations have been adjusted manually to achieve fits between modeled temperatures and measured temperature data in the vernal pool bed.
Pool Topographic Surveys

Pool morphology was characterized by surveying poolbeds during the summer when they held little to no open water. Pools were surveyed using a CTS/Berger 32X autolevel and stadia rod. Elevation measurements of the pool bottom were collected relative to the top of the monitoring well positioned in each pool. These data were then converted into an x, y, z data set, interpolated onto a Cartesian grid, and then contoured (figure 1). These data will be used to estimate the volume of water held in each pool, based on water levels recorded with data logging pressure sensors.

![Topographic map of Emerald Pond (Osborn, ME). Elevations (meters) are shown on the colorbar, and displayed in an Easting and Northing (meters) grid. The monitoring well location is indicated with a star and was assigned and arbitrary elevation of 100. m and a location of 0 m Easting, 10 m Northing.](image)

Snow Pack Measurements

Snow surveys were completed several times in each basin over the winter and compared to snow survey data published by the Maine Geological Survey (http://www.maine.gov/rfac/rfac_snow.shtml). Snow surveys were performed by collecting snow cores along transects that crossed each vernal pool basin. Snow cores were collected in thin plastic tubes that were pushed into the snow pack, gently compacted with a Styrofoam cylinder to force snow against the sides to the cylinder (increasing friction), and extracted (figure 2). Snow was the extruded into a plastic bag and weighed using a portable digital scale (figure 3). The thickness of the snowpack was measured in the core hole with a metal retractable tape measure. Because snow pack was so thin in the 2015/2016 winter, corers were cut to short lengths to facilitate transporting them into the field. Snow water equivalent depths were calculated by dividing the mass of the snow measured in a snow core by the density of water and the cross-sectional area of the core.
Time Lapse Photography

Small (Mobius Mini Sports Cam) Cameras were installed at three vernal pool locations and programmed to collect photographs at one hour intervals. An external battery was attached to each camera to prolong their life, and this battery has changed about once a month. Cameras and external batteries were placed in PVC tubes to protect them from precipitation and fastened to trees (figure 4).
These cameras sporadically stopped recording, resulting in large gaps in our data collection. We continue to experiment with the settings on the camera to see if we can get continuous photographs using these relatively inexpensive cameras. Collected images have been assembled into movies using the Python programming language to automate this process. An example of a time-lapse video for Emerald Pond (Osborn, ME) accompanies this report.

Time lapse images are being used to monitor changes within the pools, particularly changes in snow pack over time. Rods marked at 10 cm intervals have been installed in the field of view of cameras and have been monitored to assess the thickness of the snow pack (figure 5). Snow density measured from snow cores are being used to determine the snow water equivalent.
Ongoing Work

Several items described in the initial work plan are ongoing portions of the Masters Thesis research being completed by a graduate student (Kelli Straka). These items include: [(1)] 1. Piezometer tests will be completed in each of the groundwater wells to measure the hydraulic conductivity of pool bed sediments (Hvorslev, 1951), 2. Temperature and humidity data have been obtained from data archives to be used to calculate evapotranspiration from the vernal pools (McMahon et al., 2013; Rosenberry et al., 2007; Oudin et al., 2005). 3. True elevations of the monitoring wells installed in wells and elevations of nearby surface waters will be collected using a dual frequency GPS units deployed for static surveys. Once these data have been assembled and combined with other collected data, monthly water budget will be calculated for each pool.

Principle Findings and Significance

Analysis and collection of data for this project is ongoing and final results for this project will be presented in an M.S. thesis anticipated by Kelli Straka. Our preliminary results based on hydraulic head and vertical temperature data collected in the sediments indicate there is discharge (upflow) of groundwater into all pools during the Fall, 2015. For example, in Emerald Pond a best fit was obtained between a one-dimensional heat transport model and data collected at a depth of 16 cm below the pools bed using an upward specific discharge of $-5 \times 10^{-7} \text{m sec}$. Calibrated heat transport models indicate specific discharge from August to November, 2015 range from $-8 \times 10^{-8} \text{m sec}$ to $-9 \times 10^{-7} \text{m sec}$. Note that specific discharge values with magnitudes below about $1 \times 10^{-7} \text{m sec}$ are likely indistinguishable from zero (no flow) vertical groundwater flux because convection begins to dominate the heat transport model (Ferguson and Bense, 2011). The specific discharge rates with the greatest magnitude were measured in Emerald and Duck Pond, the two vernal pools located in Osborn, Maine.
Figure 6: Temperature data measured at a depth of 8 cm (green circles) has been fit with a piece-wise continuous linear interpolations (green line) and is used as the upper boundary of the saturated zone in the model (pool was dry). Modeled temperatures (black line) have been and fit to measured temperature data at a depth of 16 cm by adjusting the specific discharge in a heat transport model. Results indicate there is slow ($-2 \cdot 10^{-7}$msec) upflow into the vernal pool in August 2015.

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<td>Nov. 06-Nov. 22, 2015</td>
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Table 1: Specific discharge (m/sec) for each pool that produced the best fit between measured temperature data and temperatures simulated using a one-dimensional heat transport model. Best fits were done over entire time frame, as shown in figure 6. Negative values indicate discharge (upflow) conditions.
Water level data collected with data logging transducers illustrates the transitions in water level over time (figure 7). Similar data has been collected for all other pools and is being processed to generate similar results. Results from hydraulic head and temperature sensors are consistent, both indicating discharge of groundwater to the pools in the Fall 2015.

![Graph showing water levels over time, with green line representing monitoring well water levels and blue line representing surface water levels.](image)

Figure 7: Surface water (blue) and monitoring well (green) water levels in Penobscot Experimental Forest vernal pool. The pool dried down in mid-August 2105 and recovered in late September. Note that when both loggers are under water, the water level in the monitoring well is about 1 cm higher than surface water levels, indicating upflow.

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Table 2: Snow water equivalent (cm) collected at the six vernal pool locations. The number of samples collected was increased in the second round of sampling to improve the estimate of water within each basin.
Snow pack measurements were collected from Jan. 8 to Jan. 10 and from Jan. 30 to Feb 02 (table 2). Snow pack changed little between these sampling periods at Emerald and Duck Ponds, changing from average of 2.2 to 2.4 and 2.0 to 2.1 cm snow water equivalent, respectively. The Bangor pools (B01, B06, and B13) all decreased their snow pack by 1 to 2 cm snow water equivalent (3.4 to 1.3 cm for B1, 2.8 to 0.7 cm for B6 and 2.9 to 1.3 cm for B13), perhaps related to the warmer conditions associated with an urban area. B1 is located very close to a road and the large change in snow pack may be related to this factor.

These data are being shared with others (Amirbahman and his graduate student) working on the aquatic chemistry within vernal pools in support of Dr. Calhoun’s NSF proposal linking vernal pools with humans. This work will feed into Dr. Calhoun’s research on vernal pool ecology and management strategies.

Student Support

One graduate student and one undergraduate student have been supported with funds from this project. Kelli Straka is completing an M.S. thesis based on the data being collected for this project. One undergraduate student was hired to assist Kelli Straka with field work.

Presentations

Three presentations were delivered related to work completed for this project:


References


Information Transfer Program Introduction

Information Transfer activities for the Maine Water Resources Research Institute (MWRRI) are an important part of our mission. Information Transfer activities can be categorized as: (1) Conferences- Hosting the Maine Sustainability and Water Conference; (2) Producing web-based water information (http://umaine.edu/mitchellcenter/); (3) Participation on state-wide boards and committees; (4) Leading educational outreach such as K-12 STEM projects (GET WET!); (5) Publishing newsletters (electronic versions); and (6) Providing direct responses to inquiries from citizens. In addition to the effort made directly by the MWRRI, we require researchers funded through the 104b program to include information transfer activities in their projects. Required information transfer activities include presenting research results at the Maine Water and Sustainability Conference, production of a one-page project fact sheet written for a general audience, a progress report, and a manuscript for publication. We also encourage researchers to link research outputs to direct stakeholder interactions and K-12 curricula.
Maine Information Transfer

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Publications

There are no publications.
Information Transfer

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The Maine Sustainability and Water Conference is a leading event in the state and brings together a very broad array of water-interest groups. This conference is very popular and continues to be the most important information transfer event for the MWRRI. In FY15 our plenary speaker was Whitney King, Miselis Professor of Chemistry at Colby College, speaking on: How can we stop loving our lakes to death? Building a Community-Based Action Plan to Keep our Lakes Golden. This topic centered on the Belgrade Lakes. The Belgrade Lakes chain is typical of many Maine watersheds providing exceptional recreational value, a close-knit community, and millions of dollars in economic activity. Declining water quality is threatening many of our iconic Maine lakes. Developing sustainable solutions for the Belgrade Lakes has required community partnerships to define the change in lake ecosystem function, agree on a plan for collective action, and implement broad-based watershed restoration projects.

The 2015 conference included 13 concurrent sessions:
A. Ocean Acidification – What is it, what have we learned, what are the critical knowledge gaps, and what can we do about it?;
B. Safe Beaches and Shellfish;
C. Communities Facing Climate Change Adaptation: Their Challenges and Successes;
D. Stream Connectivity;
E. Urban Sustainability & Climate Change;
F. Developing Water Supplies in Challenging Environments;
H. Sebago Lake – A Trillion Gallons of Challenge;
I. Sustainable Engagement with the Food System;
J. Sustainability of Maine’s Working Forest in the Face of a Changing Climate: Varying
Perspectives and Solutions;
L. Sustaining Maine’s Water Resources; and,
M. Rivers: Streamflow Quantity and Quality.
Details about the conference and presentations can be found online at:

Our web page (http://umaine.edu/mitchellcenter/) is the location to find information for
current issues, activities, and publications. The web page is co-located with the Mitchell
Center's and is updated on a regular basis to include project outputs such as publications
and presentations. Also, it serves as a notice board for meetings, student opportunities, and
calls- for-proposals. Periodic messages are also web-broadcasted to alert our community to
important news, events, and opportunities.

The Water Institute Co-Directors serve on several state-wide and national boards and
committees (e.g. Maine Water Utilities Association, New England Interstate Water Pollution
Control Commission, Penobscot River and Bay Institute, American Water Works
Association, National Institutes for Water Research). These activities provide opportunities
to promote relevant institute-sponsored research and education. Also, it provides a process
for the MWRRI to collect information about the concerns and challenges of water resources
in the state and region. This effort helps to keep the MWRRI at the core of water resources
in the state and region.

Finally, the MWRRI receives public inquiries on a regular basis. Typically, someone is
searching for outcomes from funded projects that can help solve their water-related
problem, or that new research can be focused on a topic important to them. Responding to
these inquiries is a priority and we make every effort to help citizens in finding answers and
solutions to their problems. Although most inquiries come from Maine, we have received
requests from around the globe.

Seminars are a regular feature of the Mitchell Center. In FY15 the following seminars
were supported in part by the MWRRI.

of Biology & Ecology and Senator George J. Mitchell Center Decision Support Systems
(DSS) provide computer based information and analysis to aid decision-making. DSS has
been a common tool in industry for decades. This seminar showed how DSS can be used in
sustainability solutions

April 2015. Making Sense of Place in Maine: Sustainability Insights from the Humanities.
Carol Toner, Maine Studies Director "In times of climate change and threats to our
environment, the more we know about place the more we may empathize with the place
and attempt to protect it." -Yi Fu Tuan. This seminar explored what do we mean by a
sense of place?

September 2015. Upstream. Dr. Bill Sheehan is a biologist who has focused on
environmental policy for the past two decades. He has been at the forefront of two
sustainability movements. In the mid-1990s Bill helped launch and lead the civic
movement for Zero Waste as co-founder and executive director of the GrassRoots
Recycling Network. In 2003 he founded UPSTREAM (originally as the Product Policy Institute) which lead the policy movement for Extended Producer Responsibility in the United States. Bill helped local government officials in eight states form independent Product Stewardship Councils to work for state producer responsibility legislation — Councils that played a role in passing many of the 60-plus state EPR laws adopted since 2004. Sustainability is a master term used by all parties who are concerned with climate change and related global ecological and social “wicked problems.” But sustainability has different historical and cultural meanings that grew out of contrasting worldviews of scarcity and abundance. Applying the frames of scarcity and abundance to the fields of resource conservation and waste management is useful for understanding current efforts and movements to foster sustainable production and consumption. Specifically scarcity and abundance provide a framework for comparing the Circular Economy and New Economy movements, among others. This presentation will examine competing worldviews underlying solutions-oriented approaches to achieving a sustainable materials economy.

October 2015. Applied geomorphology and river restoration. Jack Schmidt is Professor of Watershed Sciences at Utah State University, where he has been on the faculty since 1991. The Rio Grande and the Colorado River are the two great rivers of western North America shared by Mexico and the United States. Both rivers carry snowmelt from the Rocky Mountains across semiarid and arid regions where water demand is high, and both rivers are fully utilized in places, such that no stream flow remains in the channel. In the face of declining runoff in a warming climate, the challenges to river rehabilitation are daunting, yet small successes have occurred in different parts of these watersheds. These small successes are notable, but a large-scale vision of the opportunities and constraints is needed if the native ecosystems of these rivers are to be maintained or rehabilitated in the future. Jack’s research has focused on describing the century-scale changes to river channel form and the causes of those changes. He has been actively involved in numerous efforts to rehabilitate large rivers, especially of the Colorado River in Grand Canyon. He was awarded the 2009 National Park Service Directors Award for Natural Resources Research for his work throughout the National Park system concerning the management of large regulated rivers. He was a member of the bi-national team who received a 2013 Partners in Conservation Award from the Secretary of Interior for their work in implementing the pulse flow release into the delta of the Colorado River. Between 2010 and 2014, Jack served as Chief of the US Geological Survey Grand Canyon Monitoring and Research Center, the science arm of the Glen Canyon Dam Adaptive Management Program.

October 2015. Mitchell Lecture Keynote speaker was Dr. Roger Pielke Jr. Dr. Pielke is Director of the Center for Science and Technology Policy Research, University of Colorado. His research focuses on science, innovation and politics. He received the Eduard Brückner Prize in Munich, Germany in 2006 for outstanding achievement in interdisciplinary climate research. He is author, co-author or co-editor of seven books, including The Honest Broker: Making Sense of Science in Policy and Politics and The Climate Fix: What Scientists and Politicians Won’t Tell you About Global Warming.

November 2015. Fish, Dams and Resilience: Dam(ned) tradeoffs between nature and technology. Karen Wilson, University of Southern Maine. Alewife are small prey fish that
spawn in lakes but spend much of their lives in the marine environment and are the subject of restoration efforts throughout Maine. Many alewife today access their spawning habitat through small dams that maintain lake water levels or produce hydropower. These dams can significantly impede access to spawning habitat, expose alewife to predators and prevent many from spawning. At the same time these dams provide convenient points at which to monitor populations and harvest alewife for bait. Some dams increase the area available for spawning or even create spawning habitat where no habitat existed in the past. This seminar explored the tradeoffs associated with dams and alewife, and how the choices we make regarding dam management may impact the future resilience of these species and the freshwater and marine ecosystems in which they play an important role.

November 2015. When water quality is the easy problem: An untold story of sustainability and human well-being. Bridie McGreavy, Assistant Professor, Communication & Journalism, UMaine. Intertidal mudflat ecosystems along Maine’s coast provide an income for approximately 1,700 licensed commercial shellfishermen in the State. These ecosystems and the individuals and communities that depend on them face threats due to unsustainable land use practices that cause bacterial contamination and make shellfish unsafe to eat. Fecal contamination of mudflats causes short and long-term closures that negatively impact shellfishermen. This presentation shared research from the New England Sustainability Consortium’s (NEST) Safe Beaches and Shellfish project that studies how communication shapes shellfishing resilience to water quality contamination. An ongoing ethnography in Frenchman Bay has yielded insights about the complex communication and decision making factors that shape individual and collective abilities to detect and respond to water quality issues. Further, this research has identified how drug addiction in Downeast Maine increases shellfishermen’s vulnerability to water quality and other types of change. This presentation intends to advance a conversation about how to partner with organizations, seek additional funding, and work with the legislature to meaningfully address drug addiction in fishing communities as a pressing issue of sustainability and human well-being.


February 2015. A Sediment Budget in a Glaciated Region: Tackling the Nonpoint Source Pollution Problem with a Different Kind of Sediment Model. Dr. Peter Wilcock Dept. of Watershed Sciences, Utah State University. Dr. Peter Wilcock is a professor and head of the Department of Watershed Sciences at Utah State University. His career spans over three decades focused on watershed erosion and sedimentation.
USGS Summer Intern Program

None.
## Student Support

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Notable Awards and Achievements

Awards to graduate student working with Sean Smith. 2016-2017 Micheal J. Eckardt Dissertation Fellowship: This fellowship was awarded to Brett Gerard for outstanding academic performance (related to research activities described by this project).

2016 Maine Sustainability and Water Conference Graduate Poster Competition (Honorable Mention): This award was given for a poster presentation of this research by Brett Gerard.

2015 University of Maine Graduate Student Exposition Poster Competition (2nd Place): This award was given for a poster presentation of this research by Brett Gerard.

2014 Senator George Mitchell Center Undergraduate Mentorship Award: This award acknowledge the mentoring activities of an undergraduate student assisting on this project by the PI and graduate student over the summer of 2013.
Publications from Prior Years


