New Hampshire Water Resources Research Center
Annual Technical Report
FY 2016
Introduction

The New Hampshire Water Resources Research Center (NH WRRC), located on the campus of the University of New Hampshire (UNH), is an institute that serves as a focal point for research and information on water issues in the state. The NH WRRC actually predates the Federal program. In the late 1950s Professor Gordon Byers (now retired) began a Water Center at UNH. This Center was incorporated into the Federal program in 1965 as one of the original 14 state institutes established under the Water Resource Research Act of 1964. The NH WRRC is currently directed by Dr. William McDowell with administrative and technical assistance from Associate Director Ms. Michelle Shattuck and Mr. Jody Potter (Water Quality Analysis Lab (WQAL) Manager). The NH WRRC is a standalone organization, in that it is not directly affiliated with any other administrative unit at UNH, and it reports to the Dean of the College of Life Sciences and Agriculture (COLSA). The NH WRRC has no dedicated laboratory or research space, and instead relies on space allocated for the research activities of the WRRC director by COLSA. The NH WRRC does have administrative space on campus, which houses WRRC files and short-term visiting staff and graduate students. The WRRC website (www.wrrc.unh.edu) serves as a focal point for information dissemination and includes NH WRRC publications and results from past research, as well as links to other sites of interest to NH citizens and researchers.
Research Program Introduction

The NH WRRC supported four research projects with its 2016 104b funding:

1. Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds

2. Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems

3. Salt and Streams: Assessing ecological stress in New Hampshire watersheds at community, population, and molecular levels

4. Ecosystem Indicators for Freshwater Streams

The NH WRRC supported four research projects with 2016 104G funding:

1. Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems

The Water Quality Analysis Lab (WQAL) is affiliated with the NH WRRC and facilitates water resources research through technical assistance and sample analysis. The WQAL was established by the Department of Natural Resources in 1996 to meet the needs of various research and teaching projects both on and off the UNH campus. It is currently administered by the NH WRRC and housed in James Hall. The mission of the Water Quality Analysis Laboratory is to provide high-quality, reasonably priced analyses in support of research projects conducted by scientists and students from throughout the University, state, and nation. Past clients have included numerous research groups on the UNH campus, Federal agencies, scientists from other universities, and private firms. Many thousands of analyses are conducted each year.
Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds

Basic Information

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Publications

3. Buyofsky, Lauren A. May 2006. Relationships between groundwater quality and landscape characteristics in the Lamprey River watershed, MS Dissertation, Department of Natural Resources, College of Life Sciences and Agriculture, University of New Hampshire, Durham, NH.
Futures Initiative Ecosystem Services Conference, Irvine, CA, November, 2011.


45. Shonka, N. 2014. Water quality sensors provide insight into the suspended solids dynamics of high flow storm events in the Lamprey River. M.S. Dissertation, Department of Natural Resources & the Environment, College of Life Science and Agriculture, University of New Hampshire, Durham, NH, 93 pages.


Publications 4
Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds

Statement of Critical Regional or State Water Problem

New Hampshire’s surface waters are a very valuable resource, contributing to the state’s economic base through recreation (fishing, boating, and swimming), tourism and real estate values, and drinking water supplies. New Hampshire has experienced rapid growth in several counties. From 1990 to 2004 the state grew twice as fast as the rest of New England, with a state-wide average population increase of 17.2% during that period (Society for Protection of NH Forests 2005). New Hampshire’s population growth has slowed slightly and from 2000-2010 the state experienced a 6.5% population gain. This was still the largest gain among northeastern states and the fastest-growing areas in New England are concentrated in southern and central NH (Johnson 2012). New Hampshire watersheds rank among the most highly threatened watersheds in the nation because of the high potential for conversion of private forests to residential development. In fact, three of the four most threatened watersheds in the US which could experience the largest change in water quality as a result of increased residential development in private forests occur at least partially in New Hampshire (Stein et al. 2009).

The long-term impacts of this rapid population growth and the associated changes in land use on New Hampshire’s surface waters are uncertain. Of particular concern are the impacts of non-point sources of pollution such as septic systems, urban runoff, stormwater, application of road salt and fertilizers, deforestation, and wetland conversion. Long-term datasets that include seasonal and year-to-year variability in precipitation, weather patterns and other factors are needed to adequately document the cumulative effects of land use change and quantify the effectiveness of watershed management programs. No other agency or research program (e.g. NH Department of Environmental Services (NH DES), US Geological Survey (USGS) or Environmental Protection Agency (EPA)) has implemented such a long-term program.

Statement of Results or Benefits

This project provides detailed, high-quality, long-term datasets which allow for a better understanding of the impacts of land use change and development on surface water quality. These surface water datasets could support the development, testing and refinement of predictive models, accurately assess the impacts of watershed management practices on drinking water supplies, assess efforts to reduce surface water quality impairments, and be potential early warning signs of dramatic changes to surface water quality in the region resulting from rapid development. Long-term datasets from this project will be essential to adaptive management strategies that strive to reduce non-point sources of nitrogen pollution in New Hampshire’s Great Bay watershed where several estuarine waters are currently impaired by elevated nitrogen and in violation of the Federal Clean Water Act. A list of selected recent presentations, publications and press releases that utilize long-term datasets supported by NH WRRC funding for this project is included at the end of this report.
Objectives of the Project

This project allows for the continued collection of long-term water quality data in New Hampshire. It will use University of New Hampshire (UNH) staff, students and volunteers from local communities to collect samples from the Lamprey and Oyster River watersheds located in southeast NH and the Ossipee River watershed in central NH. All three watersheds are located in counties experiencing high population growth rates (Figure 1). Both the Lamprey and Ossipee watersheds are predicted to more than double in population from 1998 to 2020 (Sundquist and Stevens 1999). Surface water sites within each of the 3 watersheds and details on long-term datasets collected are described below. Together these 3 watersheds capture a broad range of urban, rural and agricultural land uses as well as a range of forests and wetland cover types.

Figure 1. Projected population growth in New Hampshire (Figure from Sundquist and Stevens 1999; A) and study watersheds experiencing high population growth (B).

Methods, Procedures and Facilities

Lamprey River Hydrologic Observatory

The Lamprey River watershed (479 km²) is a rural watershed located in southeastern NH and is under large development pressure as the greater area experiences the highest population growth in the state. The Lamprey River Hydrologic Observatory (LRHO) is a name given to the entire Lamprey River basin as it serves as a platform to study the hydrology and biogeochemistry of a suburban basin and is used by the UNH community as a focal point for student and faculty research, teaching and outreach. Our
goal for the long-term Lamprey water quality monitoring program is to document changes in water quality as the Lamprey watershed becomes increasingly more developed and to understand the controls on N transformations and losses.

The Lamprey River has been sampled weekly and during major runoff events since September 1999 at site LMP73 which is co-located with the Lamprey River USGS gauging station (01073500) in Durham, NH. Two additional sites were added to the long-term Lamprey River monitoring program in January 2004. One site (NOR27) was located on the North River, the Lamprey River’s largest tributary, less than 1 km downstream from the USGS gauging station (01073460) in Epping, NH. The other site (Wednesday Hill Brook; site WHB01) drains a small suburban area in Lee, NH where residents rely solely on private wells and private septic systems for water supply and waste disposal. A stream gauge at WHB01 is operated by UNH staff and/or students. Sites NOR27 and WHB01 were sampled on a weekly basis through 2010 and in January 2011, the North River sampling frequency (site NOR27) was reduced to monthly because accurate measures of river discharge were no longer possible. Site WHB01 along with LMP73 remain at a weekly and major storm event sampling frequency. Several other sites have been sampled for multiple years on a less frequent basis to assess the spatial variability of water quality in sub-basins with various land uses and development intensities. In the past year, 14 additional sites were sampled on a monthly basis. All LRHO stream water samples are collected by UNH staff and/or students.

**Oyster River watershed**

The Oyster River watershed (80 km²) is a small watershed in southeast NH where land use ranges from rural to urban. Two urban sub-basins, College Brook (CB) and Pettee Brook (PB), were selected for long-term sampling in January 2004. Both sub-basins are dominated by the University of New Hampshire (UNH) and receive a variety of non-point pollution from several different land uses. Three sites (CB00.5, CB01.5 and CB03.0) are sampled along College brook which drains the center of campus and one site (PB02.0) is located on Pettee Brook which drains the northern section of campus. Both sub-basins drain areas with high amounts of impervious surface and College Brook also drains the UNH dairy farm and athletic fields. Historic water quality data for these two sites are available from 1991. UNH staff and/or students currently sample these sites on a monthly basis.

**Ossipee River watershed**

The entire Ossipee River watershed (952 km²) is classified as rural due to its low but increasing population. Seven sites in the watershed were selected for long-term monitoring in May of 2004. These sites are monitored monthly by volunteers and staff of the Green Mountain Conservation Group (GMCG) and were chosen to capture the areas of concentrated growth and monitor the major inputs and outputs from Ossipee Lake. Additional sites are selected by GMCG for volunteer monitoring during non-winter months (May to November). WRRC staff assist GMCG in site selection and data interpretation. In 2006, the GMCG worked with the Department of Environmental Services to establish a Volunteer Biological Assessment Program (VBAP) for the Ossipee Watershed. Numerous volunteers, including students from five local schools, assist with invertebrate sampling at a total of eleven sites.
Water Quality Analysis

Field parameters (pH, conductivity, dissolved oxygen (DO) and temperature) are measured at all sites. Water samples are filtered in the field using pre-combusted glass fiber filters (0.7 µm pore size), and frozen until analysis of dissolved constituents. Samples collected at all LRHO, CB, PB and the 7 long-term GMCG sites are analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrate (NO3-N), ammonium (NH4-N), dissolved organic nitrogen (DON), orthophosphate (PO4-P), chloride (Cl−), sulfate (SO4−2), sodium (Na+), potassium (K+), magnesium (Mg2+), calcium (Ca2+), and silica (SiO₂). Water chemistry is also analyzed on a sub-set of the GMCG seasonal sites and turbidity is measured in the field at all GMCG sites. Samples collected since October 2002 from LMP73 are also analyzed for total suspended sediment (TSS), particulate carbon (PC), particulate nitrogen (PN) and dissolved inorganic carbon (DIC). All samples are analyzed in the Water Quality Analysis Laboratory (WQAL) of the NH WRRC on the campus of UNH, Durham, NH. Methods for analyses include ion chromatography (Cl−, NO3−, SO4−2 and Na+, K+, Mg2+, Ca2+), discrete colorimetric analysis (NH4, PO4, NO3/NO2), and High Temperature Oxidation (DOC, TDN). All methods are widely accepted techniques for analysis of each analyte.

The WQAL was established by the Department of Natural Resources in 1996 to meet the needs of various research and teaching projects both on and off the UNH campus. It is currently administered by the NH Water Resources Research Center and housed in James Hall. Dr. William McDowell is the Laboratory Director and Mr. Jody Potter is the Laboratory Manager. Together, they have over 43 years of experience in water quality analysis, and have numerous publications in the fields of water quality, biogeochemistry, and aquatic ecology.

Principal Findings and Significance

Lamprey River Hydrologic Observatory

Analysis of samples collected in 2016 from the LRHO is approximately 75% complete. Results of stream chemistry to date show a significant increase in weekly nitrate concentrations during the first 10 years (Water Years (WY) 2000-2009) of monitoring at LMP73 based on the Seasonal–Kendall Test (SKT; seasons set to 52) flow-adjusted nitrate concentrations (SKT t = 0.28, p < 0.01). There has also been a statistically significant increase in nitrate concentrations at LMP73 (Figure 2) over the entire study period (2000-2016), but not at WHB01. We have shown previously that stream water nitrate is related to watershed population density (Daley 2002) and since suburbanization continues to occur throughout the greater Lamprey River watershed, population growth is likely responsible for the increase in stream water nitrate over the study period. The watershed population density increased from 53 to 60 people/km² or by 12% from 2000 to 2010 (2000 and 2010 Census). The highest levels of nitrate at LMP73 occurred in 2014. We are uncertain if nitrate levels in LMP73 will remain relatively constant, increase or decrease with changing climate, land use and management in the watershed. Wednesday Hill Brook watershed is near its development capacity, unless the Town of Lee, NH changes its zoning regulations, and the lack of increase in WHB01 nitrate may be due to the limited population growth in this watershed, that this
watershed has reached nitrogen saturation or that the current time period of data collection is not reflective of long-term trends. Changes in Lamprey River nitrogen, especially nitrate, can have significant impacts for the downstream receiving water body, the Great Bay estuarine system which is impaired by elevated nitrogen and is currently in violation of the Federal Clean Water Act. Tidal tributaries to the bay are experiencing dangerously low dissolved oxygen levels and the bay is experiencing a significant loss of eelgrass which provides important habitat for aquatic life. The Lamprey River is the largest tributary to Great Bay, and thus the long-term data provided by the NH WRRC from the LRHO are of considerable interest for watershed management.

When we combine our specific conductance data (2003 – 2015) with data collected by the USGS (1978 - 1999), we see a long-term increase in specific conductance in the Lamprey River with a slight decline in recent years (Figure 3). Sodium and chloride concentrations are directly related to specific conductance ($r^2 = 0.95$, $p<0.01$ for Na$^+$; $r^2 = 0.93$, $p < 0.01$ for Cl$^-$) and we conclude that this increase in specific conductance indicates a corresponding increase in Lamprey River NaCl. Since Na$^+$ and Cl$^-$ are strongly correlated with impervious surfaces in southeast NH (Daley et al. 2009) and road pavement among southeastern and central NH basins, we conclude that the associated road salt application to these surfaces is responsible for this long-term
increase in stream water NaCl. The slight decline in recent years is likely due to the flushing effect of the 2006 and 2007 100-year flood events (Daley et al. 2009), but we are uncertain how long this slight decline will persist and thus continued monitoring is necessary to better understand how the interaction between human activities and climate variability affects water quality.

Figure 3. Mean annual specific conductance in the Lamprey River at LMP73 (co-located with the USGS gauging station in Durham, NH. (modified from Daley et al. 2009).

**Oyster River watershed**

Laboratory analysis of the monthly CB and PB samples collected in 2016 is still in progress, but will be completed soon. Recent data show that DO is lowest at the CB upstream station (CB00.5) where it does drop below 5 mg/L (level that is necessary to support in-stream biota) during the summer months. The downstream stations do not drop below 5 mg/L and this difference is due to the hydrologic and biogeochemical properties of the upstream sampling location which has slow stream flow, high dissolved organic matter content and resembles a wetland. DO increases downstream as flow becomes faster and the stream is re-aerated.

Data from 2000 until now indicate that the steam is strongly impacted by road salt application at its origin, which is essentially a road-side ditch along the state highway leading to a wetland area, and by road salt applied by UNH and the town of Durham which drains to the middle and lower reaches of the brook (Figure 4). Average sodium and chloride concentrations, as well as specific conductance, appear to have remained reasonably constant since 2001, but are much higher than in 1991 (Daley et al. 2009). Concentrations are highest at the upstream stations and tend to decline downstream as the stream flows through the campus athletic fields and then increase as the stream passes...
through the heart of campus and downtown Durham. Concentrations are also highest during years of low flow. Data from this project have been used to list College Brook as impaired for excess chloride.

Figure 4. Median annual chloride in College Brook from the headwaters (CB00.5) to the mouth (CB03.0).

College Brook and Pettee Brook have noticeably higher nitrogen concentrations than many other local streams draining less developed or undeveloped watersheds. As College Brook flows from upstream to downstream where it becomes more aerated, ammonium decreases and nitrate increases (Figure 5) indicating that nitrification is occurring in the stream channel. However, an increase in total dissolved nitrogen (Figure 6) indicates that there are additional sources of nitrogen entering the stream as it flows downstream though UNH and Durham. This is possibly from fertilization of the athletic fields, storm water runoff or exfiltration from sewage lines. There is no statistically significant change in nitrate or TDN concentrations from 2000 to 2015 at the station with the longest record (CB01.5).
Figure 5. Median annual dissolved inorganic nitrogen (DIN) in College Brook from the headwaters (CB00.5) to the mouth (CB03.0).

Figure 6. Median annual total dissolved nitrogen (TDN) in College Brook from the headwaters (CB00.5) to the mouth (CB03.0).
Ossipee Watershed

Collaboration with the Green Mountain Conservation Group (GMCG) and their sampling of the Ossipee River watershed provides much benefit to the NH WRRC and the long-term monitoring of rapidly developing suburban watersheds. Volunteers sampled streams within the watershed every 2 weeks from April through October, and monthly winter sampling was conducted by volunteers and GMCG staff at 9 sites. Over 100 samples were collected for analysis in the WQAL and additional field data were collected at over 40 sites throughout 6 towns using the help of many volunteers. Many presentations were made to planning boards, conservation commissions and other local government groups (see information transfer section below). The impact of road salting in this central NH watershed is similar to what we see in coastal NH (Daley et al. 2009). Data have been used to heighten awareness of the impacts of excessive road salting and snow dumping in local streams. Communication with local road agents has led to the remediation in one development where road salting was an issue. Samples collected and data generated from this funding have shown an improvement in water chemistry following reduced salting and snow dumping. Data have also been useful in promoting low impact development techniques and best management practices where new development has been proposed in proximity to lakes, rivers and streams within the watershed.

Notable awards and achievements

Lauren Koenig was awarded the Mulholland Award from the Society for Freshwater Science. March 2016.

Rodriguez-Cardona, B. 2016. Received a fellowship from the CZO SAVI program (a NSF funded program) to support her proposal “Cross-biome comparison of nitrogen uptake and carbon dynamics in streams”. March 2016. $7,000.

McDowell, W.H. 2017. Received the 2017 Distinguished Professor Award. The purpose of this award is to identify and honor longstanding members of the faculty. This singular university-wide award will be given each year to the faculty member whose overall record of excellent teaching, caring about students, devotion to the university community and substantial record of scholarly achievement exemplifies what we would call a ‘distinguished career’.

Wymore, A. and Kaushal, S. were awarded a Long Term Ecological Research Network Communications Office working group proposal “Stream Elemental Cycling: Global Patterns in Stream Energy and Nutrient Cycling”. National Center for Ecological Analysis and Synthesis (NCEAS) working group. $78,000 from the LTER National Office.

Wymore, A. was awarded $4,000 from the Luquillo Long Term Ecological Research: Research Experience for Undergraduates (REU) program. Using field-based manipulative biogeochemistry to introduce undergraduates to environmental research in a tropical landscape.
Number of students supported

Three PhD students (Lauren Koenig, Bianca Rodriguez, Rich Brereton) and 7 undergraduate hourly employees from the Department of Natural Resources & the Environment (John Ciaburri, James Casey, Margaret Phillips, Christina Mroz, Justin Sherman, Liam Waldron, Christina Lyons). Two post-doctoral students were also supported by this project (Adam Wymore and Ashley Coble).

References


Information transfer activities that utilize long-term datasets supported by 
NH WRRC and matching funds

Publications


Conference Proceedings & Abstracts:


Presentations/Information Transfer

Shattuck, M.D. 2016. Shared Wednesday Hill Brook and organic dairy farm data with Katie Slebodnik for use in Aqueous Geochemistry class project and directed her to the NH EPSCoR DDC. September 2016.
Koenig, L. 2016. For the fourth consecutive year, Koenig served as the instructor for the STEM mini-course offered August 22-26th, 2016 through the CONNECT program at UNH (http://www.unh.edu/connect/). The objective of the course is to provide an opportunity for incoming freshmen that come from groups with historically low retention in STEM majors (e.g. low-income, multicultural, first-generation college students) to build community, discover college resources, and bolster skills that are needed to succeed in their academic programs (e.g. writing of lab/research reports, basic math and statistics for analyzing scientific data). There were 12 students in the class, but the broader CONNECT program serves approximately 100 students.


Press Releases


Green Mountain Conservation Group meetings, workshops and presentations supported by matching funds

2016

Saturday, April 16th 10:00am RIVERS Annual Water Quality Monitoring volunteer training. This RIVERS training and refresher will teach volunteers how to use the testing meters, sample collection procedures and other data collection protocols according to GMCG’s and Saco River Corridor Commission’s shared Quality Assurance Project Plan (QAPP).

Friday June 17th 6:30pm. How the Ossipee Aquifer was formed: Learn about how the glacier formed this extensive aquifer with Dr. Robert Newton, Geologist from Smith College. The evening presentation will also be GMCG’s kickoff meeting to form the Ossipee Watershed Groundwater Protection Advisory Committee. Meeting will take place at Runnells Hall in Chocorua at 6:30 pm. Call GMCG for more information at 539-1859.

Saturday August 27th 2pm-8pm Summer Music Festival “Loons and Tunes” at Camp Calumet in West Ossipee. Family friendly fun event. A selection of local music and local produce will be available. There will also be Pontoon boat tours of Ossipee Lake and a presentation on Loon Habitat by the Loon Preservation Association.

Thursday July 7th at 7pm No Impact Man Please join us at the GMCG office for a tree-hugging, polar bear saving, bicycle composting movie about how one man dragged his family to live a life of zero impact. This is a sensational, funny, and consciousness-raising story of how they managed this in the middle of Manhattan. After the showing, Aislinn Pluta from Global Awareness Local Action (GALA) will be leading the discussion about how someone can live a less impactful life like the one Colin did through personal experience with him.

Monday July 25th Hike up Mt. Katherine with the Wonalancet Out Door Club Come join GMCG and WODC for a guided hike starting at the Wonalancet Chapel. This is about a 2.5 mile round trip with a great view and picnic at the top. We will be talking about the conservation easements that GMCG holds in the area.

Thursday August 8th at 7pm Gasland You are invited to the GMCG office for the last of our summer movies. Gasland is a urgent, cautionary and sometimes darkly comic that looks at the largest domestic natural gas drilling campaign in history, which is currently sweeping the county by promising landowners a quick payoff.” This film describes how Josh Fox reacts to being offered $100,000 for the natural gas drilling rights to his property in the Delaware River Basin. Join us for a closer look at how Fox traveled through 24 states to investigate the environmental effects of hydraulic fracking. What he uncovers is mind-boggling.

Thursday August 11th Volunteer BBQ 5 pm: GMCG invites all Board, Committee, WQM, and special projects volunteers and GMCG supporters to the annual volunteerism party at the GMCG Office. Help us celebrate all the hard work you put in for this organization. Bring yourselves and a chair. GMCG will provide the BBQ and plenty of conversation about the summer’s successful projects.
Wednesday, December 7th: Youth Water Quality Presentation 6:30 at Effingham Elementary School

Monday, December 19: Annual Cookie Swap and global celebration of “Take a Break from Plastic” 3-6 pm. GMCG will partner with local and international friends on this event—details to follow on GMCG Website.

2017

Wednesday, January 11, 2017: Aquifer Protection Committee Meeting Are you interested in learning more about how you can protect the Ossipee Aquifer? Attend the next Aquifer Protection Committee to learn how you can make a difference.
Basic Information

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Publications


Overview of activities during 2016-2017. A schematic summary of progress on the project plan is provided below (Table 1) and discussed on the following pages. We have concluded the final year of five for the most current project agreement, which supports the continuing needs of EPA to assess the effectiveness of the Clean Air Act Amendments of 1990 (CAAA). Field work and data assessment continue on schedule. Project coordination as well as most analytical chemistry, and some field sampling are conducted by the University of New Hampshire. Additional field sampling, data quality assurance, and data reporting are conducted by the University of Maine. This year the project is partially funding a Postdoctoral Researcher who is evaluating biotic and abiotic changes in the LTM and TIME lakes. One graduate student at the University of Maine was partly funded through this project. Publications by three graduate students who were supported last year by this funding or leveraged research were completed. One publication by the current graduate student is in review. One research faculty at the University of Maine was partly supported during this project year to coordinate sampling, develop R code for data QA and analysis, and begin to transition data management to a new secure server. Additionally, this project continues to fund a portion of the base program of stream chemistry monitoring at Bear Brook Watershed in Maine (BBWM), for the reference watershed, East Bear. BBWM has just completed a three-year NSF DEB grant that is evaluating nitrogen dynamics in both watersheds using $^{15}$N tracer studies, and has transitioned to a NSF RAPID grant that funds observation of ‘recovery’ from acidification. The base funding through this IAG project created continuity that was key in securing the NSF award.
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= project plan  = in progress  = completed  = cancelled (weather)

### Project background

**Objectives.** This research is part of EPA CAMD programs that are verifying the effectiveness of emission controls at reducing acidification of surface waters. Our approach is to collect long-term high-quality data that characterize the trends and patterns of response in low ionic-strength surface waters. We have specifically targeted waters that have been classified as being sensitive to acidic deposition and will represent lakes across the Northeast in varying landscape settings. The goals and methods are hierarchical, ranging from intensive site-specific investigations to regional assessment of sites that have been chosen to provide a statistically rigorous sample of regional surface waters. The objectives are to:

1. document the changes and patterns in aquatic chemistry for defined sub-populations and sites that are known to be susceptible to acidification or recovery;
2. evaluate the extent to which changes in surface waters, if any, can be linked to changes in deposition that are driven by regulatory actions;
3. characterize the effectiveness of the CAAA in meeting goals of reducing acidification of surface waters and improving biologically-relevant chemistry in the northeastern US;
4. provide information for assessment of the need for future reductions in atmospheric deposition based on the long-term trajectories of the systems under study; and
5. assess the extent to which increased variability in precipitation events will play a role in the long-term sustainability of CAAA success in these sensitive surface waters. This is leveraged through other funded research.
**Approach.** The schedule of tasks ranges from weekly to annual, continuing data records that now range from 23 to 34 years. We evaluate chemistry on a weekly basis year-round at the small watershed-scale at BBWM, quarterly in LTM, and annually during the historical index period for the TIME and HELM lakes. These project components provide a statistical framework for inferring regional patterns in chemistry using TIME and LTM (and ELS-II under separate funding). The long-term records of LTM, HELM and BBWM provide information on seasonal and annual variability, and thus provide a seasonal context for the annual surveys.

**Expected Results.** This information is needed for EPA to meet its Congressional mandate to assess the effectiveness of the CAAA. The combination of site-specific data within the regional context provides a rigorous assessment of the effects of declining pollutant emissions on SO₄ concentrations, base cation depletion, and changes in N-saturation or DOC contributions to acid-base status. The results are also central to assessing whether additional emission reductions may be needed to produce recovery.

**Project Status: Water Chemistry**

**Field sampling.** All project field objectives in 2016 were accomplished as planned. A summary of the annual field schedule for this project is provided below (Table 2). HELM sampling was previously accomplished through helicopter access, but in 2016 a limited set of HELM sites were sampled during day hikes.

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**Analytical.** Analyses are complete for all samples collected through 2016. All laboratory analyses for TIME, RLTM, and HELM are conducted at the University of New Hampshire Water Quality Analysis Laboratory (WQAL) except for color and closed cell pH for LTM samples and aluminum. Total and organic aluminum samples are processed on an ICP at the USDA Forest Service Region 1 laboratory in Durham, NH. All analyses for TIME, RLTM, and
HELM continue to be conducted by, or under the supervision of, Jody Potter as has been the case since 2012.

Samples from East Bear Brook at BBWM, which are collected on a regular basis year-round, continue to be analyzed at the University of Maine Sawyer Water Research Lab. The LTM color and closed cell pH samples were also analyzed there.

**Data reporting.** All data collected through 2015 have been delivered to EPA. The next delivery of data to EPA is expected by August 31 2017, after evaluation of inter-laboratory comparisons and regular QA analyses by UNH and UMaine.

**Presentation of findings.** Several publications and presentations continue to result from this project and are listed at the end of this report. Recent leveraged funding supported portions of two M.S. theses and a Ph.D. dissertation at UMaine under the supervision of co-PI Saros; results of those projects are now published (Strock et al. 2014, 2016; Boeff et al. 2016; Brown et al. 2016).

**New developments:** During the past five years we were able to make routine two new sets of analyses to continue to extract new and innovative information from these study sites. A subset of lakes were analyzed for DOC quality using SUVA and fluorescence (EEMS) analysis, as well as concentrations of the dissolved greenhouse gases (CH4, CO2, and N2O) in surface waters. Moving forward, these data will provide valuable insight into changes in organic sources to acid-base status as well as the influence of precipitation event variability on long-term changes in surface water chemistry.

**Publications using related project information (recent publications in bold):**


Dissertations/theses:


Presentations using related project information (recent presentations in bold):


Kahl, J.S., 2005 (invited). The intersection of environmental science and environmental policy. NH Charitable Foundation Lakes Region annual meeting, Meredith, NH, September, 2005.


Kahl, J.S., and Catherine Rosfjord, 2005 (invited). Acid rain and the Clean Air Act in the northeastern US. Annual meeting of the NH-ME Androscoggin River Watershed Council, Bethel, June, 2005


Kahl, J.S., 2004 (invited). The Clean Air Act Amendments of 1990; testing a program designed to evaluate environmental policy. Lecture, Colby College. April, 2004


Bear Brook publications and presentations that include “base program” data (East Bear Brook stream chemistry partly funded through this grant; recent publications and presentations in bold):


MacRae, J.D., C. Tatariw, D. Rothenheber, S. Nelson, I.J. Fernandez. The effects of nitrogen enrichment on forest soil microbial communities and their activities, 2013 AEESP 50th Anniversary Conference, July 14-16, Golden, CO.


Minocha, Rakesh, Swathi A. Turlapati, Stephanie Long, Mohammad M. Bataineh, Aaron Weiskittel, Ivan Fernandez, and Lindsey Rustad. 2013. Chronic N and S additions impact foliar physiology of forest trees at the Bear Brook Watershed in Maine, USA. Hubbard Brook Experimental Forest Annual Meeting, Thornton, NH.


Salt and Streams: Assessing ecological stress in New Hampshire watersheds at community, population, and molecular levels

Basic Information

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Publications

There are no publications.
Salt and Streams: Assessing ecological stress in New Hampshire watersheds at community, population, and molecular levels

Problem

New Hampshire’s climate is expected to resemble that of the US Mid-Atlantic by 2100 (USGCRP 2009). With this shift comes increased air temperatures, less snow pack, more ice storms, and more rain on snow events. From a freshwater ecology perspective, much of central and northern New Hampshire’s streams are currently populated by coldwater species (e.g., Brook trout; Neils 2009). As a result of increasing air temperatures, stream temperature will likely increase; however, the increase will vary among streams (Kelleher et al. 2011). For many species, this thermal shift may be within their fundamental tolerance range (e.g., 21°C thermal maxima for Brook Trout), barring additional physiological stress. However, growth in development (e.g., roads, housing) and energy production (mining, fracking) in northeastern states is causing additional stress on freshwater biota (Van Meter et al. 2011, Kelting et al. 2012). Among emerging concerns are the short-term and cumulative impacts of thermal and salinity stress on freshwater resources and biota (Findlay and Kelley 2011, Cuffney et al. 2010, Van Meter et al. 2011, Dalinsky et al. 2014, Stitt et al. 2014).

Recently it has been reported that salts are infiltrating into subsurface flow and groundwater before reaching streams (Daley et al. 2009). The infiltration of salts into soil and retention in groundwater systems adds a lag to the emergence of salts in streams, elevating Cl concentrations into summer months (Williams et al. 2000, Findlay et al. 2011, Kelting et al. 2012). The impacts of thermal variability and salt loading on freshwater biota have garnered attention and study in northern states, but it remains unclear how the synergy of salt and thermal stressors impact biota across the community, population and molecular levels.

Traditionally, biotic response to water quality degradation is measured using broad-based community metrics (e.g., Simpson’s Index of Diversity) and/or assessing populations of select bio-indicator species (e.g., EPT= the macroinvertebrate orders of Ephemeroptera, Plecoptera, and Tricoptera). Rapid biological assessments examine community composition and the presence of indicator species to assess overall stress (Friberg et al. 2011); however, these methods are largely reliant on the loss of individuals and/or species, which could have cascading effects on biodiversity and the ecological function of streams. In order to avoid the potentially cataclysmic effects of osmo-thermal stress on NH streams, we need studies that investigate the biotic response along a gradient of salt and thermal stress. However, to truly avert the loss of species and ecosystem function, we need to develop techniques that will provide an early-warning signal of ecosystems in jeopardy.

Objectives

The goal of this project was to enhance biomonitoring efforts and early detection of thermal and salt stress on stream biodiversity in New Hampshire. To achieve this, our objectives were to:

1) Evaluate differences in stream macroinvertebrate communities along a thermal-salt stress gradient. We assessed macroinvertebrate community composition in ten 1st to 4th order wadeable streams across NH that vary along a thermal-salt stress gradient (Figure 1).

2) Evaluate sub-lethal osmotic stress in mayfly larvae by quantifying HSP expression in mayflies. This objective was pursued by first conducting in-lab salt exposure trials using nymphal mayflies to create salt-stress response curves. The in-lab exposure trials were
followed by snapshot expression profiles from field caught individuals. Mayflies are a sensitive, yet very important source of prey in northern streams; therefore, the development of stress protein expression metrics in mayfly nymphs holds promise as a sensitive, early stage, and rigorous measure of the biotic impacts of salt load on freshwater habitats (Bauernfeind and Moog 2000, DeJong et al. 2006).

3) **Compare and evaluate benthic macroinvertebrate sampling techniques and potential indicator taxa for salt stress.** The NH Department of Environmental Services (NHDES)–Biomonitoring program has adopted a rock basket approach for assessing water quality using indicator taxa and community metrics. We set out to compare the rock basket approach to kicknetting over the months of May – October to evaluate their ability to detect small changes in community composition that may be attributed to elevated salt or temperature.

**Methods**

**Site selection**
Field sites were selected by using GIS to overlay the LoVoTECS network of stream monitoring sites with fish sample sites between 2009 and 2015. From this subset of NH streams, we selected sites based on median chloride concentrations derived from snapshot water chemistry data collected in May and July 2013 and July, Sept, Oct 2014. Our ten sites ranged from 4.35-52.6 mg/L of Chloride during this period. The ten sites represent a range of human impact; some sites have roads and development, and some sites have little to no human impact. Two of our sites, Mad River in Waterville Valley and Douglas Brook near the Kancamangus
Highway are located in the White Mountain National Forest. The other stream sites are located near minor and major road systems, with minimal to moderate influence from road salts and other anthropogenic influences. The ten sites include: Halfway Brook and Shannon Brook in Moultonborough, Mad River in Waterville Valley, Douglas Brook in Bartlett, Beaver Brook in Keene, Wednesday Hill Brook in Lee, Pemigewasset River in Woodstock, Clay Brook in Plymouth, Otter Brook in Peterborough, and Sucker Brook in Franklin.

Our research team adopted NAWQA and EPA Rapid Bio Assessment Macr
invertebrate Sampling protocols for multi-habitat kicknet sampling. We sampled each study stream once every month beginning in mid-May to September/October, 2016. At each site, we selected a 100-meter reach that was largely representative of the stream habitat. This 100-m reach was established in close proximity to continuously logging specific conductance, water temperature, and water level sensors; most sites consisted of sample reaches that were 50-meters upstream and 50-meters downstream, or, where that was not feasible, 25-meters and 75-meters. We sampled total of 10 kicks over the 100-meter stream reach, sampling different habitats in approximate proportion to their representation of the total surface area of the reach. We determined this by assigning a percentage of each habitat type (cobble, sand, or large woody debris) totaling 100%. In cobble substrate/habitat, we chose to kick in riffles or runs. In sand substrate and habitat, we mainly kicked in runs and slow moving water since that is the main stream morphology for this type of habitat. We placed all macroinvertebrates in labeled containers with 70% ethanol for preservation. If there were any predator macroinvertebrates, such as the family Corydalidae, then we used an additional container to store the predators.

In addition to kicknetting, we adopted the New Hampshire Department of Environmental Services (NHDES) biomonitoring program rock basket approach for macroinvertebrate sampling. At each site, we deployed 3 rock baskets side-by-side in a cobble and riffle habitat in close proximity to the continuously logging sensors. We collected rock baskets roughly every four weeks to collect macroinvertebrates from June to July for identification and enumeration. We left rock baskets in study streams for eight weeks from July/August to September/October to better compare results with NH DES Biomonitoring Program’s annual assessments. Our rock basket collection was similar to the NHDES sampling protocol, which included four, 5-gallon buckets, 3 of which will hold the rock baskets themselves, and one bucket to rinse and store the rocks that have been examined. We filled three buckets with stream water a quarter full and facing upstream with the opening facing towards the rock basket. One person lifted each basket into the bucket, making sure to catch any debris that comes loose from the basket. The research team thoroughly examined every rock in each basket, and the water in the bucket were filtered through a sieve. We placed all macroinvertebrates in rock basket labeled containers separately to the kick net samples, and stored in 70% ethanol to be preserved. We labeled containers with the correct site name and date sampled. Rocks were cleaned and put back into the baskets. The three baskets were then re-deployed in the same location.

Macr
invertebrate Identification

We preserved our field samples in 70% ethanol, transported them to the laboratory, and sorted and identified by family using NAWQA and EPA protocol for macroinvertebrate sorting and identification. References used to identify macroinvertebrates by family were from online sources from New England, and book sources, such as A Guide to Common Freshwater Invertebrates of North America, An Introduction to the Aquatic Insects of North America 4th Edition, and Freshwater Macroinvertebrates of Northeastern North America.
Community analysis
We calculated community composition metrics for each site visit, including: EPT family richness, % EPT, % Plecoptera, Philopotamidae (fingernet caddisfly; Tolerance Value: 0-4 according to NHDES) relative abundance, % Chironomidae (non-biting midges) and compared these metrics to the chloride concentration from the same sample visit. In addition, we used multiple linear regression to assess the relationship between the given community metric and a suite of potentially influential environmental factors: discharge, reach area, latitude, elevation, water temperature, pH, dissolved oxygen, as well as chloride and sodium. Finally, we explored the data using Canonical Correspondence Analysis to investigate the influence of chloride and other environmental conditions on community composition.

Salt exposure trials & HSP70 expression:
This portion go the student focused on three primary research objectives: 1) examine the concentration/distribution of HSP70 across the mayfly body; 2) quantify dosage-dependent response curves of HSP70 expression to gradients of sodium chloride using in-lab mesocosms; and 3) examine in-situ levels of HSP70 expression among mayflies in 10 New Hampshire streams across a chloride gradient (Figure 2). For the first objective, individuals were collected from nearby streams and dissected into four body regions: 1) head, 2) legs, 3) gills, and 4) abdomen. For salt trials, individuals were collected and transported to micro aquaria setups using one-liter beakers as tanks. The source water for micro aquaria originated from the site itself in order to keep baseline ionic conditions constant. Leaf pack was also collected from sites along with specimens to provide a substrate for attachment and a food source. To best mimic running water conditions, battery-powered bubblers were placed in each beaker to create an oxygenated environment. Specimens were exposed to a gradient of salt concentrations following a three-day acclimation period in order to rule out the possibility of stress protein expression due to handling/travel. Preceding salt dosage, several specimens were immediately extracted for proteins to provide a measure of baseline HSP70 expression. Applied concentrations of salt have included 150mg/L, 300mg/L, 400mg/L, 2000mg/L, and 4000mg/L; during these exposures, individuals were selected and proteins were extracted at the 1, 2, 4, and 168hr marks. In order to examine HSP70 levels across different field sites in NH, specimen collection occurred once per month May-September with a goal of no less than 15 specimens per site, per month. All protein extractions were carried out using physical homogenization coupled with T-PER extraction buffer. Quantification of proteins was then carried out by use of a bicinchonininc acid assay (BCA) assay to determine the concentrations of total protein extracted via a nanodrop spectrophotometer. Finally, HSP70 expression was observed by western-blotting technique, exposing proteins separated by size (electrophoresis through a gel medium) to a primary monoclonal HSP70 antibody for specific binding of the protein of interest.
**Principal Findings & Significance**

*Chloride concentrations*

The summer of 2016 was an unusually dry season, leaving many of our study sites with water levels much lower than normal. Monthly snapshot water chemistry samples confirmed the initial classification of streams (based on snapshot sampling in 2013 and 2014). Our sites in southern NH (Keene and near Durham), generally had higher chloride concentrations than the other sites, but all sites had chloride levels below 60 mg/L (Figure 3). Even our highest chloride concentration was substantially below the EPA’s chronic toxicity concentration of 230 mg/L. Chloride concentrations increased at most sites throughout the sampling season, which we believe is attributed to low water levels. Low surface water inflow suggests that groundwater likely comprised a larger portion of stream water. Thus, increasing concentrations throughout the summer may help support the findings of Daily et al. (2009).

*Figure 3: Snapshot sampling of chloride in 10 sample streams between May/June and September/October 2016.*
Chloride and Community Composition

We found that chloride rarely explained a significant portion of the observed variation in the aforementioned community metrics used by NH DES Biomonitoring Program (Table 1 and graphs in Appendix A). We found the mean percent EPT at each site throughout the summer was inversely related to chloride; however, this relationship was weak and not statistically significant (Figure 4A-B). Interestingly, chloride was only a significant predictor of some metrics in late summer (late August through early October; Table 1). We also found an unexpected positive relationship between chloride concentration and the percent of the community comprised of Plecopterans. We are investigating this more to determine if the relationship was driven by a single family or if the unexpected positive relationship (more chloride, more Plecopterans) is consistent across the order. Either way, it is important to reconcile because percent of individuals from EPT orders are considered indicators of good water quality.

Figure 4A: Linear regression comparing average EPT % community composition and chloride for kick nets ($r^2=0.18, p=0.22$)

Figure 4B: Linear regression comparing average EPT % community composition and chloride for kick nets ($r^2=0.17, p=0.24$)
Table 1: Summary of regression analyses of chloride and various macroinvertebrate community composition metrics.

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<th>Month</th>
<th>EPT Family Richness R²</th>
<th>% EPT R²</th>
<th>% Plecoptera R²</th>
<th>Philopotamid Abund. R²</th>
<th>% Chironomidae R²</th>
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<td>0.12</td>
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<td>0.00*</td>
<td>0.28</td>
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<td>0.09</td>
<td>0.00*</td>
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<tr>
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</table>

Relationship was as hypothesized and is significant

The lack of a clear and consistent relationship between chloride and the community metrics prompted us to take a multiple linear regression approach to better understand the influencers of the observed macroinvertebrate communities. We took a backwards parameter selection approach, starting with the full (global) model that included discharge, stream area, as well as snapshot measures of water temperature (snapshot), pH, dissolved oxygen, chloride, and sodium. Sample month was also included because there is uncertainty in the timing of emergence for all families observed. Table 2 below provides a summary of the best model for each community metric. Again, chloride was a significant predictor of Plecopteran abundance, but the relationship was unexpectedly positive. Water temperature was the factor most frequently included in significant best models. Month was also an important explanatory variable for percent Ephemeroptera and percent Tricoptera. We are in the process of investigating these patterns more thoroughly at the family level.
We have yet to find a consistent model that explains the individual community metrics discussed. However, many of these metrics are intended to be used as indicators of water quality rather than explicit measures of aquatic biodiversity. To better understand how chloride may be influencing community composition and structure, we conducted a series of Canonical Correspondence analyses (CCAs). CCAs are a multivariate approach to identify the suite of variables that best explain the composition and structure of a given community. We conducted CCA analysis for families within each Order separately because we did not have a large enough samples size to allow proper CCA for all families identified. Our results are preliminary at this time, and will be updated at the conclusion of this project.

**Temperature and Community Composition**

Using the continuously logging air and water temperature sensors, we were able to calculate the sensitivity of stream temperature to changes in air temperature at each study stream (i.e., stream thermal sensitivity). Sensitivity is reflected in the slope of the air to water temperature relationship. Sensitivity ranged between 0.41 and 0.68. This can be interpreted as for every degree (F) increase in air temperature there was an observed increase in stream temperature
between 0.41 and 0.68 degree (F). Table 3 provides a summary of the slope and \( r^2 \) values for each relationship. There was not a direct relationship between thermal sensitivity and chloride concentrations, suggesting other factors may be affecting stream temperature aside from roadway density within the stream’s catchment.

The influence of stream temperature on macroinvertebrates was explored by focusing on the same key community composition metrics described for chloride analyses. We calculated the monthly mean, 7-day mean, and the mean daily max stream temperature in correspondence to the macroinvertebrate sampling events. Table 4 below provides the \( r^2 \) values for the linear regression between each as well as the slope of the relationship. Several of the observed linear relationships were significant, albeit weak, and for some metrics the relationship was positive suggesting that warmer temperatures were associated with greater proportional abundance of specified taxa (e.g. Corydalidae and Philopotamidae). We found no notable relationships between common diversity metrics, such as Simpson’s Diversity, and stream temperature.

We also explored the relationship between each of the community metrics and the sensitivity of the stream to air temperature changes. The rationale for this analysis was that temperature sensitive streams may experience drastic diurnal and weekly temperature changes during summer months. These drastic temperature shifts can be a disturbance to some aquatic organisms. Table 5 below provides the \( r^2 \) values for the linear regression between each as well as the slope of the relationship. Interestingly, we found that the only community metrics to be significantly related to the thermal sensitivity of the stream were common diversity metrics Simpson’s Index of Diversity and Shannon’s Diversity Index, both of which were not significantly related to temperature itself. For both metrics, the relationship was negative suggesting that the greater thermal sensitivity the less diverse the community’s composition of macroinvertebrates would be. We plan to explore this result more with more extensive family level analyses and multivariate approaches.

### Table 3 Summary of stream thermal sensitivity to changes in air temperature.

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<td>DGB</td>
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Table 4: Summary of the linear relationships between stream temperature and community composition metrics. Bolded values note statistical significance ($p \leq 0.05$).

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<th>Explanatory Variable</th>
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<td>R-sq</td>
<td>Slope</td>
<td>R-sq</td>
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<tr>
<td>% EPT</td>
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<td>% Plecoptera</td>
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<td>% Philopotamidae</td>
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<td>Simpson's Index (1-D)</td>
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Table 5: Summary of the linear relationships between stream thermal sensitivity (measured as the linear slope between air and water temperatures (Table 3) and various community composition metrics.

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<th>Explanatory Variable</th>
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<td>% Philopotamidae</td>
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<tr>
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<td>Shannon's Evenness</td>
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**HSP Analysis**

The first year of work has been largely devoted to developing a field to lab protocol for assessing HSP70 in macroinvertebrates, first with a focus on mayflies and later stoneflies. Thus far, the HSP70 stress response has been identified in both mayfly and stonefly nymphs across several regions of the body (Figure 5), as well as in whole insect based extractions. However, after little HSP70 expression was observed in several western blots (additional examples of HSP70 western blotting results are available in Appendix C) we have taken a series of approaches to rule out any
possible researcher-based error. These included eliciting the HSP70 stress response, ensuring proteins were not degraded or aggregated prior to analysis, and exploring the possibility of minimal HSP70 expression being present. We are working through each step of the protocol to be sure that the method is appropriate and that the lack of HSP70 expression is a true result suggesting low stress environments for mayflies, rather than one influenced by procedural decisions or chemical choices.

HSP70 EXPRESSION ACROSS LEGS, HEAD, ABDOMEN
Glove Hollow, Plymouth, NH

Figure 5: Western blot results of HSP70 expression across the legs, head, and abdomen of mayflies collected in spring 2016.

Recent western blots have begun to assert the possibility that this molecular response to salt stress may not reflect the true biological stress (or lack of stress) in the organisms. Current and future work involves pursuing longer lab exposures to mimic exposure to elevated salinity in the field (24hrs – 5 days), simultaneous exposures to different stressors (salt, heat, heat + salt), extractions of individuals at more frequent and longer time periods following exposure, and probing western blots with an additional HSP60 antibody (also known to be part of the stress response system). We will continue to explore differences in HSP expression across body parts. Work on the HSP70 protocol and lab trials continues and additional samples will be taken in summer 2017 for future snapshot assessment.
**Future work:**

This research will continue through summer of 2017 with funding from NH WRRC during which the same 10 sites will be revisited and the HSP70 lab protocol development and vetting will continue. HSP70 protocol will expand to include stoneflies and will focus on combining salt and thermal stress. We will specifically be examining interannual variability potential attributed to precipitation and stream water levels, which impact chloride concentrations and water temperature. Likewise, we will begin to assess the relationship between longer term exposure to elevated chloride and temperature using data from installed temperature and conductivity sensors.

**References**


**Notable Awards:**
Dr. Amy Villamagna was honored with the Helen Abbott Endowed Professors of Environmental Studies (2016-2020) for her research on the environment and engagement of students in research.

**Publications and presentations:**

**2016**

**2017**
Lafortune, T., A. Villamagna, B. O’Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire*. New England Association of Environmental Biologists annual meeting (Hartford, CT) [poster printed but not presented due to poor blizzard travel conditions]
Fruit, R., A. Villamagna, B. O’Donnell. 2017. *Quantification of HSP70 Expression in Mayflies: A Novel Bioindicator of Road Salt Pollution*. New England Association of Environmental Biologists annual meeting (Hartford, CT) oral presentation
Mazzone, M. A. Villamagna, B. O’Donnell. 2017. *Assessing Salt Stress In Selected NH Streams at the Community Level For Macroinvertebrates*. New England Association of Environmental Biologists annual meeting (Hartford, CT) [oral presentation prepared but not presented due to poor blizzard travel conditions]


Fruit, R., A. Villamagna, B. O’Donnell. 2017. *Quantification of HSP70 Expression in Mayflies: A Novel Bioindicator of Road Salt Pollution.* Plymouth State University Showcase of Excellence (oral presentation)


**Number of students supported:** 2 master’s students, Matthew Mazzone and Roy Fruit. 2 undergraduate students, Ryan Duquette and Thomas Lafortune, were affiliated with the project through university match and research collaboration, but did not receive direct funding from NH WRRC.

**Number of faculty supported:** Assistant professor, Amy Villamagna (Ph.D.) received direct funding for this project and Associate professor, Brigid O’Donnell (Ph.D.) was affiliated with the project through university match and research collaboration.
APPENDICES
Appendix A: Within season comparison of family level abundance observed through kicknet sampling for benthic macroinvertebrates at all ten study sites.
Appendix B: Univariate relationships between chloride and common benthic macroinvertebrate biomonitoring metrics. The graph illustrates the observed relationship across all sampling months in 2016. Yellow highlighted boxes in table suggest the relationship was significant and as hypothesized.

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Appendix C: Preliminary results from HSP70 lab trials.

Salt Trial (4000mg/L)

Figure above: Preliminary results from HSP70 lab analysis of mayflies exposed to an acute dose of 4000 mg/L of sodium chloride. The top blot captures the total protein in the sample, and the lower blot in each panel reflects the HSP70. The lane on the far left reflects the positive control for each blot.
Salt & Temperature + Increased P.I.

Figure above: Preliminary results from HSP70 lab analysis of mayflies and stoneflies exposed to an acute dose of (potential) salt and temperature stress. The top blot captures the total protein in the sample, and the lower blot in each panel reflects the HSP70. The lane on the far right reflects the positive control for each blot.
Ecosystem Indicators for Freshwater Streams

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<td><strong>Principal Investigators:</strong></td>
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Publications

There are no publications.
Improved Ecosystem Indicator Tools for Water Quality Management - NH WRRC Annual Report – Alison Watts, University of New Hampshire

Problem
Water resource managers, such as state and federal agencies, municipalities, and watershed groups, must identify and manage multiple interconnected stressors within an individual watershed. Primary stressors include nutrient inputs, invasive species, water clarity, low dissolved oxygen, contaminants in water and sediment, increased impervious cover, and loss of aquatic buffers and wetlands. Many of these are interrelated, and may be temporally and spatially variable. Ideally, assessment of biotic condition will provide information that allows managers to identify loss of ecosystem function, indicate the relative importance of primary stressors, identify measures or methods to reduce the stress and repair the system, and ultimately track progress towards management goals. Misidentification of stressors may lead to expenditure of management resources without benefit, and contribute to further degradation of the system. Current approaches to assessing the biological integrity of surface waters rely on manual identification of individual species of fish, invertebrates or other organisms. While effective, this approach is labor-intensive and expensive. Furthermore, it assumes a priori knowledge of which groups of aquatic biota are most likely to be impacted by water quality; these are the target groups for which identification of individual specimens are obtained. Advances in DNA methods and rapid reductions in analytical costs present an opportunity to harness this new technology and fundamentally improve our capacity to monitor biological communities and individual species (Bista et al., 2017; Thomsen and Willerslev, 2015). Environmental DNA (eDNA), or DNA present in an environmental sample, includes whole microorganisms (microalgae, bacteria etc.) and fragments of tissue, reproductive and waste products, and other cellular material.

Objectives
This study has two major objectives: (1) Pilot a sampling program to develop statistical correlations between causal parameters (including nutrients, land use and chloride) and microbiotic species attributes for wadeable streams in New Hampshire; (2) to assess the value of genomic analyses of eDNA as an additional tool to evaluate the ecological health of streams.

Methods
The study is being conducted at wadeable streams across New Hampshire, representing a range of land use and stream characteristics. Samples are collected at existing NH Department of Environmental Services (NHDES) Volunteer River Assessment Program (VRAP) and Long Term Monitoring sites, in coordination with NHDES staff and volunteers. The NHDES VRAP program engages over 150 volunteers to sample 30 sites, and provides data that contributes to stream assessment associated with more than 2,900 miles of rivers and streams in New Hampshire. Volunteers have been trained to collect samples for genomic analysis, and the methods, results, and implications will be shared with volunteers.
and watershed groups. Water samples collected from these sites 10 times (monthly) in 2016 are being analyzed by amplicon sequencing to provide data on stress response, seasonality, replicability, and trends.

Sampling was conducted from June –October 2016, and over 340 samples were collected. Samples were conveyed to UNH and frozen pending extraction and sequencing of DNA. Most of these samples have been sequenced to identify bacteria, while a smaller subset have been sequenced for animal species. Initial analysis and taxonomic identification was performed via QIIME2 (Quantitative Insights Into Microbial Ecology; Caporaso et al, 2010) with the Genbank database.

Initial findings and significance
We identified approximately 40,000 named bacterial species, and 300 animal species (primarily representing fragments, not whole organisms) in the sample set. Initial analysis of microbial populations indicates strong correlation with water quality parameters (Figure 1).

Additional sampling will be performed at selected sites in 2017 to provide information on the annual variability of microbial populations in streams. 2016 was an unusually dry year, which provides an opportunity to compare results under drought conditions and (potentially) more normal conditions.

More detailed analysis of the correlation between site characteristics and microbial conditions will be conducted on the full data set in 2017.
Publications and presentations


Outreach or Information Transferred

We have engaged volunteer organizations in sampling collection in NH streams. Outreach materials and fact sheets have been prepared for volunteers (Figure 2).

Figure 2. Fact sheet distributed to VRAP volunteers.
Number of students supported
This project has provided partial support for one undergraduate student (Kendra Dow, senior in Environmental and Civil Engineering), and will support up to two additional students in the summer of 2017.

Number and names of faculty and staff supported
Alison Watts, Research assistant Professor
Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems

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Publications

There are no publications.
Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems (2016NH205G)

Problem: Neurotoxic methylmercury bioaccumulates through aquatic food webs and is a primary cause for fish consumption advisories in the Northeast. The mobilization, transport and bioavailability of mercury in aquatic ecosystems is strongly tied to organic matter dissolved in the water, yet levels of methylmercury in fish are difficult to predict. Previous studies have noted that relationships between stream methylmercury and dissolved organic carbon (DOC) in streams change over time. There is also a seemingly contradictory effect of DOC on uptake by the biota: at low concentrations of organic carbon, methylmercury bioaccumulation increases, whereas at higher concentrations, uptake into fish is attenuated. This project is testing the hypothesis that differences in the chemical structure of the DOC that is transporting MeHg in streams lead to the temporal changes and non-linearity in bioaccumulation noted in previous studies.

Objectives:
1) Identify and characterize fractions of DOC that associate with MeHg and Hg in streams,
2) Determine the effects of DOC quality and quantity on MeHg uptake by primary producers at the base of the stream food web.

Methods:
DOC isolation: Dissolved organic carbon was isolated from two streams with contrasting watershed landscape features (wetland/no wetland) in September 2016, using methods described in Dittmar (2009). Briefly, large (40 L) samples were filtered through 0.45 µm cartridge filters, then acidified to pH 2 with HCl and passed through a reverse phase cartridge at a rate of 4 ml/min. Cartridges were rinsed with dilute HCl, then eluted in 10 mL MeOH. The extracts were dried first under nitrogen, then freeze dried. These samples are being characterized and used for MeHg uptake experiments with periphyton biofilms at the base of the foodweb, to understand the effects of DOC from different sources on MeHg bioavailability.

Biofilm growth: Racetrack-style artificial streams were assembled in 25 gallon troughs using aquarium pumps for circulation, with ceramic tiles placed along the bottom. Streams were housed in an environmental chamber (25°C, 16:8 L:D), inoculated with biofilm scraped from rocks collected from several tributaries, and maintained by periodic addition of nutrients (diluted COMBO medium).

Mapping: Watershed landscape features were assessed along stream buffer zones using high resolution aerial imagery from 2009 (NAIP, true-color) and high-resolution lidar elevation models (NH Granit) in ArcGIS. Landscape characteristics will be related to water chemistry components on completion of field sampling (summer 2017).

Field work: Water samples were collected from 27 sites in the Lake Sunapee watershed in May, 2017, and will be sampled again in August, 2017. Sites were selected to cover a range of watershed features (%wetland, %forest, %open canopy, and open water connectivity). Samples are filtered to give dissolved (<0.45µm) and whole water fractions, and will be analyzed for methylmercury and total mercury, and for dissolved organic carbon quality and quantity.
We will be deploying *in situ* ceramic tiles for biofilm growth in streams in late May, 2017, for collection later in the summer. Invertebrates (mayflies, which are grazers), will also be collected from the streams in August.

**DOC quality and quantity:** Filtered water samples from field collection are analyzed by 3D excitation-emission fluorescence spectroscopy, and by ultraviolet spectroscopy within 48 h of collection. Whole and filtered water samples are also analyzed for total organic carbon within 28 days of collection. Fluorescence excitation-emission matrix (EEM) data will be analyzed in Matlab using the “Dr. EEM” toolbox, and correcting for instrument and inner filter effects.

**Principal findings:** As we are in the first year of the award and have not yet completed a field season, we have not reached a stage of reporting findings. Our preliminary data on dissolved organic carbon isolated from streams and on field collection of stream water suggests there is a wide range of organic carbon quality in this system, indicating we will have suitable data to address the problem stated above.

**Publications and presentations:** We are in the early stages of this project and have not yet published or presented our findings. We have organized a meeting in late May, 2017, to meet with our USGS collaborators and the Lake Sunapee Protection Association to discuss the goals of this research, progress to date, and proposed future plans.

**Notable awards and achievements:** Co-PI Celia Chen and investigator Vivien Taylor received a CompX grant through the Neukom Institute at Dartmouth to compare findings from Lake Sunapee with other watersheds. The goal of the CompX grant is to model the effects of landscape and organic carbon on methylmercury cycling in streams from different watersheds, using data collected in this study and by our USGS collaborators. The CompX grant also enables us to increase our sampling coverage on Lake Sunapee, and to improve our GIS and modeling capabilities.

**Outreach and information transferred:** We are in the early stages of this research and have not yet undertaken any outreach activities. We are in contact with the Lake Sunapee Protective Association and will be meeting with them later in May 2017 to talk with them about our prior studies and current research, and to determine whether certain findings are likely to be of particular interest to their members in order to develop targeted outreach activities for this time next year.

**Students supported:** (1) Paul Vickers (2nd year undergraduate), is working full-time on the project spring term, and part-time in the winter and summer terms.

**Faculty and staff supported:** (4)

Kathy Cottingham (Professor)

Celia Chen (Research Professor)

Vivien Taylor (Research Scientist)

Kate Buckman (Research Scientist)
The NH WRRC supported one information transfer project with its 2016 104b funding:

1. New Hampshire WRRC Information Transfer
New Hampshire WRRC Information Transfer

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Publications


Information Transfer

Unbridled development and population growth can have detrimental impacts to water resources and ecosystem services. Rapid population growth is occurring in New Hampshire and state regulations, planning board decisions and zoning classifications all attempt to minimize the environmental impact of this rapid population growth. Most land use planning decisions are made at the local level on a town by town basis, often by volunteers who serve on various boards, commissions and committees. Decisions by these various resource managers are often made without a full understanding of the consequences that their decisions will have on water resources or ecosystem services.

This project provided salary for the Center’s Director and Associate Director to meet with state representatives, local town officials, watershed groups, school groups, the general public and scientists to discuss WRRC findings that relate to population growth, land use change and climate variability. Over the past year, the NH WRRC meet with the following groups to discuss water resource issues: NH Fish and Game, Natural Resources Conservation Service (NRCS), Trout Unlimited (TU), Southeast Watershed Alliance, The Nature Conservancy, Piscataqua Region Estuaries Partnership (PREP), NH Department of Environmental Services (DES), the US Geological Survey and the US Environmental Protection Agency (EPA). The NH WRRC website (http://www.wrrc.unh.edu/) is also used to disseminate information on water resources, and is updated and maintained by salary provided by this project. The Director and Associate Director dedicate time discussing current and future research in the Lamprey River Hydrologic Observatory, which is partially funded by the longstanding 104B project “Water Quality and the Landscape: Long-term monitoring of a rapidly developing suburban watershed”. On January 9, 2017 the NH WRRC funded and organized the Tenth Annual Lamprey River Symposium (see also below). Presentations focused on nutrients and other solutes, bacteria, sediment, hydrology, groundwater, climate and land use change, water quality indicators and monitoring programs in coastal New Hampshire. The symposium attracted approximately 90 attendees, including scientists, regional leaders, town officials, members of state agencies, and federal agencies. The agenda can be found on the NH WRRC Lamprey River Hydrologic Observatory Symposium website. This annual symposium and other discussions in which the Center’s Director and Associate Director participate further the research and information transfer goals of the NH WRRC.

2016 Information Transfer Activities Supported by Section 104b Funding and Matching Funds

Data sharing with Lamprey River watershed local advisory committee

The Lamprey River Advisory Committee (LRAC) is undergoing a long-term analysis of Lamprey River water quality data collected by both the Lamprey River Watershed Association’s (LRWA) volunteer monitoring program and the NH WRRC 104B project “Water Quality and the Landscape: Long-term monitoring of a rapidly developing suburban watershed”. The NH WRRC associate director serves on the LRAC and is a member of the water quality sub-committee which is advising a LRAC funded intern who is conducting the long-term water quality analysis. Temporal and spatial trends in dissolved oxygen, pH and nitrate have been examined thus far and further analysis is underway.
Nitrogen Data in New Hampshire’s Great Bay watershed

Over the last eight years, there has been significant focus on nitrogen loading to New Hampshire’s largest estuary, the Great Bay estuary, and the impairment to aquatic life it has caused. In August 2009, Great Bay, Little Bay and the tidal rivers were added to the New Hampshire 2008 303d list of impaired waters rendering them in violation of the federal Clean Water Act. Based on the most recent “State of Our Estuaries Report” prepared by PREP (2013), 32% of the nitrogen entering Great Bay and Little Bay is from point sources; the majority (68%) enters via non-point sources of pollution. The Lamprey River is the largest tributary to Great Bay, and thus the long-term data provided by the NH WRRC from the LRHO are of considerable value for watershed management. The NH WRRC provides the best dataset in NH for assessing the spatial and temporal variability in N concentrations and export in response to suburbanization and changes in land use. These 16+ years of data will be instrumental in assessing the success of current and future efforts to reduce non-point sources of nitrogen pollution reaching Great Bay. There is much interest in LRHO datasets from NH DES, PREP, EPA and other municipal, regional, state and federal agents. Many of the presentations listed below and meetings attended focused on transferring information on nutrient cycling to stakeholders throughout NH’s coastal watershed and beyond. The NH WRRC has received several phone calls and meeting requests to discuss the Great Bay nitrogen issue. The NH WRRC has also been asked by PREP to help update the nutrient loading indicator for the 2017 State of Our Estuaries report.

Water quality monitoring advice for wood restoration projects in NH streams

The Natural Resources Conservation Service and TU have selected 23 Wetlands Reserve Program (WRP) properties in NH for wood loading restoration work. The project involves adding wood into small segments of 1st and 2nd order stream channels (averaging about 1,000 feet) with a primary goal of recreating and increasing fish spawning and rearing habitat as well as preventing bank erosion and improving stream geomorphology. A supplemental goal of this work is to study the changes in water quality and nutrient uptake which may be enhanced by adding carbon (in the form of wood) to streams. The NH WRRC Director, Associate Director and the WQAL manager have been advising the NRCS and TU on how to best understand changes in water quality and nutrient dynamics with existing financial resources. With collaboration between the NRCS, TU and the NH WRRC, baseline water quality monitoring began in 2014. Wood installations occurred in 2015 and 2016 and a few remaining properties have been scheduled for restoration in 2017.

Drinking water quality in New Hampshire

The recent Perfluorooctanoic Acid (PFOA) and lead contamination of southern NH drinking water has prompted several inquiries to the NH WRRC and the Water Quality Analysis Laboratory (WQAL) from residents and local media concerned with drinking water quality in the state.
Symposia, Conferences and Seminars Organized and Funded

The NH WRRC funded and organized the "Tenth Annual Lamprey River Symposium" held January 9, 2017 in Durham, NH. The symposium is dedicated to exchanging the results of recent research on the water quality, hydrology, water resources issues, and management of the Lamprey River basin. The Symposium is a vehicle for researchers to share data and insights with other researchers, as well as those in the management and policy arena who would benefit from exposure to the latest research on the watershed. The symposium drew approximately 90 attendees, including researchers, legislators, water system operators, town officials, regional leaders and government officials. The symposium contained 13 presentations split up over three sessions. There was a poster session during and after lunch where 4 posters displays were exhibited. The day ended with an open discussion on research priorities in the Lamprey watershed and southeast NH. This event was funded and organized by the NH WRRC. NH EPSCoR assisted with registration and printing. Survey results indicate that most of the attendees found the topics covered to be either helpful or very helpful.

The NH WRRC sponsored the “NH Water and Watershed Conference” which was held on March 18, 2016 in Plymouth, NH. This event was designed to meet the information and networking needs of lake, river, and watershed groups; environmental organizations; volunteer monitors; municipal board and staff members; elected officials; local and regional planners; policy makers; scientists; educators; consultants and students. The focus for the 2016 conference was “Managing New Hampshire’s Water for a More Resilient Environment”. The NH WRRC co-sponsored this conference along with Plymouth State University and the Center for the Environment, NH EPSCoS, NH DES, US Geological Survey New England Water Science Center and a few others. The conference contained 5 concurrent sessions including stormwater management and MS4 Permits, floods and flood hazards, water and infrastructure, modeling New Hampshire’s water and watersheds and measuring and modeling New Hampshire’s water and watersheds. The conference drew approximately 250 people, including researchers, legislators, water system operators, land use planners, and government officials. The Center’s Associate Director also serves on the planning committee for the annual NH Water and Watershed Conference.

Publications


“Continuous nutrient sensing in research and management: applications and lessons learned across aquatic environments and watersheds”.


Conference Proceedings & Abstracts:


Presentations/Information Transfer

Shattuck, M.D. 2016. Shared Wednesday Hill Brook and organic dairy farm data with Katie Slebodnik for use in Aqueous Geochemistry class project and directed her to the NH EPSCoR DDC. September 2016.

Koenig, L. 2016. For the fourth consecutive year, Koenig served as the instructor for the STEM mini-course offered August 22-26th, 2016 through the CONNECT program at UNH (http://www.unh.edu/connect/). The objective of the course is to provide an opportunity for incoming freshmen that come from groups with historically low retention in STEM majors (e.g. low-income, multicultural, first-generation college students) to build community, discover college resources, and bolster skills that are needed to succeed in their academic programs (e.g. writing of lab/research reports, basic math and statistics for analyzing scientific data). There were 12 students in the class, but the broader CONNECT program serves approximately 100 students.


Press Releases

McDowell, W.H. 2016. Spoke with reporter from the Manchester Union Leader on chemical perfluorooctanoic acid, or PFOA, which has been found in water sources in Merrimack and Litchfield. Article: “Bedford water samples detect low levels of PFOA contamination” by KIMBERLY HOUGHTON. March 25, 2016.

➤ http://www.unionleader.com/Bedford-water-samples-detect-low-levels-of-PFOA-contamination


USGS Summer Intern Program

Basic Information

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Additional Remarks

This internship has been an incredibly enjoyable and educational experience for me. Working at USGS has taught me more about how scientists collect, analyze, and present data in the past year than I have learned in years of school education. I learned safe and effective lab techniques, the functionality of numerous machines, and techniques for the organization, analysis, and presentation of data. I have also learned how bureaucratic, legal, and technical problems can roadblock science, which, although frustrating at times, has been nonetheless valuable. I loved going out into the field to collect samples and carrying those samples through the process of analysis. It has been fulfilling to understand the importance of my project and learn about other scientists' projects and their cumulative conclusions. Other scientists were more than happy to teach me about their problems and findings. Robin Stewart has been an incredible mentor for me -- she is a superb scientist and a wonderful person.
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Notable Awards and Achievements

Ursula Jongebloed served as an USGS intern on the project “Investigations into the bioavailability and bioaccumulation of selenium (Se) and mercury (Hg) in the San Francisco Bay Estuary”. Ursula began the internship at the USGS National Research Program office in Menlo Park, California in June 2015 after completing her sophomore year at Dartmouth College (located in Hanover, New Hampshire). The internship experience was very rewarding for both Ursula and her USGS mentor Robin Stewart. The internship experience was so successful that Ursula is preparing a manuscript along with co-authors Robin Stewart and Amy Kleckner on the trends in dissolved and particulate selenium concentrations with respect to bivalve Se concentrations and water year in the San Francisco Estuary. This manuscript is in preparation for a special issue “Undergraduate Research in Water – Training the Next Generation of Water Scientists” in the Journal of Contemporary Water Research and Education which is scheduled for publication in 2017.

Director Dr. William H. McDowell received the 2017 Distinguished Professor Award. The purpose of this award is to identify and honor longstanding members of the University of New Hampshire faculty. This singular university-wide award will be given each year to the faculty member whose overall record of excellent teaching, caring about students, devotion to the university community and substantial record of scholarly achievement exemplifies what we would call a ‘distinguished career’.

Currently NH has numerous watersheds listed as impaired due to elevated chloride levels resulting from salt use in winter road maintenance with most those watersheds located in the southern part of the state. College Brook is one of the impaired watersheds and the impairment listing was based on data produced from the 2003NH21B project.

Paul Vickers is a sophomore at Dartmouth College and has become an integral part of the research team for the 104G project “Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems” (2016NH205G). The grant has allowed him to learn how to collect and process trace-metal clean water samples, use multiple types of spectroscopy to measure dissolved organic carbon in the water he collected, maintain algal cultures, and put his interest in engineering to use designing better tanks for growing periphyton. It is unusual for an undergraduate to take on as large a role as Paul has in carrying out the goals of a research grant, but he has stepped up to the task with enthusiasm. As part of our team, Paul is learning skills that will help him become more competitive as he finishes college and begins his career. Paul Vickers states that “Being part of the Dartmouth WRRC team has given me invaluable experience studying the characteristics of organic carbon in a lake environment, which provides me with a critical base of knowledge as I hope to work on minimizing the degradation of marine ecosystems in the Pacific Northwest after graduation.”