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

Basic Information

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There are no publications.

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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 mayfiles. 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

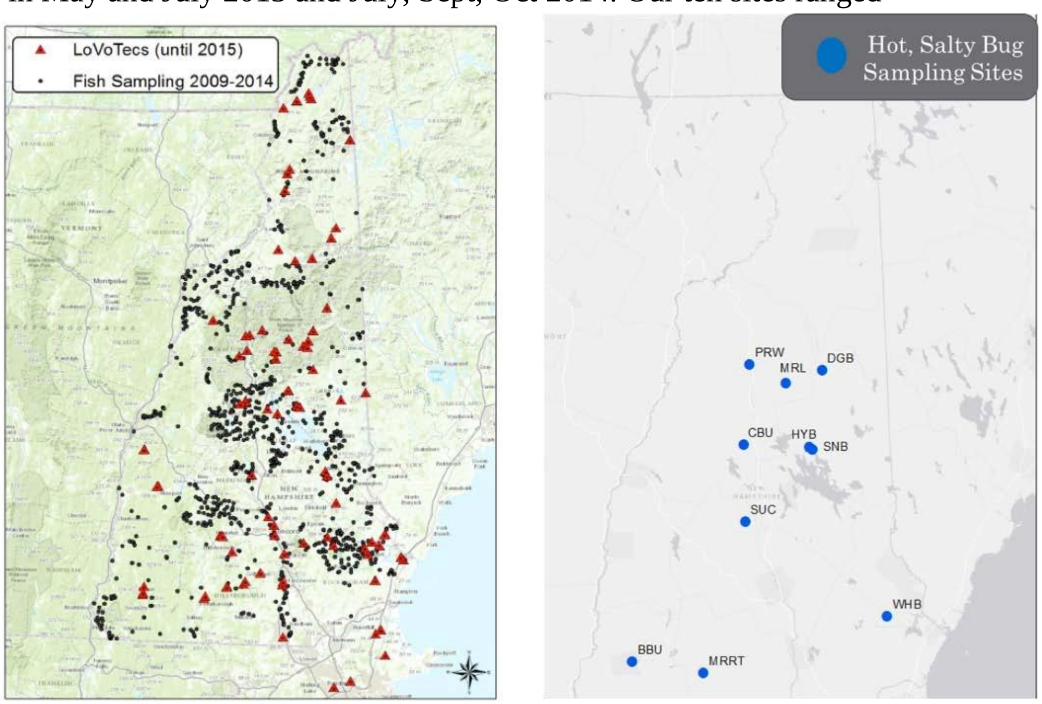

Figure 1: Network of LoVoTEC monitoring sites overlaid with NH Fish and Game Dept fish sampling sites between 2009-2014 (LEFT); Ten Hot, Salty Bugs sampling sites between May and October 2016.

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 Macroinvertebrate 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 100meter 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 Septemeber/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.

Macroinvertebrate 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 bicinchoninic 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.

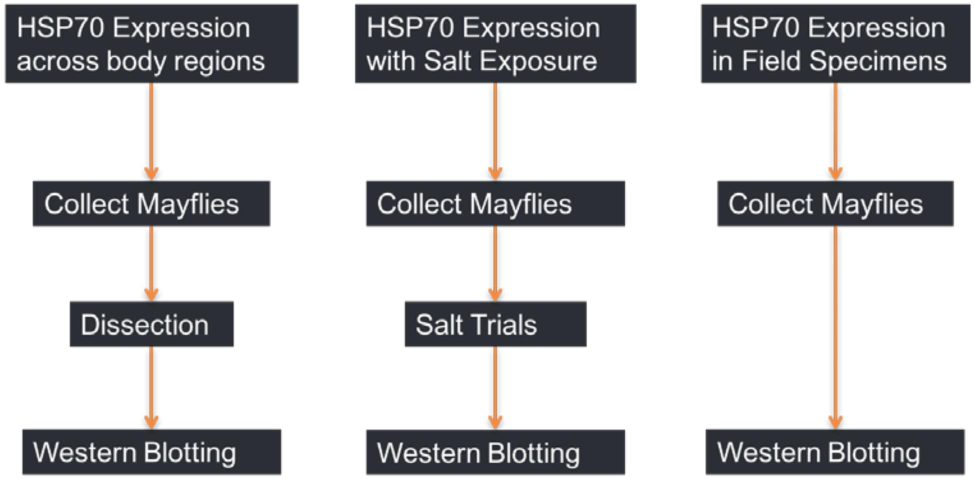

Figure 2: Overview of HSP70 analysis.

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 that 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).

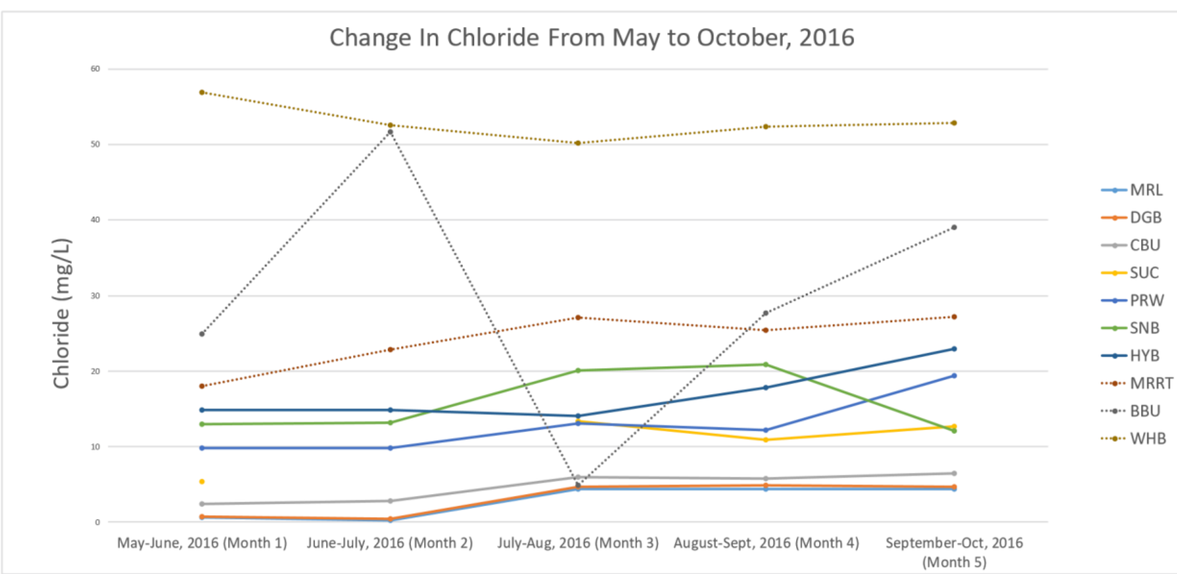

Figure 3: Snapshot sampling of chloride in 10 sample streams between May/June and September/October 2016.

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.

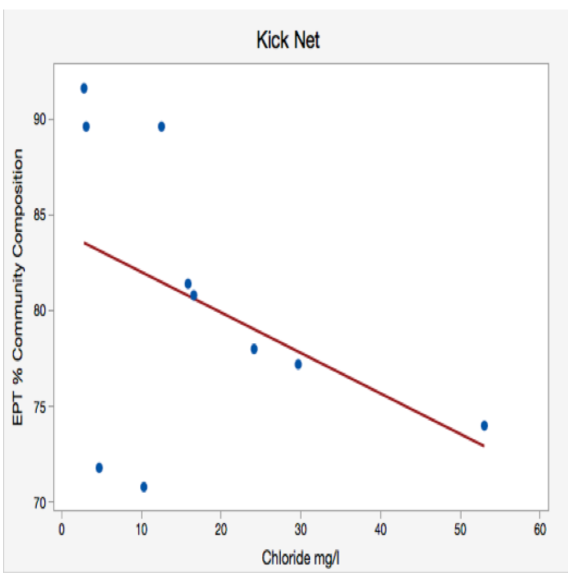

Figure 4A: Linear regression comparing average EPT % community composition and chloride for kick nets (r^2=.18, p=0.22)

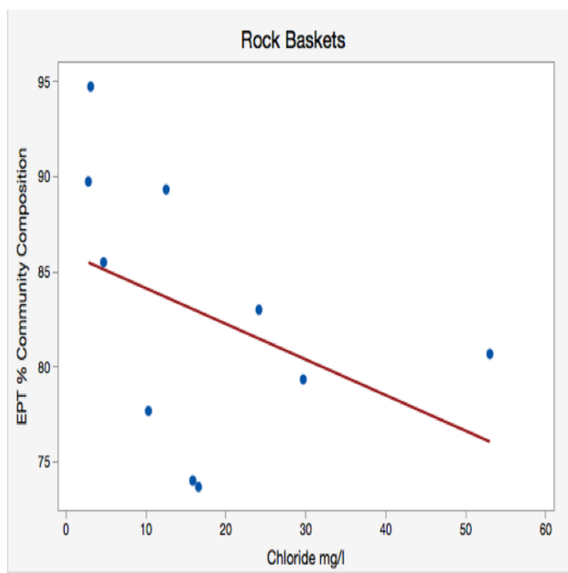

Figure 4B: Linear regression comparing average EPT % community composition and chloride for kick nets (r^2=.17, p=0.24)

Table 1: Summary of regression analyses of chloride and various macroinvertebrate community composition metrics.

	EPT Family Richness	% EPT	% Plecoptera	Philopotamid Abund.	% Chironomidae
Month	R^2	R^2	R^2	R^2	R^2
May-October	0.22*	0.11*	0.12	0.02*	0.03*
May-June	0.22*	0.00*	0.28	0.01	0.32*
June-July	0.07*	0.18*	**0.53**	0.14*	0.16*
July-August	0.13*	0.05*	0.09	0.00*	0.07
August-September	**0.53***	0.25*	0.06*	0.19*	**0.51***
September-October	**0.56***	**0.56***	0.01*	0.02*	0.25*
Bold= $p< 0.05$ and * means relationship was as hypothesized					

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.

Table 2: Summary of the 'best' multiple linear regression models for each community composition metric.

Multiple Linear Regression Models to Predict Community Composition

Dependent variable	Independent variable	R^2	P value
% EPT	Elevation	0.19	<0.0001
	Sodium	0.11	0.004
% Ephemeroptera	Month	0.24	<0.0001
% Plecoptera	Chloride	0.30	<0.0001
% Tricoptera	Month	0.24	<0.0001
	Water Temperature	0.22	<0.0001
Total Family Richness	Water Temperature	0.13	0.005
	Latitude	0.16	0.002
Philopotamidae Relative Abundance	Water Temperature	0.24	0.001
Rhyacophilidae Relative Abundance	Month	0.11	0.044

The single best independent variable is listed first, followed by the second most, if applicable, by its unique variance (R^2) and p-value.

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.

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

Linear Regression - Mean Daily Water vs. Air		
Site	R sq	Slope
BBU	0.39	0.424
CBU	0.67	0.586
DGB	0.74	0.584
HYB	0.72	0.681
MRL	0.54	0.412
MRRT	0.60	0.520
PRW	0.88	0.509
SNB	0.70	0.612
SUC	0.78	0.605
WHB	0.95	0.506

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 Simpsons 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 4: Summary of the linear relationships between stream temperature and community composition metrics. Bolded values note statistical significance ($p \leq 0.05$).

Explanatory Variable	**Monthly Mean**		**168 Hour (7 day) Mean**		**Average Daily Max**	
Response Variable	R-sq	Slope	R-sq	Slope	R-sq	Slope
% EPT	0.04	-0.673	0.07	-0.964	0.05	-0.726

% Plecoptera	**0.13**	**-1.673**	**0.13**	**-1.731**	**0.13**	**-1.643**
% Philopotamidae	**0.26**	**3.045**	**0.14**	**2.275**	**0.26**	**2.982**
% Leptophlebiidae	**0.27**	**-0.922**	**0.19**	**-0.739**	**0.27**	**-0.905**
% Corydalidae	**0.14**	**0.615**	**0.23**	**0.781**	**0.14**	**0.611**
Simpson's Index (1-D)	0.03	-0.005	0.01	-0.003	0.03	-0.005
Shannon's Index (H)	0.02	-0.013	0.00	-0.003	0.01	-0.010
Shannon's Evenness	0.06	-0.006	0.06	-0.006	0.07	-0.006

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.

Air vs. Water Slope Values as Explanatory Variable		
Explanatory Variable	**Slope**	
Response Variable	R-sq	Slope
% EPT	0.00	3.830
% Plecoptera	0.09	-22.290
% Philopotamidae	0.03	23.620
% Leptophlebiidae	0.02	5.473
% Corydalidae	0.01	3.663
Simpson's Index (1-D)	**0.15**	**-0.280**
Shannon's Index (H)	**0.14**	**-0.867**
Shannon's Evenness	0.00	-0.090

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.

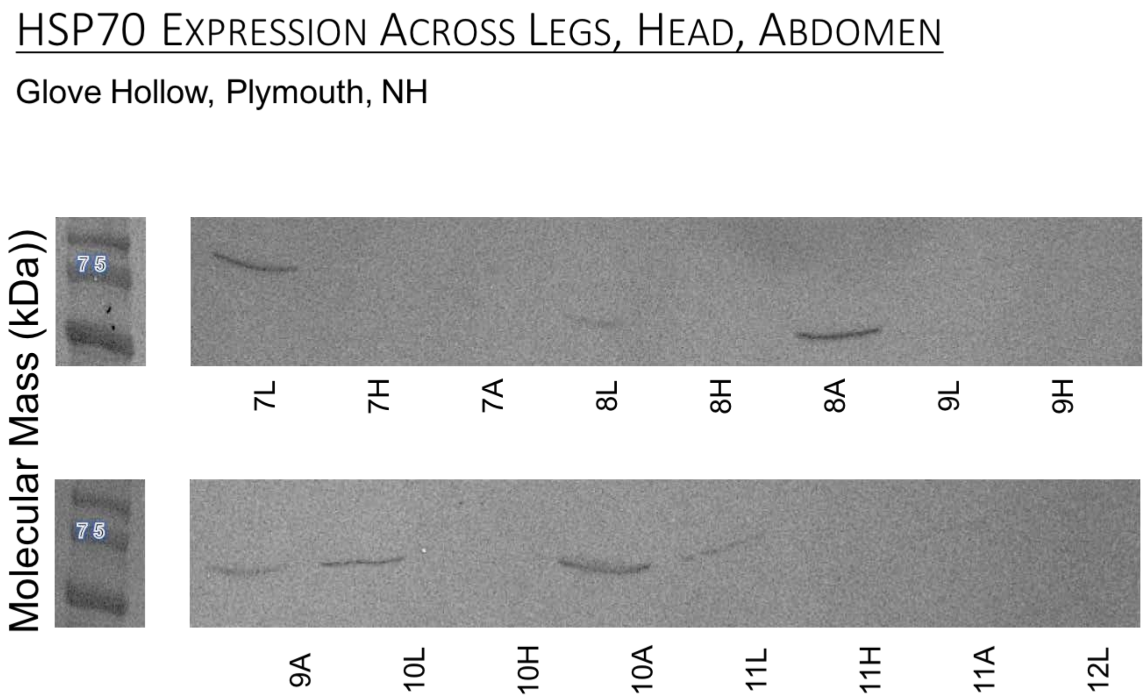

Figure 5: Western blot results of HSP70 expression across the legs, head, and abdomen of mayflies collected in spring 2016.

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.

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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

Fruit, R. , A. Villamagna, B. O'Donnell. 2016. *Stress Protein Expression: An Early Warning Sign of Freshwater Community Degradation via Road Salt Runoff in New Hampshire* (poster), 2016 NH Water and Watersheds Conference in Plymouth, New Hampshire

Duquette, R., A. Villamagna, B. O'Donnell. 2016. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* Hubbard Brook Research Experience for Undergraduates Symposium (Thorton, NH)

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* Hubbard Brook Research Experience for Undergraduates Symposium (Thorton, NH)

Duquette, R., A. Villamagna, B. O'Donnell. 2016. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* EPSCoR Research for Undergraduates Symposium at University of New Hampshire (Durham, NH)

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* EPSCoR Research for Undergraduates Symposium at University of New Hampshire (Durham, NH)

2017

Duquette, R., A. Villamagna, B. O'Donnell. 2017. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* New England Association of Environmental Biologists annual meeting (Hartford, CT) *[poster printed but not presented due to poor blizzard travel conditions]*

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]*

Duquette, R., A. Villamagna, B. O'Donnell. 2017. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* Plymouth State University Showcase of Excellence (poster)

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* Plymouth State University Showcase of Excellence (poster)

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)

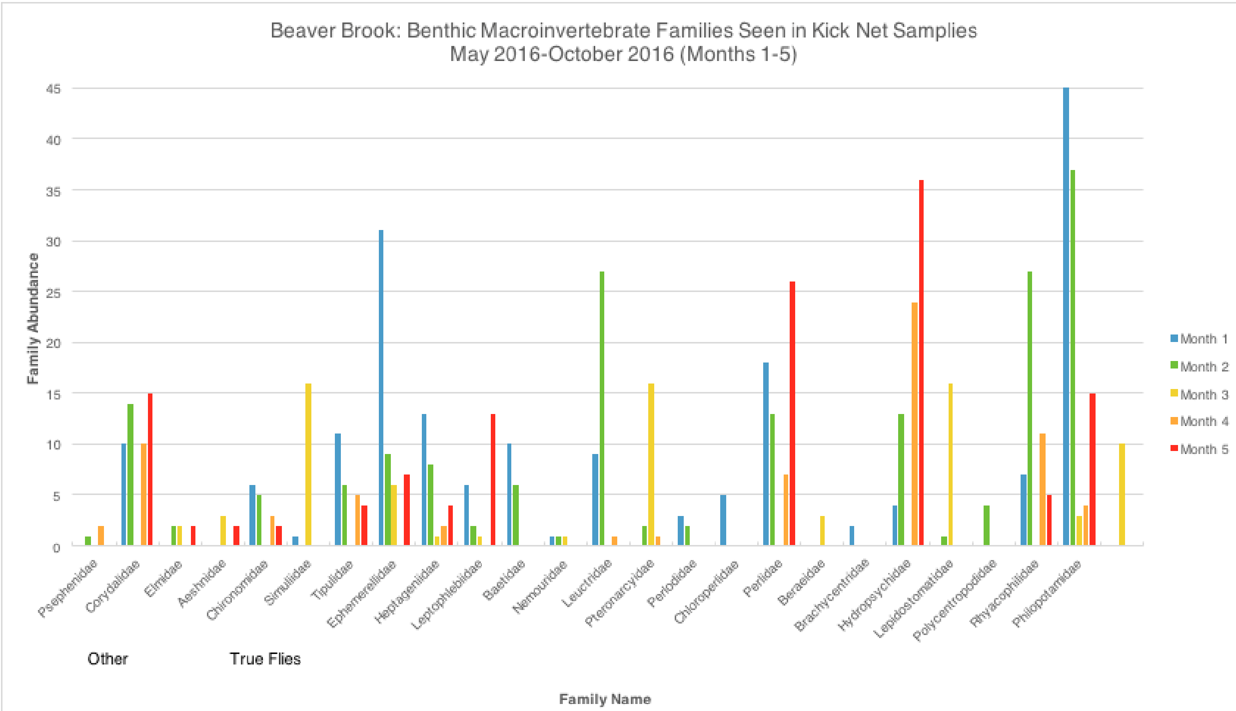
Mazzone, M. , A. Villamagna, B. O'Donnell. 2017. Assessing Salt Stress on Macroinvertebrate Communities in NH Streams. Plymouth State University Showcase of Excellence (oral presentation)

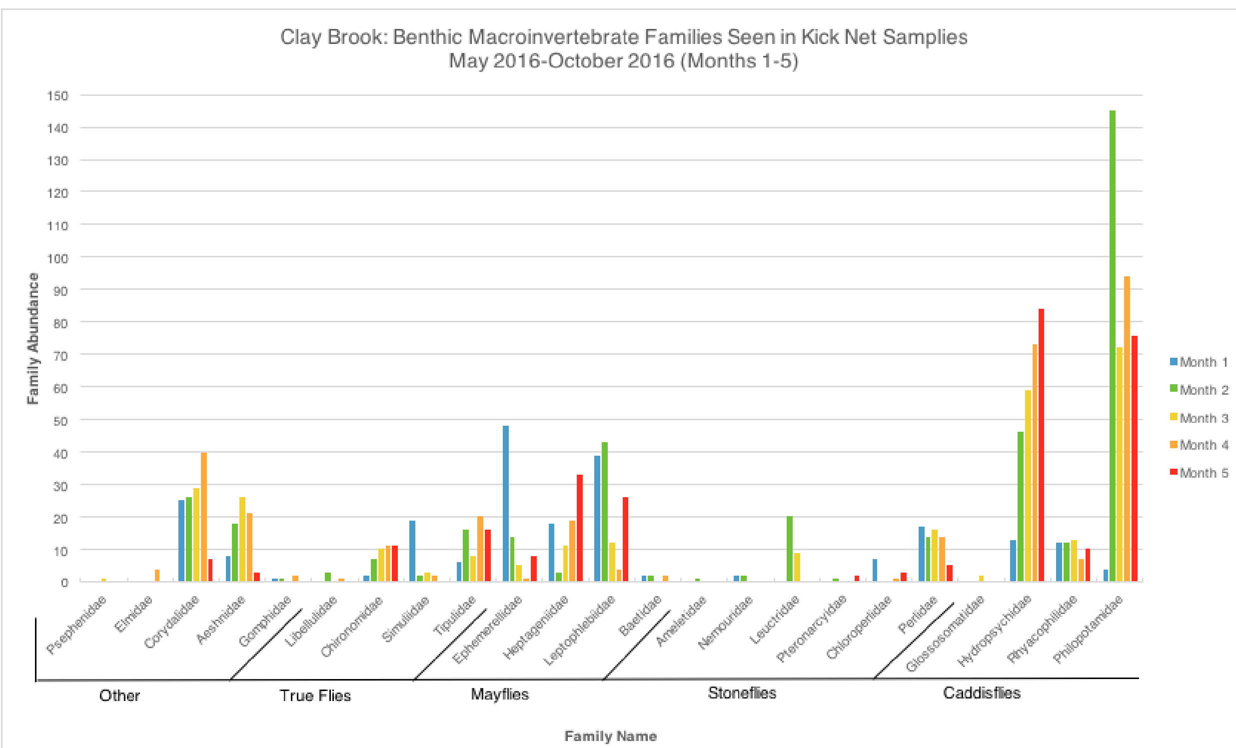
Number of students supported*:* 2 master's students, Matthew Mazzone and Roy Fruit. 2 undergraduate students, Ryan Duquette and Thomas Lafortune, were affiliated with the project through university match and research collaboration., but did not receive direct funding from NH WRRC.

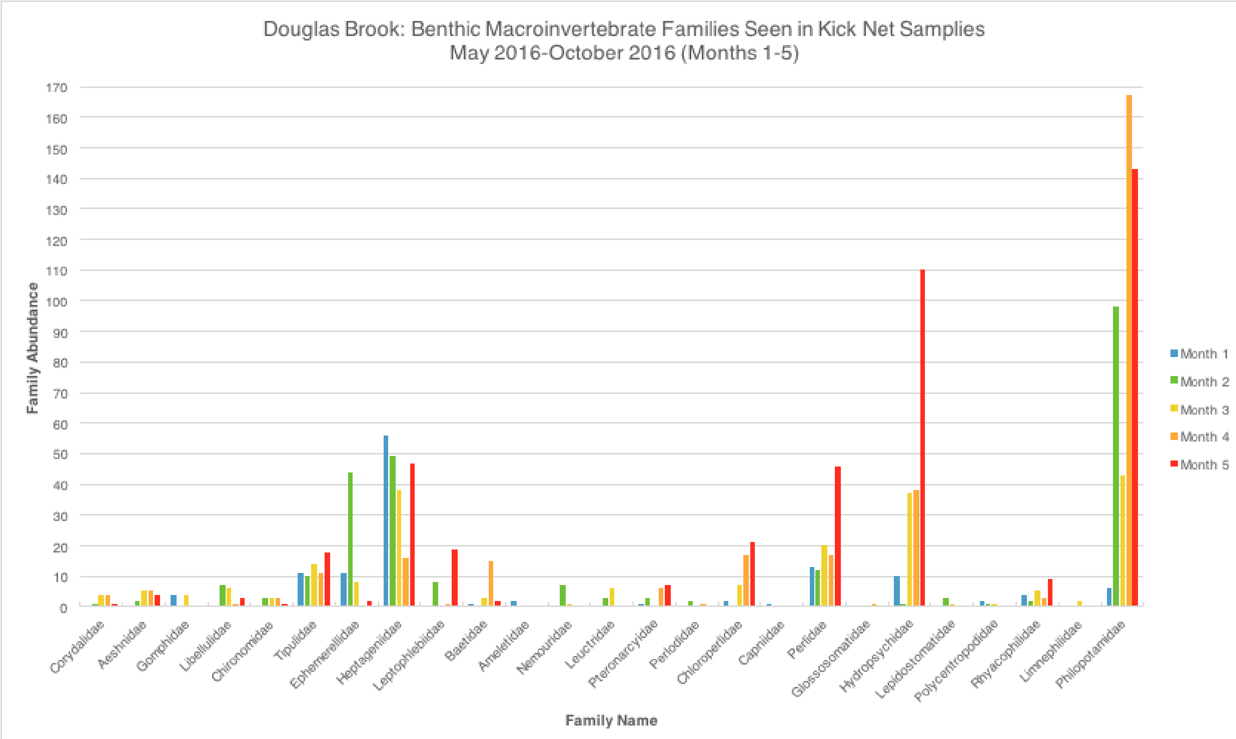
Number of faculty supported: Assistant professor, Amy Villamagna (Ph.D.) received direct funding for this project and Associate professor, Brigid O'Donnell (Ph.D.) was affiliated with the project through university match and research collaboration.

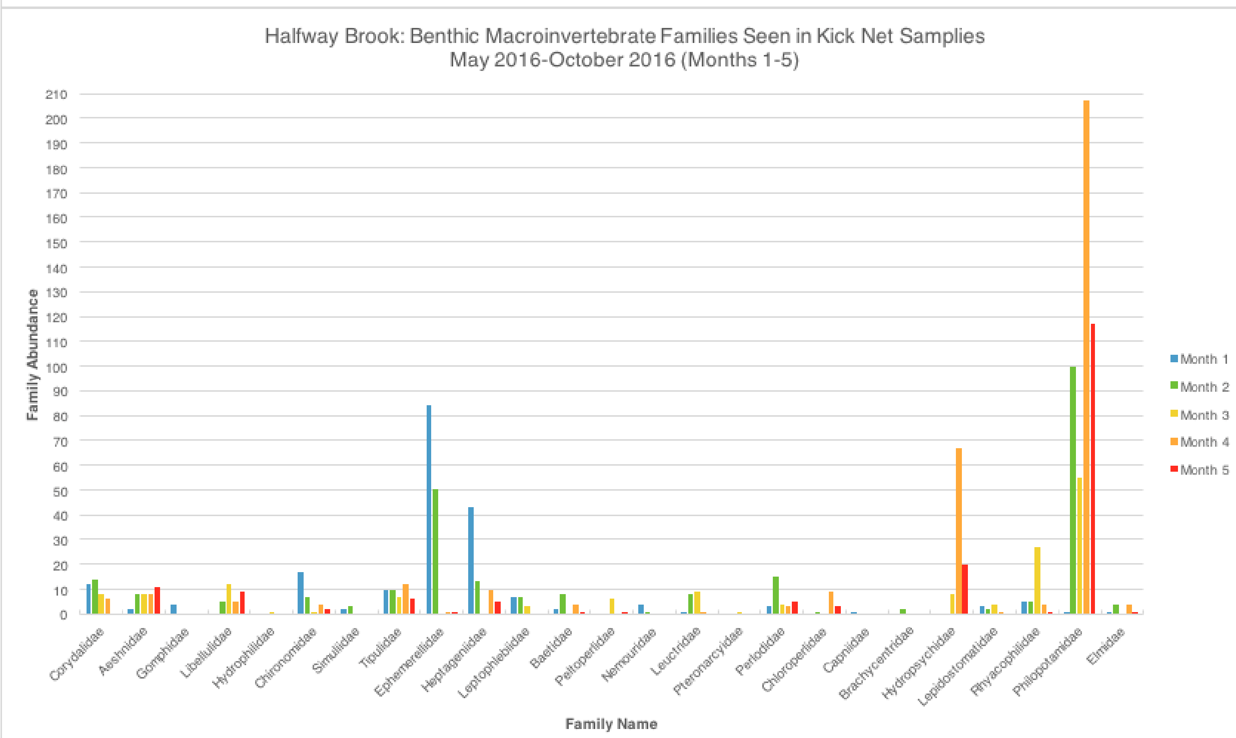
APPENDICES

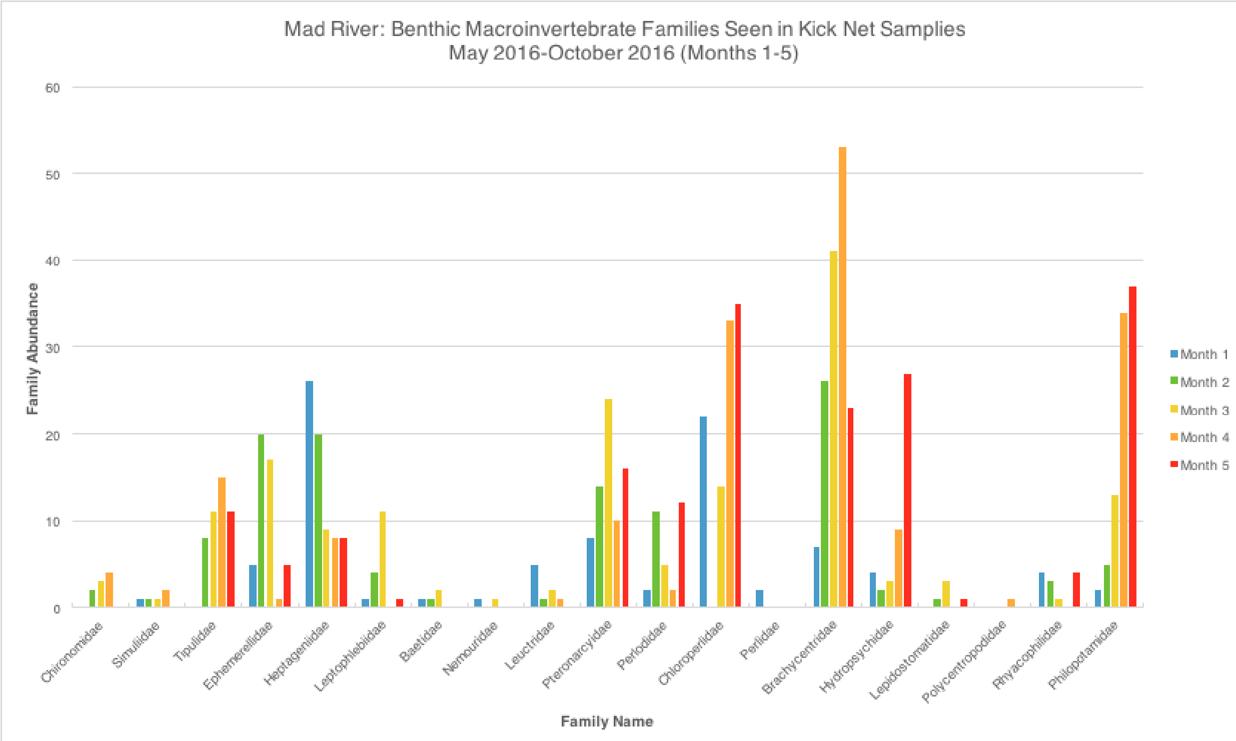
Appendix A: Within season comparison of family level abundance observed through kicknet sampling for benthic macroinvertebrates at all ten study sites.

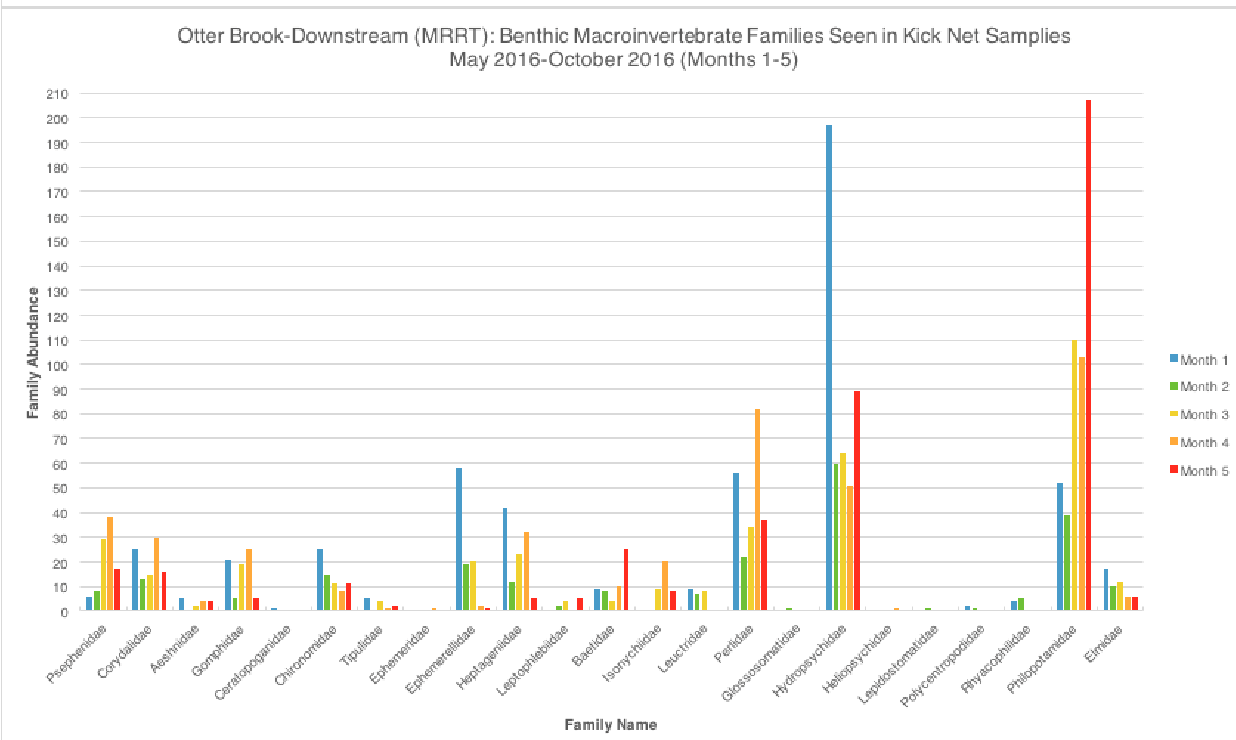


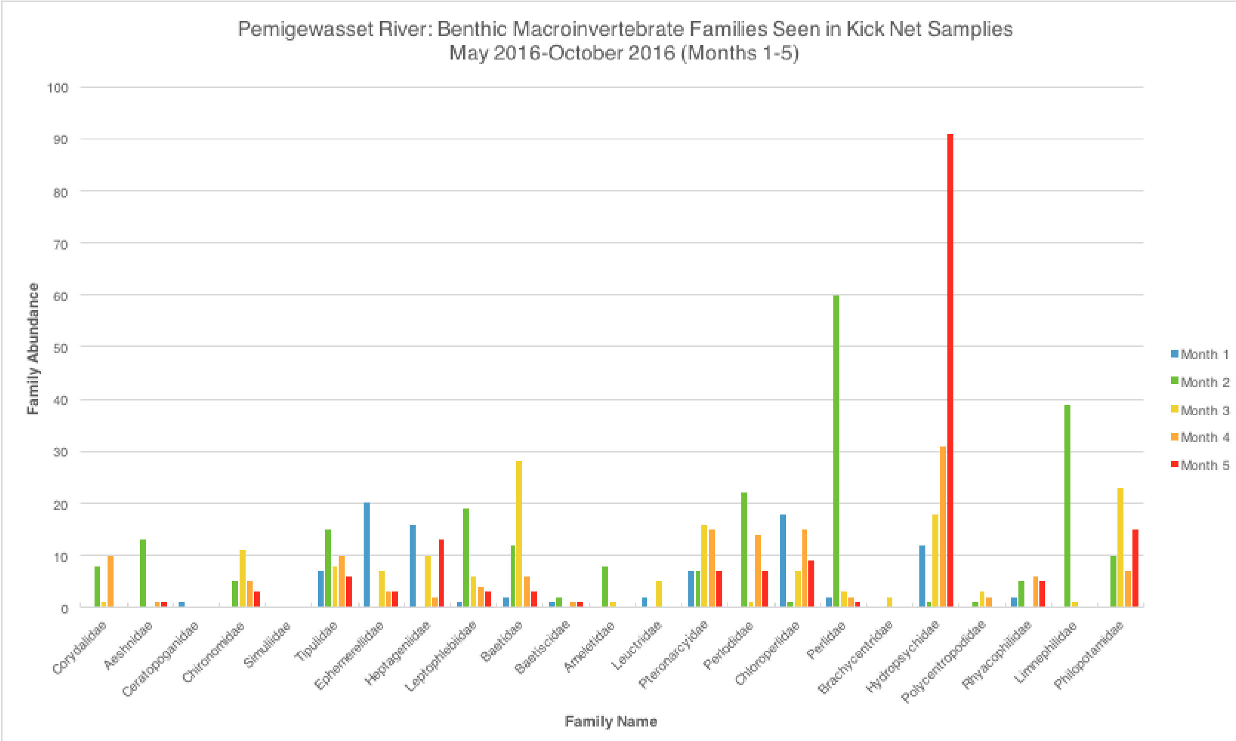



Douglas Brook: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Corydalidae
Aeshnidae
Gomphidae
Libellulidae
Chironomidae
Tipulidae
Ephemerellidae
Heptagenlidae
Leptophlebiidae
Baetidae
Ameletidae
Nemouridae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Capniidae
Perlidae
Glossosomatidae
Hydropsychidae
Lepidostomatidae
Polycentropodidae
Rhyacophilidae
Limnephilidae
Philopotamidae

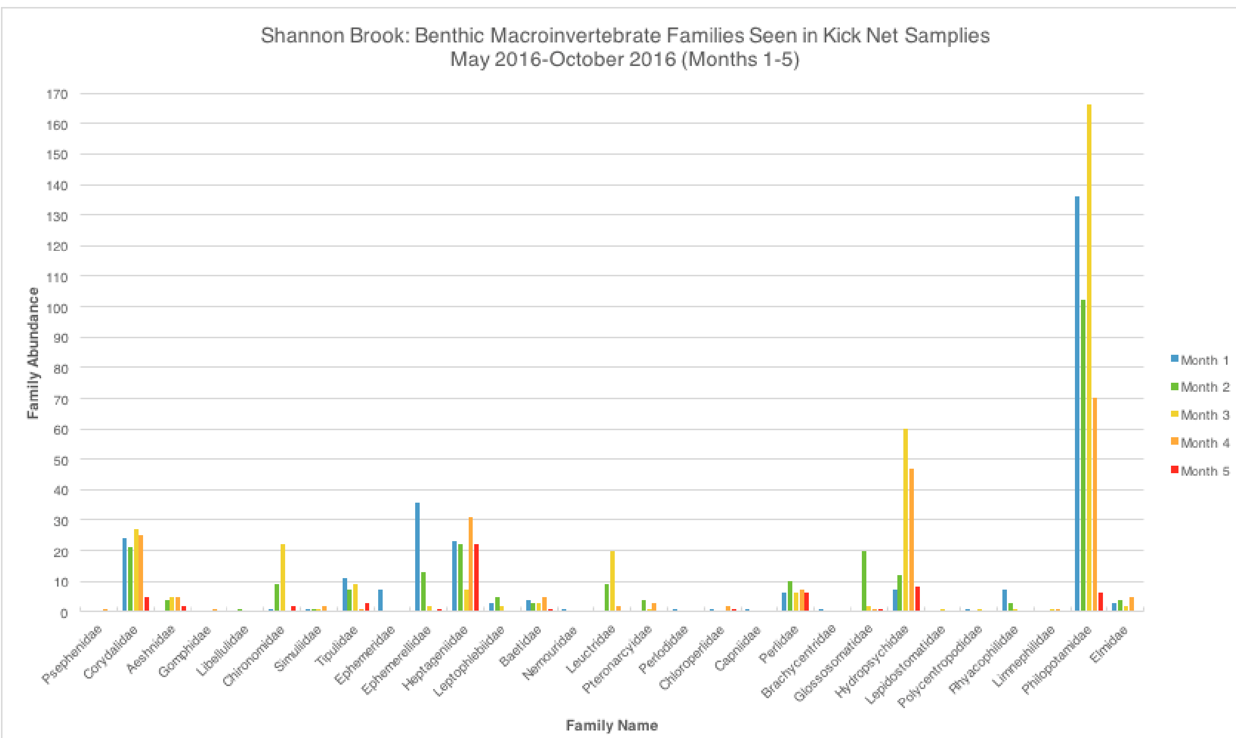

Halfway Brook: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Corydalidae
Aeshnidae
Gomphidae
Libellulidae
Hydrophilidae
Chironomidae
Simulidae
Tipulidae
Ephemerellidae
Heptagenidae
Leptophlebiidae
Baetidae
Peltoperlidae
Nemouridae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Capniidae
Brachycentridae
Hydropsychidae
Lepidostomatidae
Rhyacophilidae
Philopotamidae
Elmidae


Mad River: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
0
10
20
30
40
50
60
Chironomidae
Simulidae
Tipulidae
Ephemerellidae
Heptagenidae
Leptophlebiidae
Baetidae
Nemouridae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Perlidae
Brachycentridae
Hydropsychidae
Lepidostomatidae
Polycentropodidae
Rhyacophilidae
Philopotamidae
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5

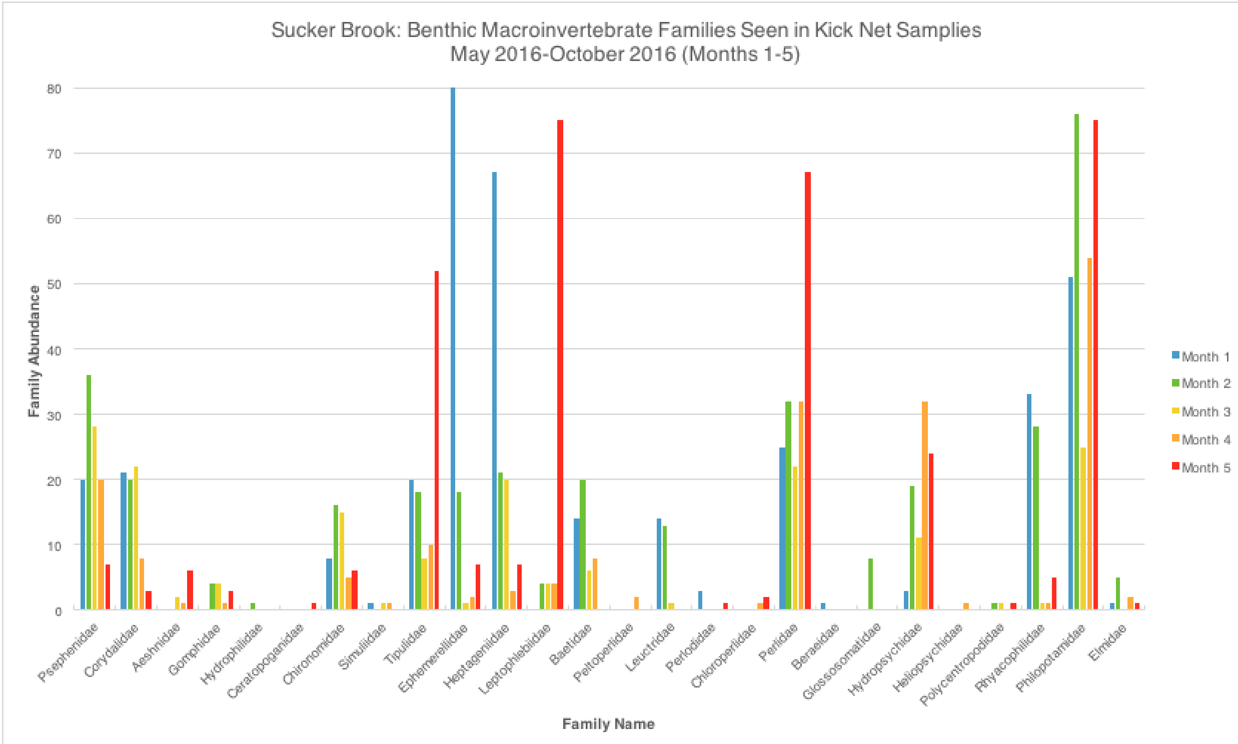

Otter Brook-Downstream (MRRT): Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
Psephenidae
Corydalidae
Aeshnidae
Gomphidae
Ceratopoganidae
Chironomidae
Tipulidae
Ephemeridae
Ephemerellidae
Heptagenidae
Leptophlebiidae
Baetidae
Isonychidae
Leuctridae
Perlidae
Glossosomatidae
Hydropsychidae
Heliopsychidae
Lepidostomatidae
Polycentropodidae
Rhyacophilidae
Philopotamidae
Elmidae
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5



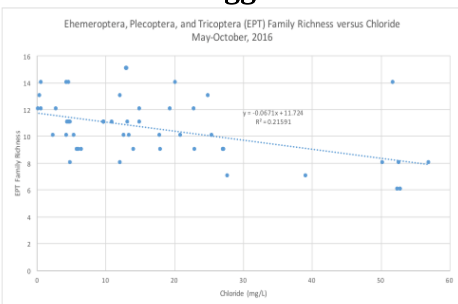
Pemigewasset River: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Corydalidae
Aeshnidae
Ceratopoganidae
Chironomidae
Simuliidae
Tipulidae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Baetiscidae
Ameletidae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Perlidae
Brachycentridae
Hydropsychidae
Polycentropodidae
Rhyacophilidae
Limnephilidae
Philopotamidae



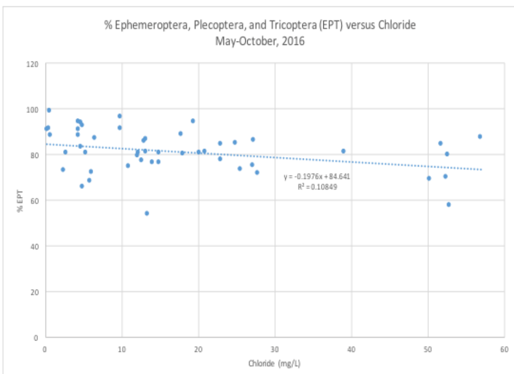
Shannon Brook: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Psephenidae
Corydalidae
Aeshnidae
Gomphidae
Libellulidae
Chironomidae
Simuliidae
Tipulidae
Ephemeridae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Nemouridae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Capniidae
Perlidae
Brachycentridae
Glossosomatidae
Hydropsychidae
Lepidostomatidae
Polycentropodidae
Rhyacophilidae
Limnephilidae
Philopotamidae
Elmidae


Sucker Brook: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
80
70
60
50
40
30
20
10
0
Month 1
Month 2
Month 3
Month 4
Month 5
Psephenidae
Corydalidae
Aeshnidae
Gomphidae
Hydrophilidae
Ceratopoganidae
Chironomidae
Simuliidae
Tipulidae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Peltoperlidae
Leuctridae
Perlodidae
Chloroperlidae
Perlidae
Beraeidae
Glossosomatidae
Hydropsychidae
Heliopsychidae
Polycentropodidae
Rhyacophilidae
Philopotamidae
Elmidae
Family Name

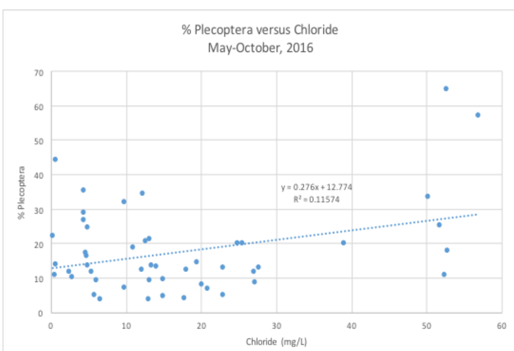
Appendix B: Univariate relationships between chloride and common benthic macroinvertebrate biomonitoring metrics. The graph illustrates the observed relationship across all sampling months in 2016. Yellow highlighted boxes in table suggest the relationship was significant and as hypothesized.



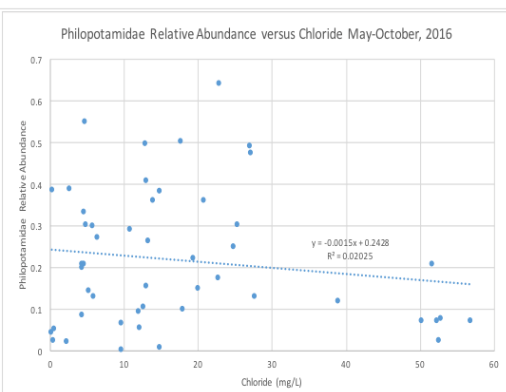
Month	Equation	R^2
May-June	y = -0.0603x + 12.183	0.21106
June-July	y = -0.0221x + 12.08	0.07356
July-August	y = -0.0733x + 12.158	0.13203
August-September	y = -0.099x + 11.506	0.52586
September-October	y = -0.0878x + 10.971	0.55617



Month	Equation	R^2
May-June	y = -0.0009x + 84.463	3.90E-06
June-July	y = -0.1335x + 87.1	0.17894
July-August	y = -0.1632x + 77.697	0.04555
August-September	y = -0.292x + 84.044	0.24641
September-October	y = -0.5147x + 93.763	0.5565



Month	Equation	R^2
May-June	y = 0.5558x + 12.819	0.28156
June-July	y = 0.6511x + 6.6956	0.53375
July-August	y = 0.1862x + 14.897	0.09404
August-September	y = -0.1573x + 18.015	0.05514
September-October	y = -0.0471x + 16.253	0.00675



Month	Equation	R^2
May-June	y = 0.0009x + 0.1004	0.0105
June-July	y = -0.003x + 0.2854	0.14317
July-August	y = -0.0005x + 0.226	0.00242
August-September	y = -0.005x + 0.3646	0.19266
September-October	y = -0.0018x + 0.2873	0.02316

Appendix C: Preliminary results from HSP70 lab trials.

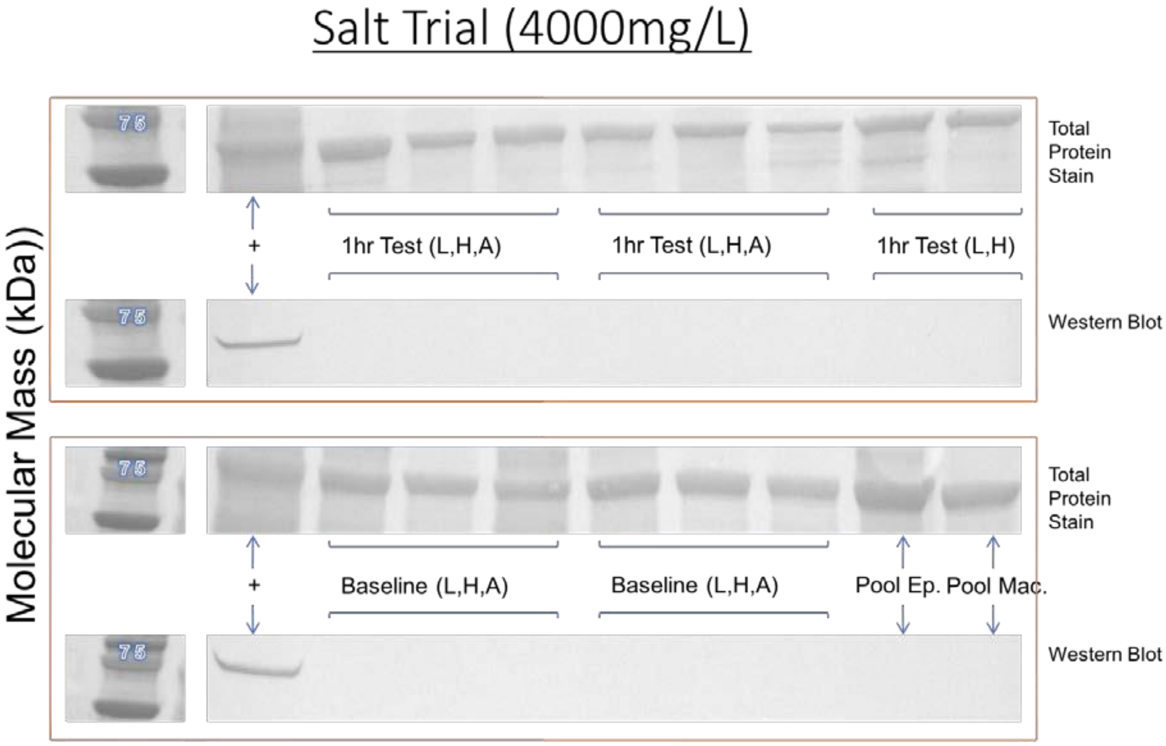


Figure above: Preliminary results from HSP70 lab analysis of mayflies exposed to an acute dose of 4000 mg/L of sodium chloride. The top blot captures the total protein in the sample, and the lower blot in each panel reflects the HSP70. The lane on the far left reflects the positive control for each blot.

Salt & Temperature + Increased P.I.

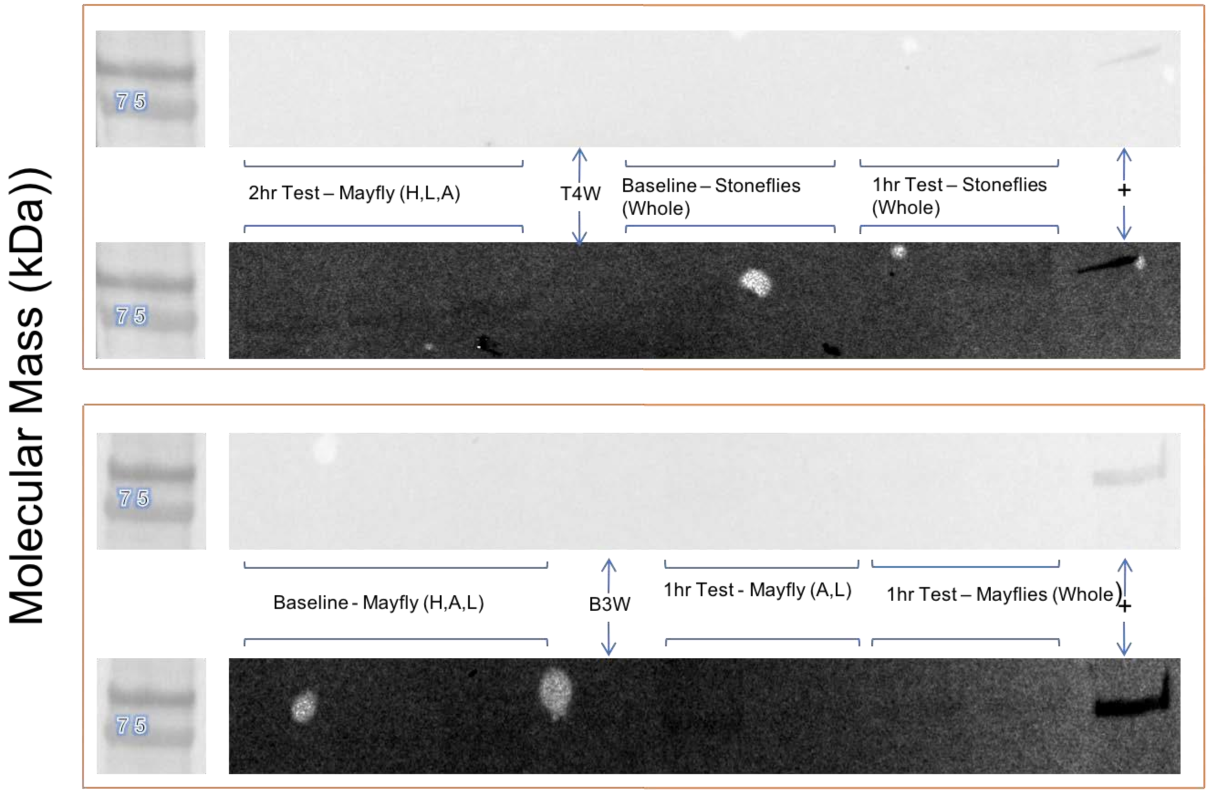


Figure above: Preliminary results from HSP70 lab analysis of mayflies and stoneflies exposed to an acute dose of (potential) salt and temperature stress. The top blot captures the total protein in the sample, and the lower blot in each panel reflects the HSP70. The lane on the far right reflects the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