

**Connecticut Institute of Water Resources
Annual Technical Report
FY 2015**

Introduction

The Connecticut Institute of Water Resources (CTIWR) is located at the University of Connecticut (UConn) and reports to the head of the Department of Natural Resources and the Environment, in the College of Agriculture, Health and Natural Resources. The current Director is Dr. Glenn Warner and Associate Director is Mr. James Hurd.

Although located at UConn, the Institute serves the water resource community throughout the state as it solicits proposals from all Connecticut universities and colleges. The Institute works with all of Connecticut's water resource professionals, managers and academics to identify and resolve state and regional water related problems and to provide a strong connection between water resource managers and the academic community.

The foundation for this connection is our Advisory Board, whose composition reflects the main water resource constituency groups in the state. Currently the Advisory Board is composed of 11 members. This past year, two members (Betsey Wingfield and Bill Hyatt) resigned their positions and were replaced by individuals from the same agencies (Robert Hust, Assistant Director of the Water Planning and Standards Division CT DEEP and Pete Aarrestad, Director Inland Fisheries Division, CT DEEP). CTIWR staff also participates on statewide water-related committees whenever possible, enabling the CTIWR to establish good working relationships with agencies, environmental groups, the water industry and academics.

The USGS 104B program is the financial core of the CTIWR. The Institute does not receive discretionary funding from the state or the university, although the CTIWR does receive funds to cover two thirds of the Associate Director's salary per year from the Dean of the College of Agriculture, Health and Natural Resources as match for our program administration and other activities.

Research Program Introduction

The majority of our 104B funds are given out as grants initiated in response to our annual RFP that is released in September of each year. The majority of these funds go to research projects. To solicit research proposals, the Institute sends an announcement to Connecticut institutions of higher learning requesting the submission of pre-proposals. These are reviewed by the CT IWR Director and Associate Director. When selecting potential projects for funding, the Institute considers three main areas: 1. technical merit, 2. state needs and 3. CT IWR priorities (use of students, new faculty, seed money for innovative ideas). Investigators submitting pre-proposals meeting the initial requirements are invited to submit a full proposal. Each full proposal received is reviewed by two to four outside individuals with expertise in the field described in the proposal. Proposals and reviewer comments are presented to the CT IWR Advisory Board, composed of 11 individuals that reflect the main water resource constituency groups in the state, and a determination is made on which projects are to be funded. This past reporting year we funded four one-year research projects, and funded the second year of one two-year research project.

Mitigating Eurasian watermilfoil invasion success and ecosystem impact using native herbivores

Basic Information

Title:	Mitigating Eurasian watermilfoil invasion success and ecosystem impact using native herbivores
Project Number:	2014CT286B
Start Date:	3/1/2014
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	CT-4
Research Category:	Biological Sciences
Focus Category:	Invasive Species, Ecology, Water Quality
Descriptors:	None
Principal Investigators:	LaTina Steele, Michele Guidone

Publications

1. Steele LaTina, Michelle Guidone. 2015. Using Native Herbivores to Mitigate Eurasian Watermilfoil Invasion in New England. Benthic Ecology Meeting 2015. Quebec City, Canada. Oral Presentation.
2. *Ray, Courtney; Jessica Michaud; LaTina Steele; Michelle Guidone. 2015. The Use of Native Herbivores as a Mitigation Strategy for Eurasian Watermilfoil (*Myriophyllum spicatum*) in Connecticut Lakes. Connecticut Association of Wetland Scientists Annual Meeting. Southbury, CT. Poster Presentation.
3. Ray, Courtney; LaTina Steele; Michelle Guidone. [in preparation]. Use of Native Aquatic Herbivores as Biocontrol Agents for Eurasian Watermilfoil (*Myriophyllum spicatum*) in New England. *Oecologia*.
4. Steele LaTina, Michelle Guidone. 2015. Using Native Herbivores to Mitigate Eurasian Watermilfoil Invasion in New England. Benthic Ecology Meeting 2015. Quebec City, Canada. Oral Presentation.
5. *Ray, Courtney; Jessica Michaud; LaTina Steele; Michelle Guidone. 2015. The Use of Native Herbivores as a Mitigation Strategy for Eurasian Watermilfoil (*Myriophyllum spicatum*) in Connecticut Lakes. Connecticut Association of Wetland Scientists Annual Meeting. Southbury, CT. Poster Presentation.
6. Ray, Courtney; LaTina Steele; Michelle Guidone. [in preparation]. Use of Native Aquatic Herbivores as Biocontrol Agents for Eurasian Watermilfoil (*Myriophyllum spicatum*) in New England. *Oecologia*.

Proposal Title: *Mitigating Eurasian Watermilfoil Invasion Success and Ecosystem Impact Using Native Herbivores*

Final Report, FY 2014-2015

LaTina Steele, Sacred Heart University, 5151 Park Ave., Fairfield, CT 06825

Introduction & Research Objectives

Eurasian watermilfoil has invaded lakes across the state of Connecticut, often becoming dominant within these submerged aquatic vegetation communities. Factors contributing to milfoil's invasion success are poorly understood and are limited mainly to nutrient conditions and the broad assertion that invasion is less likely in lakes with an established submerged macrophyte community (Smith and Barko 1990, Madsen 1998). However, evidence suggests that allelopathic interactions between milfoil and epiphytic algae may contribute to milfoil's establishment (Gross et al. 1996). These allelopathic phenolic compounds produced by *M. spicatum* are also well-known feeding deterrents in terrestrial, aquatic, and marine plants (Constabel 1999). Thus, it seems reasonable that chemical interactions may reduce milfoil herbivory and play a role in its invasion success. Increased understanding of factors leading to successful milfoil invasions is critical for effective management and prevention of milfoil invasion, highlighting the importance of studies like the one proposed here. Managers, policy-makers, and those who use our state's lakes for recreational purposes will all benefit from this study.

Common techniques for eradicating nuisance milfoil involve costly and harmful chemical application and physical removal of milfoil. Such measures often need to be repeated in order to be effective and inherently affect other members of the lake community (e.g., Delong and Mundahl 1996). Furthermore, physical removal of milfoil could increase its spread to other areas, since it is propagated via fragmentation (Maezo et al. 2010). Mitigation of *M. spicatum* using native herbivores is a much more palatable alternative to many common eradication measures.

Many studies have investigated the potential of a North American weevil to mitigate Eurasian watermilfoil impacts (e.g., Sheldon and Creed 1995). However, few have considered additional herbivores native to particular regions or the impacts of community composition (i.e., the identity and abundance of herbivores, predators, and algal species) that can also influence invasion success. For example, herbivorous snails may either directly or indirectly affect milfoil populations, as some gastropods feed on *M. spicatum* (Boland et al. 2008), while others positively impact milfoil growth by limiting the growth of algal competitors (Chase and Knight 2006). Predator identity and abundance is also vital to our understanding of milfoil success. In lakes where predators are abundant, herbivore populations may be suppressed to levels that inhibit their control of milfoil growth (Ward and Newman 2006). This last point is particularly important for making informed mitigation choices, as there are a number of predatory fish species that are commonly stocked for recreational fishing.

Most studies proposing herbivory as a milfoil control measure have been conducted in the Midwest or the southeast United States. Few have been conducted in New England, and none of those have considered the use of multiple native herbivores to mitigate milfoil impacts. Nor have those studies considered the role of chemical deterrents in determining when and where milfoil will invade, despite evidence that *M. spicatum* produces many allelopathic chemicals

(Gross et al. 1996, Spencer and Ksander 1999), which commonly contribute to plant invasion success (Callaway and Ridenour 2004). Connecticut lakes are home to many potential herbivores, including crustaceans (amphipods and crayfish), insect larvae, gastropods, and herbivorous fishes. Previous studies in other regions suggest that crayfish (Parker and Hay 2005, Maezo et al. 2010) and insect larvae (Johnson et al. 1998) are milfoil consumers, with some insects leading to shifts in community structure from milfoil-dominated systems to dominance by *Elodea canadensis* (Gross et al. 2001).

The objectives of the project were to 1) investigate the role of chemical interactions between plants and herbivores in determining milfoil invasion success, 2) identify native consumers with the potential to successfully mitigate milfoil invasions, and 3) measure the effects of milfoil invasion on community structure by comparing community composition and diversity between Eurasian watermilfoil and native aquatic plants.

Methods & Progress

Field Sampling

During summer 2014, five throw trap samples were collected in milfoil-dominated areas of Osbourndale Pond in Derby, Connecticut and another five throw trap samples were collected from *Elodea*-dominated areas of the same pond. All animals within each trap sample were identified to the lowest possible taxon and enumerated. Primer-E software was used to conduct Analysis of Similarity (ANOSIM) on a Bray-Curtis similarity matrix constructed using abundance of all taxa per m² to determine if community composition differed in milfoil and *Elodea* dominated areas. Results were considered significant at $p < 0.05$.

Upon identifying an appropriate reference pond without Eurasian watermilfoil (Colony Pond, Ansonia, CT), an additional five throw trap samples were collected from each of three areas: 1) milfoil-dominated areas of Osbourndale Pond, 2) *Elodea*-dominated areas of Osbourndale Pond, and 3) Colony Pond, where milfoil is not present. All plants within these traps were identified and the wet weight was recorded. Animals from these trap samples were preserved in 10% formalin for two weeks, then rinsed and stored in 70% isopropyl alcohol. Processing of these preserved samples is currently underway. Rose Bengal stain is being added to each sample prior to identifying and counting all animals within the sample.

Samples of Eurasian watermilfoil and three native aquatic plant species (*Elodea canadensis*, *Ceratophyllum demersum*, and *Potamogeton berchtoldii*) were collected during the summer in order to compare the chemical deterrent content of invasive milfoil and the native plant species. These samples were rinsed, placed in sample vials, flash frozen in liquid nitrogen, and stored at -80°C prior to freeze drying. Samples were ground to a fine powder in liquid nitrogen in their tubes and returned to the -80°C freezer until chemical analysis was performed.

Eurasian watermilfoil was sampled in the early morning hours (before sunrise) and in the late afternoon (just before sunset) to assess diurnal differences in chemical deterrent production; chemical analyses on these samples have not yet been conducted. The vacuum pump on the freeze dryer at Sacred Heart University is currently being replaced. When the pump has been replaced, samples will be dried, and chemical analyses will be performed.

Field Experiments

Diurnal differences in milfoil and *Elodea* consumption in the field were examined using tethering experiments. Five tether lines consisting of two feet of sisal rope with six pre-weighed milfoil fragments each and another five tether lines with six pre-weighed *Elodea* fragments each were deployed at approximately 08:00. Tethers were collected after 36 hours. The first 24 hours allowed time for animals to colonize the tethered fragments, and the following 12 hours allowed time for herbivores to feed on the plants during the day. These tethering methods were then repeated, deploying the tethers at approximately 19:00, with 24 hours to allow animals to colonize the tethered plants and another 12 hours to allow for additional feeding during the night. All plants were weighed a second time after tethers were collected from the field, and the change in weight (taking into account both consumption and growth) was calculated. Because the change in weight data were not normally distributed and did not meet the assumption of equal variance, a two-way ANOVA could not be used to determine if there were differences in consumption between the two plant species during the day and at night. Instead, two Mann-Whitney U tests were performed to determine if there were differences in consumption during the day and at night, with one test run on the milfoil data and a second test run on the *Elodea* data. Results were considered significant at $p < 0.025$ to account for multiple tests.

Laboratory Experiments

A series of four separate laboratory experiments were conducted to quantify milfoil consumption by the following native herbivores: amphipods (*Hyaella azteca*), snails (*Physella* sp.), mayfly larvae (*Caenis* sp.), and milfoil weevils (*Euhrychiopsis lecontei*). Choice feeding experiments were used to test the palatability of invasive milfoil and native *E. canadensis* to *H. azteca*, *E. lecontei* and *Physella* sp. T-tests and Mann-Whitney U tests were used, as appropriate, to determine if there were differences between control treatments (milfoil only) and herbivore treatments (milfoil + one herbivore species). Paired t-tests were used to determine if amphipods, weevils, and snails consumed different quantities of milfoil and *Elodea* in choice feeding experiments, since the data from all choice experiments were normally distributed and had homogenous variances. Results were considered significant at $p < 0.05$ in all cases.

Chemical Analyses

A simple colorimetric assay, the Folin-Denis assay (cf. Steele et al. 2005), was used to quantify total reactive phenolics in freeze-dried and ground samples of milfoil, *E. canadensis*, and two additional native plant species, *Potamogeton berchtoldii* and *Ceratophyllum demersum*. A one-way ANOVA was used to determine if there were differences in phenolic concentrations among plant species, since the phenolic data were normally distributed and variances among groups were equal. A post-hoc Tukey test was used to identify which plants had significantly different phenolic concentrations from each other. Results were considered significant at $p < 0.05$.

Results and Discussion

Results from the throw trap sampling suggest that in Osbourndale Pond, invasive milfoil has not had a detrimental impact on the lake community (Figure 1). A similar suite of animals seems to take up shelter in both milfoil and the native *Elodea*, which are the two dominant aquatic plants in Osbourndale Pond. In areas such as this where milfoil has not caused a noticeable effect on the consumer community, costly and ecologically harmful removal methods for the invasive plant may not be necessary. Likewise, the particular herbivore community found at this site may be acting to prevent milfoil from overgrowing the area. Additional experiments will be performed during summer 2015 (Year 2 of the project) to help address this question. However, the information gained at this one site may still be useful in determining lake characteristics that, when absent, may lead to greater effects of milfoil invasion (e.g., lack of plant competitors, lack of amphipods and snails).

The field tethering experiment suggests that herbivores may be more active during the night than during the day, though the difference in biomass reduction at night compared to during the day was only significant in *Elodea* and not milfoil (Figure 2). Plant samples were collected to determine if chemical deterrent production in milfoil and *Elodea* changes during the night and during the day to match times of greatest herbivore activity. These samples have not yet been analyzed.

Data obtained from the feeding experiments suggest that locally abundant native herbivores like the amphipod *Hyaella azteca* and the snail *Physella* sp. may be effective in controlling milfoil biomass and in mitigating its effects (Figure 3). Data from feeding preference tests are consistent with those results, since they showed that amphipods will still consume milfoil, even in the presence of other, less chemically defended plant species (Figures 4 & 5). Likewise, snails also consumed milfoil in the presence of alternative plant prey, showing no preference for either milfoil or native *Elodea canadensis* (paired t-test, $t = 0.29$, $p = 0.78$, $n=10$).

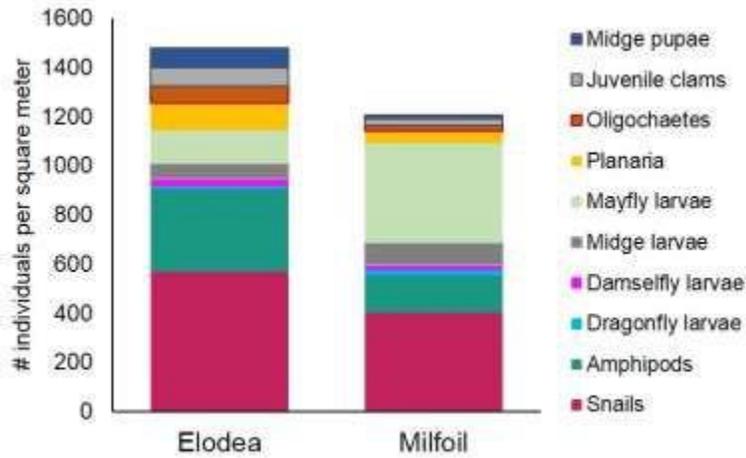


Figure 1. Most abundant taxa in throw trap samples (# individuals/m²) collected in *Elodea*- and milfoil-dominated areas of Osbourndale Pond (Derby, CT). Analysis of similarity (ANOSIM) detected no significant differences in community composition between habitats (Global R = 0.036, p = 0.333).

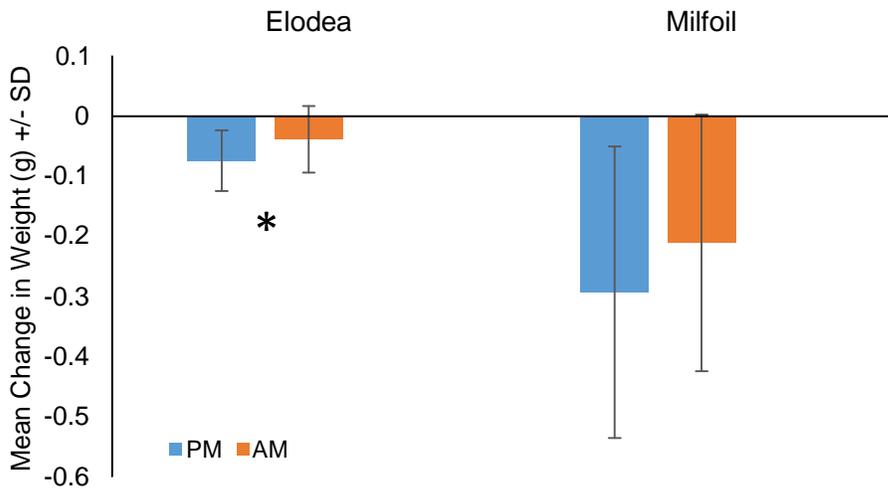


Figure 2. Mean change in weight of *Elodea canadensis* and Eurasian watermilfoil (*Myriophyllum spicatum*) after being deployed in the field in the morning (AM) or evening (PM) and remaining in the field for 36 hours. Mann-Whitney U tests indicated that significantly more *E. canadensis* was lost during the night than during the day (U = 159, p = 0.001), while there was not a significant difference in milfoil loss during the night and day (U = 315, p = 0.146). Asterisk indicates a significant difference in weight change between AM and PM.

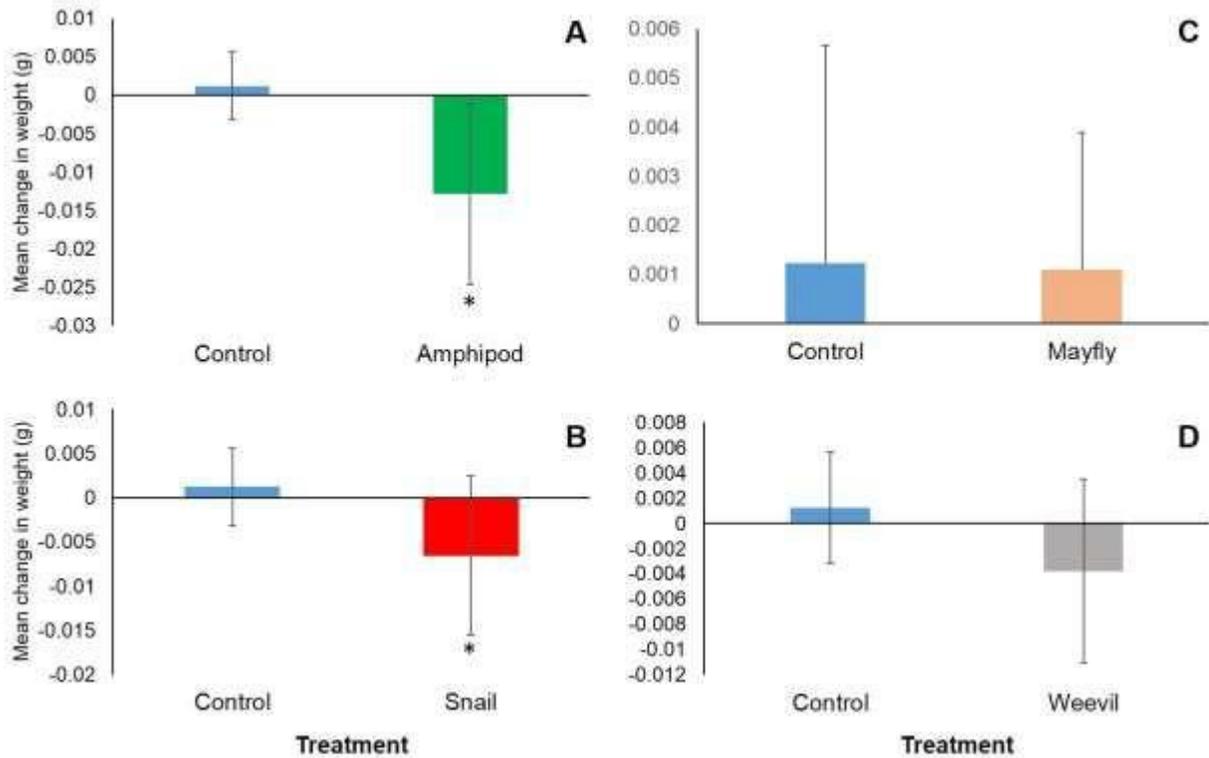


Figure 3. Change in *Myriophyllum spicatum* weight (g) after one week alone (controls) and following feeding by A) 10 individuals of the amphipod *Hyalella azteca* (Mann-Whitney test $W = 61.0$, $df = 13$, $p = 0.0010$, $n = 10$), B) 4 Physidae snail individuals (t-test $t = 2.45$, $df = 13$, $p = 0.029$, $n = 10$), C) 4 mayfly larvae (t-test $t = 0.08$, $df = 15$, $p = 0.938$, $n = 10$), and D) one milfoil weevil individual (t-test $t = 1.72$, $df = 11$, $p = 0.114$, $n = 8$). Each panel represents one experiment. Asterisk next to the error bar indicates a significant difference from the control.

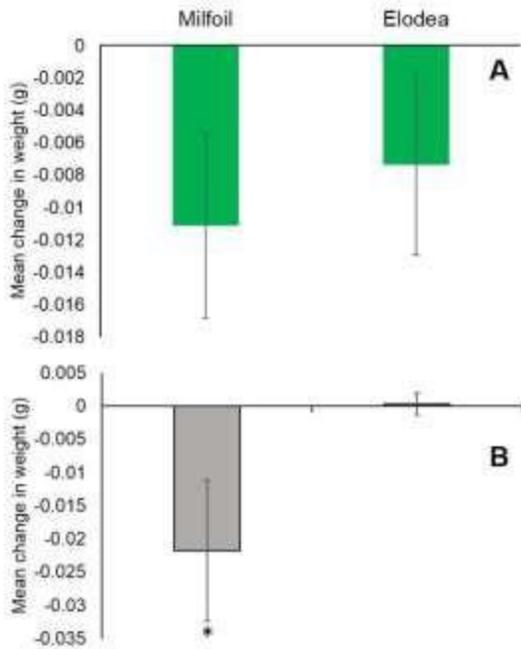


Figure 4. Mean change in weight of *Myriophyllum spicatum* and *Elodea canadensis* following one week of feeding by A) the amphipod *Hyalella azteca* (paired t-test $t = -1.93$, $p = 0.086$, $n = 10$) and B) the weevil *Euhyrchiopsis lecontei* (paired t-test $t = -6.485$, $p < 0.001$, $n = 10$) in choice experiments. Asterisk next to error bar indicates a significant difference between treatments.

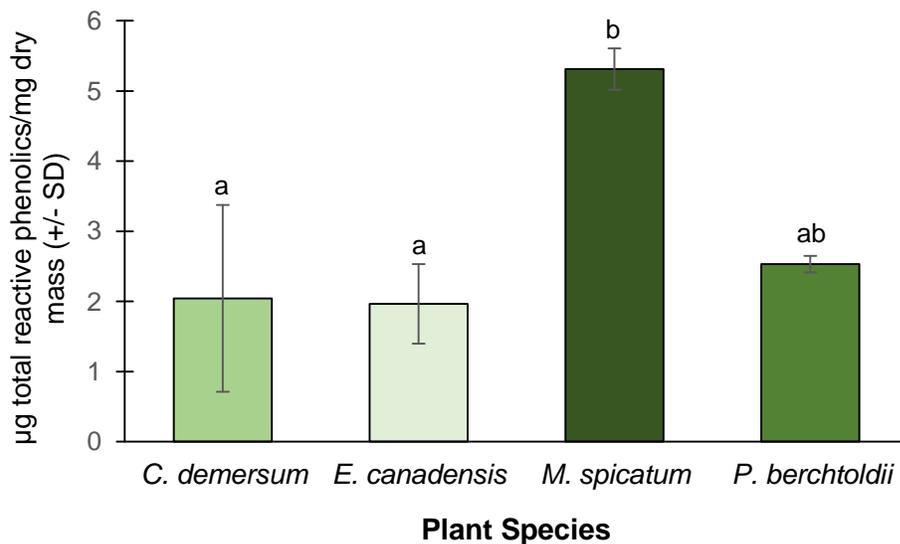


Figure 5. Total reactive phenolic content (μg phenolics/mg dry mass \pm standard deviation) of four aquatic plant species: *Ceratophyllum demersum*, *Elodea canadensis*, *Myriophyllum spicatum*, and *Potamogeton berchtoldii*. Different letters over the error bars indicate significant differences among species (ANOVA $F_{1,17} = 4.953$, $p = 0.012$).

Literature Cited

- Boland BB, Meerhoff M, Fosalba C, Mazzeo N, Barnes MA, and Burks RL. 2008. Juvenile snails, adult appetites: contrasting resource consumption between two species of applesnails (*Pomacea*). *J Molluscan Stud* 74: 47-54
- Callaway RM and Ridenour WM. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Front Ecol Environ* 2: 436-443
- Chase JM and Knight TM. 2006. Effects of eutrophication and snails on Eurasian watermilfoil (*Myriophyllum spicatum*) invasion. *Biol Invasions* 8: 1643-1649
- Constabel CP. 1999. A survey of herbivore-inducible defensive proteins and phytochemicals. In: AA Agrawal, S Tuzun, and E Bent (eds). *Induced Plant Defenses Against Pathogens and Herbivores*. APS Press, St. Paul, MN, pp 137-166
- Delong MD and Mundahl ND. 1996. Secondary effects of fluoridone treatment on invertebrate community structure in lake ecosystems. Winona State University Biology Dept., Report to the MN DNR-St. Paul, Winona, MN
- Gross EM, Johnson RL, and Hairston NG. 2001. Experimental evidence for changes in submersed macrophyte species composition cause by the herbivore *Acentria ephemerella* (Lepidoptera). *Oecologia* 127: 105-114
- Gross EM, Meyer H, and Schilling G. 1996. Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. *Phytochemistry* 41: 133-138
- Johnson R L, Gross EM and Hairston NG. 1998. Decline of the invasive submersed macrophyte *Myriophyllum spicatum* (Haloragaceae) associated with herbivory by larvae of *Acentria ephemerella* (Lepidoptera). *Aquat Ecol* 31: 273-282
- Madsen JD. 1998. Predicting Invasion Success of Eurasian Watermilfoil. *J Aquat Plant Manage* 36: 28-32.
- Maezo MJ, Fournier H, and Beisner BE. 2010. Potential and realized interactions between two aquatic invasive species: Eurasian watermilfoil (*Myriophyllum spicatum*) and rusty crayfish (*Orconectes rusticus*). *Can J Fish Aquat Sci* 67: 684-700
- Parker JD and Hay ME. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecol Lett* 8: 959-967
- Sheldon SP and Creed RP Jr. 1995. Use of a native insect as a biological control for an introduced weed. *Ecol Appl* 5: 1122-1132
- Smith CS and Barko JW. 1990. Ecology of Eurasian Watermilfoil. *J Aquat Plant Manage* 28: 55-64
- Spencer DF and Ksander GG. 1999. Seasonal changes in chemical composition of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and water temperature at two sites in northern California: implications for herbivory. *J Aquat Plant Manage* 37: 61-66
- Steele L, Caldwell M, Boettcher A, and Arnold T. 2005. Seagrass-pathogen interactions: 'pseudo-induction' of turtlegrass phenolics near wasting disease lesions. *Mar Ecol Prog Ser* 303: 123-131
- Ward DM and Newman RM. 2006. Fish predation on Eurasian watermilfoil (*Myriophyllum spicatum*) herbivores and indirect effects on macrophytes. *Can J Fish Aquat Sci* 63: 1049-1057

Effects of Road Salts on Ephemeral Wetland Ecosystems

Basic Information

Title:	Effects of Road Salts on Ephemeral Wetland Ecosystems
Project Number:	2015CT290B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	CT-002
Research Category:	Biological Sciences
Focus Category:	Wetlands, Ecology, Nutrients
Descriptors:	None
Principal Investigators:	Ashley M Helton, Tracy Rittenhouse

Publications

1. Macklem, D. 2016. Temperature variability and multiple environmental stressors: how will tadpole performance change with our climate? University Scholars Program and Honors Thesis. University of Connecticut, Storrs, CT.
2. Macklem, DC, JT Kolek, AM Helton, TAG Rittenhouse. (in preparation) Temperature interactions with road salt application alter fitness of wood frog (*Lithobates sylvaticus*) and spring peeper (*Pseudacris crucifer*) tadpoles.
3. Nelson, E, M Schoell, T Rittenhouse, AM Helton. 2015. Poster: Effects of road salts on ephemeral wetland ecosystems: Water chemistry results from mesocosm experiments. Connecticut Conference on Natural Resources. Storrs, CT.
4. Zawatski, M, M Schoell, A Doroski, AM Helton. 2016. The effects of road salts on denitrification in ephemeral wetlands. Connecticut Conference on Natural Resources. Storrs, CT.
5. Schoell, M, E Nelson, T Rittenhouse, AM Helton. 2015. Poster: Effects of road salts on ephemeral wetland ecosystems: Water chemistry results from mesocosm experiments. Society of Wetland Scientists Annual Meeting. Providence, RI.
6. Kolek, J. D. Macklem, AM Helton, T Rittenhouse. 2015. Poster: Effects of road salts and climate change on ephemeral wetland ecosystems: Amphibian response within mesocosms. Connecticut Conference on Natural Resources. Storrs, CT.

Proposal Title: *Effects of road salts on ephemeral wetland ecosystems*

Principal Investigators:

(PI) *Ashley M. Helton*, Assistant Professor, Natural Resource & Environment, University of Connecticut, Storrs, CT 06269-4087. Telephone: (860) 486-1259, Email: Ashley.helton@uconn.edu

(co-PI) *Tracy A.G. Rittenhouse*, Assistant Professor, Natural Resource & Environment, University of Connecticut, Storrs, CT 06269-4087. Phone: (860) 486-5042, Email: Tracy.rittenhouse@uconn.edu

Introduction/Research Objective:

Ephemeral wetlands, including seasonal, vernal, and other temporary ponds, are widespread in the Northeast United States (Tiner 2003). They are not connected to permanent surface water bodies, such as lakes, rivers, or streams. As a result they hold water only temporarily, periodically drying out. Although ephemeral wetlands are typically smaller in size than their permanent counterparts, in the Northeast United States they can make up a large portion of the aquatic landscape (e.g., Cormier et al. 2013), and are protected in many states, including Connecticut (Connecticut Inland Wetlands and Watercourses Act, sections 22a-36 through 22a-45 of the General Statutes of Connecticut).

Ephemeral wetlands provide critical fishless habitat for amphibian and macroinvertebrate reproduction (Semlitsch and Bodie 1998), and play particularly important roles in the processing of carbon and retention of nitrogen on the forest floor (Capps et al. 2014). Ephemeral wetlands are often filled during land development, used as storm water detention basins, and receive drainage from agricultural fields or residential areas (Tiner 2003). Human activities can severely alter population and community dynamics in isolated wetlands, by fragmentation of the surrounding terrestrial landscape and by direct surface water contamination (Gibbons 2003; Willson and Hopkins 2013).

Surrounding land use dramatically changes both the chemical and hydrologic regimes of ephemeral wetlands. Deforestation associated with development decreases leaf litter input to wetlands in developed catchments (France et al. 1996), decreasing basal carbon resources to temporary wetland ecosystems. Development increases overland runoff to aquatic systems since impervious surfaces reduce surface water infiltration to groundwater (Walsh et al. 2005). Increased runoff volume and direct routing of storm water increases the amount of time ephemeral wetlands experience standing surface water. Aquatic systems receiving runoff from developed land also typically have higher concentrations of nutrients, salt, and other pollutants (Walsh et al. 2005). Indeed, water quality is commonly degraded in temporary wetlands near human development (e.g., Atkinson et al. 2011).

In the Northeast United States, where chemical deicer is used on roadways in winter months, salinization of surface waters and groundwater (Kaushal et al. 2005; Cassanelli and Robbins 2013) is a pervasive pollutant in temporary wetlands. Road salt use has increased from 7.5 to over 22 million tons per year across the U.S. from 1975 to 2005, with the most dramatic increases across the glaciated northern U.S. (Findlay and Kelly 2011). The mean maximum concentration of chloride regularly exceeds the USEPA recommended chronic criterion for aquatic life in surface waters of urban areas of the glaciated northern United States, and chloride concentrations in surface waters are strongly correlated metrics indicative of paved areas, including percent development, road density, and impervious cover (Findlay and Kelly 2011).

Road salts have a wide range of direct impact on temporary wetland ecosystems. Elevated salinities in roadside wetlands 1) may influence microbial processing of C and N (Groffman et al. 1995), and resulting trace gas emissions, 2) are correlated with shifts in invertebrate communities to more salt-

tolerant species (Petranka and Doyle 2010), and 3) can disrupt osmoregulation of eggs (Karraker 2011) and reduce larval survival of wood frogs and spotted salamanders (Karraker et al 2008).

For this proposal, our objectives were:

1. Quantify the microbial response to salinity by measuring potential denitrification rates and in situ greenhouse gas concentrations across wetlands with a range of road salt exposure.
2. Determine the abundance and shift in species of mosquitos across wetlands with a range of road salt exposure.
3. Quantify amphibian response to road salts across a range of road salt exposure and with controlled mesocosm experiments.

Methods/Procedures/Progress:

Surveys of wetlands: We selected 16 wetlands within the towns surrounding the University of Connecticut Storrs Campus. Wetlands were sampled seven times from 10 April 2015 to 3 August 2015. On the last day of sampling all but one ephemeral wetland was completely dry. During each visit, we measured specific conductance and water temperature. Surface water samples were immediately filtered and analyzed for nitrate, ammonium, soluble reactive phosphorus, total carbon, total nitrogen, and chloride according to standard methods (APHA 1998) at the Center for Environmental Sciences and Engineering (CESE). We also filtered samples to measure chlorophyll A as a surrogate for algal biomass using standard methods (APHA 1998) with a Turner Fluorometer at CESE. We collected additional surface water samples in evacuated vials and used headspace equilibration techniques to extract dissolved gas samples (Hudson 2004; Helton et al. 2014). Gas samples were analyzed on a Perkin Elmer Clarus 580 gas chromatograph customized to measure CO₂, CH₄, and N₂O located at CESE. Soil cores were collected in six of the wetlands on 23 June 2015. Soil cores were divided into 0-5 cm and 5-10 cm depth increments, sieved (2mm, #10 mesh), and homogenized. We analyzed soil cores for potential rates of denitrification using denitrifying enzyme activity (DEA; Groffman et al. 1999), as well as soil moisture and organic matter content using the loss on ignition method.

Mosquitos were counted on four dates from 22 April 2015 to 30 June 2015 using the partial submersion technique (O'Malley 1989) to sample invertebrate larvae in the water column, where a dipper is used to sample equal volumes near the surface of the water column. All samples were concentrated through a small mesh screen using a concentrator cup and preserved with 90% ethanol. Mosquitos were identified by the Connecticut Agricultural Experiment Station for samples collected on 22 April 2015. Mosquitos collected on the three additional sampling dates are preserved and stored in A.M. Helton's lab at CESE. Wood frog and spotted salamander egg masses were also counted using the double observer method (Scherer 2008) and repeat sampling for five sampling dates from 10 April 2015 to 30 June 2015.

Mesocosm experiments: In a fully randomized and replicated experiment, we randomly assigned one of 12 treatments to each 1000-liter mesocosm (N = 48 mesocosms) such that tanks had all possible combinations of species (LISY, PSCR, and BOTH), temperature (ambient or elevated), and salt (ambient or elevated). Tanks were also organized into four spatial blocks to account for potential environmental gradients at our study site. *Lithobates (Rana) sylvaticus* (LISY; LeConte, 1825; Frost et al., 2006) is a terrestrial frog with a wide geographic range and a complex life cycle (Berven, 1990). *Pseudacris crucifer* (PSCR; Weid-Neuweid, 1838) is a semi-arboreal frog with a wide geographic range covering all of the United States east of the Mississippi and as far north as Hudson Bay (Lovett, 2013).

Each mesocosm represented a natural wetland. Thus, we filled mesocosms with ground water, which we allowed to age for two days. To each mesocosm, we added 1 kg of leaf litter collected from a mixed hardwood forest located within the University of Connecticut's Fenton Tract (Wharton et al., 2009; Parent and Volin, 2014). We covered mesocosms with 50% shade cloth lids to represent canopy

cover and to prevent other amphibian species and dragonflies from ovipositing in the water. We then inoculated each mesocosm with a concentrated mixture of phyto- and zooplankton collected from multiple natural ponds on 17 April. Zooplankton do not serve as a food source for tadpoles, but are important components of the complex communities found in natural ponds (Schell et al., 2001). We created the elevated salt treatment by adding road salt obtained from the Department of Transportation storage facility in Mansfield, CT. We dissolved road salt into 15 L of water and then stirred salt water into the mesocosms until we reached a concentration of 1600 mg/L on 21 April. We created the elevated by 3 °C temperature treatment using JBJ True Temp Heating Systems (Model T3-1000) which consists of a 1000 watt titanium heating rod and digital controller. We floated the heated rod 10 cm below the surface of the water, such that a natural temperature gradient occurred with the warmest water at the surface and coolest water at the bottom. The digital controller provided a set point temperature, but could not be programmed to follow daily temperature fluctuations, and the thermometer linked to the controller was placed at the bottom of the tank. We adjusted temperature set points in the morning, by programming the set-point temperature to be 3 °C warmer than the current morning temperature at the bottom of control tanks. Set-point temperatures were not changed daily, but rather every three to five days. Adjustments, both increasing and decreasing the set-point, were needed following weather fronts causing large shifts in daily high or low temperatures and when rain fell which added water of a different temperature to the mesocosms.

We stocked mesocosm tanks on 27 April with 30 tadpoles of LISY, PSCR, or BOTH species; mesocosms assigned to BOTH received 30 tadpoles of each species for a total of 60 tadpoles. This density is well within the range of larval anuran densities in natural wetlands (Morin, 1983). We removed animals when at least one front leg erupted (stage 42; Gosner 1960), and these individuals were identified as surviving the larval lifestage. We placed metamorphosed individuals into plastic sandwich containers with a small amount of water from the mesocosm to protect against desiccation during tail absorption and positioned the containers to allow for movement of metamorphs in and out of water. We labeled containers with the corresponding mesocosm tank number and held the animals in the adjacent animal care facility through tail resorption. We checked the containers daily to assess progress through metamorphosis. We recorded the date of metamorphosis and mass at the completion of resorption. We calculated all "days to metamorphosis" using the date of complete tail resorption, rather than capture date, because some metamorphs were captured at different stages in the tail resorption process.

Water temperature, specific conductivity, and dissolved oxygen (DO) were measured biweekly in all mesocosms using a handheld sonde (Yellow Springs Instruments, YSI 556 MPS). Water samples were collected biweekly and filtered immediately through 0.7µm GF/F Whatman filters in syringe filter holders. Filtered samples were collected in acid washed and field rinsed bottles, transported to the lab on ice, and frozen until analysis. Filtered samples were analyzed according to standard methods (APHA, 1998) for soluble reactive phosphorus (SRP; ascorbic acid method), ammonium (NH₄⁺; phenate method), and nitrate (NO₃⁻; cadmium reduction method) at the Center for Environmental Sciences and Engineering Nutrients Laboratory. Dissolved inorganic nitrogen (DIN) was calculated by summing NH₄⁺ and NO₃⁻ concentrations. Filters were preserved by freezing, and chlorophyll a concentrations were measured by acetone extraction on a Turner Designs fluorometer (Trilogy 7000-000).

Results/Significance:

Surveys of wetlands: The wetlands surveyed ranged in their average specific conductivities from 0.034 to 1.102 mS cm⁻¹ (Table 1). Wetlands also varied in their water chemistry and greenhouse gas concentrations, with several wetlands having particularly high nitrous oxide concentrations (Table 1). We are currently analyzing this dataset, and summary statistics are available in Table 1.

Table 1. Average and Standard Deviation of water chemistry, Chlorophyll A, and dissolved greenhouse gases collected from ephemeral wetlands during 2015.

Site Name	Specific Conductivity (ms / cm)		Water Temperature (°C)		Ammonium (mg N / L)		Ortho-phosphate (mg P / L)		Nitrate (mg N / L)		Dissolved organic carbon(mg / L)		Chlorophyll A (ug/L)		Dissolved methane (ug / L)		Dissolved carbon dioxide(ug / L)		Dissolved nitrous oxide (ug / L)	
	Avg.	Std. Dev.	Avg.	Std. Dev.	Avg.	Std. Dev.	Avg.	Std. Dev.	Avg.	Std. Dev.	Avg.	Std. Dev.	Avg.	Std. Dev.	Avg.	Std. Dev.	Avg.	Std. Dev.	Avg.	Std. Dev.
Andover	0.498	0.433	18.9	1.7	0.225	0.406	0.017	0.015	0.011	0.011	7.47	2.30	13.34	15.02	274.9	403.2	12057	4694	76	47
BackRd	0.047	0.013	16.1	5.3	0.009	0.017	0.006	0.006	0.000	0.017	8.10	5.10	1.71	1.41	211.2	362.2	26215	21639	66	26
BrownRd	0.382	0.129	12.4	4.2	0.013	0.009	0.005	0.005	0.658	0.890	5.29	4.41	10.51	5.92	35.9	38.4	35697	38089	508	108
Chaffee	0.044	0.012	14.1	5.6	0.075	0.086	0.027	0.027	0.000	0.004	9.49	8.12	2.79	1.63	75.0	81.6	17929	12079	72	29
Erdoni	0.037	0.009	16.8	2.5	0.037	0.044	0.004	0.004	0.034	0.073	5.76	2.19	2.52	2.88	128.1	109.5	18201	23943	47	28
GehringRd	1.102	0.321	17.3	2.9	0.020	0.019	0.013	0.005	0.000	0.022	12.95	3.80	9.08	5.87	393.7	366.0	23514	16561	66	27
GrantHill	0.618	0.351	15.5	3.2	0.109	0.098	0.013	0.013	0.194	0.279	5.67	3.01	2.21	1.25	113.3	109.2	20934	15360	368	223
Heidi1	0.432	0.095	14.3	5.9	0.040	0.048	0.015	0.011	0.016	0.064	9.01	2.89	2.90	1.97	242.5	176.4	23341	14611	64	31
Heidi2	0.491	0.066	15.4	6.1	0.061	0.047	0.011	0.008	0.088	0.167	4.57	1.16	16.05	28.43	52.4	31.2	16578	11657	59	24
MS1	0.222	0.269	11.7	5.1	0.082	0.083	0.014	0.015	0.000	0.026	20.15	12.99	3.03	1.86	273.1	126.2	32211	28097	64	30
MS2	0.034	0.005	12.4	4.6	0.068	0.142	0.020	0.023	0.000	0.013	10.45	7.56	2.73	3.46	319.7	611.3	38624	35211	67	27
NH	0.116	0.025	14.4	2.5	0.008	0.008	0.004	0.003	0.000	0.013	7.77	3.87	4.10	4.54	323.3	287.4	30081	25178	60	16
UF1	0.165	0.024	10.0	5.4	0.010	0.007	0.007	0.007	0.045	0.048	2.91	0.38	0.55	0.34	1.7	0.5	8973	1580	75	8
UF2	0.034	0.012	13.6	9.4	0.039	0.034	0.040	0.057	0.000	0.028	23.89	15.41	2.79	1.55	39.4	6.9	14830	14267	57	22
UF3	0.087	0.023	11.2	6.4	0.005	0.007	0.004	0.006	0.013	0.024	2.87	1.99	1.00	0.19	1.7	2.2	15624	10793	157	127
UF4	0.076	0.026	13.5	3.3	0.026	0.033	0.008	0.005	0.000	0.010	2.93	1.42	2.00	1.26	172.1	197.2	18261	15805	67	25

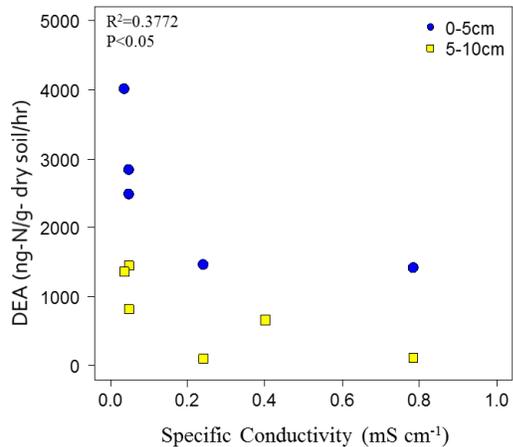


Figure 1. Potential denitrification rates versus specific conductivity for wetland soil cores.

Mesocosm experiments: Detailed results of the mesocosm experiments are available in D. Macklem (2016). Briefly, LISY and PSCR survival was significantly influenced by salinity (Figure 2). The number of LISY tadpoles surviving to metamorphosis was reduced by both elevated road salt ($p < 0.001$) and elevated temperature ($p < 0.039$). Overall, road salt additions decreased average LISY survival from 90.4% to 62.3% and average PSCR survival from 56% to 14%.

We also found elevated chlorophyll a in both LISY and PSCR salt treatments. Chlorophyll a was higher in LISY elevated salt treatments ($F_{1,26} = 18.07$, $p < 0.001$). This may be due to elevated soluble reactive phosphorus concentrations in both LISY and PSCR salt treatments, due to sand with elevated phosphorus commonly added to road salts.

Soil cores were collected from six of the 16 wetlands that covered the full range of measured average salinities. We found that potential denitrification was significantly positively related to organic matter content ($r^2 = 0.50$, $p < 0.05$) and soil moisture content ($r^2 = 0.79$, $p < 0.05$). We also found that potential denitrification rates were significantly negatively correlated with specific conductance (Figure 1), *suggesting that elevated salinity suppresses potential rates of denitrification in ephemeral wetlands.*

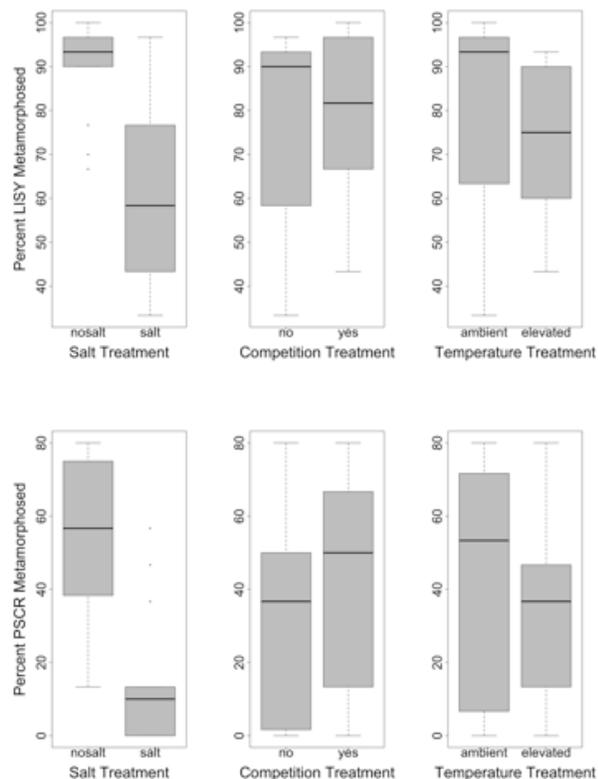


Figure 2. The percent of LISY and PSCR tadpoles surviving to metamorphosis under the various treatments.

References:

- APHA (1998) Standard methods for the examination of water and wastewater, Washington DC, American Public Health Association Publication, APHA, AWWA, WEF
- Atkinson CL, Golladay SW, and First MR (2011) Water quality and planktonic microbial assemblages of isolated wetlands in an agricultural landscape. *Wetlands* 35:885-894.

- Capps KA, Rancatti R, Tomczyk N, TB Parr, Calhoun AJ, Hunter M (2014) Biogeochemical hotspots in forested landscapes: The role of vernal pools in denitrification and organic matter processing. *Ecosystems*. doi 10.1007/s10021-014-9807-z
- Cassanelli JP and Robbins GA (2013) Effects of road salt on Connecticut's groundwater: A statewide centennial perspective. *Journal of Environmental Quality* 42(3): 737-748.
- Cormier TA, Congalton RG, and Babbitt KJ (2013) Spatio-statistical predictions of vernal pool locations in Massachusetts: Incorporating the spatial component in ecological modeling. *Photogrammetric Engineering & Remote Sensing* 79 (1): 25–35.
- Findlay SEG, Kelly VR (2011) Emerging indirect and long-term road salt effects on ecosystems. *Annals of the New York Academy of Sciences, The Year in Ecology and Conservation Biology* 1123: 58-68.
- France R, Culbert H, and Peters R (1996) Decreased carbon and nutrient input to boreal lakes from particulate organic matter following riparian clear-cutting. *Environmental Management*. 20 (4). 579-583.
- Frost, D.R., T. Grant, J. Faivovich, R.H. Bain, A. Haas, C.F.B. Haddad, R.O. De Sá, A. Channing, M. Wilkinson, S.C. Donnellan, C.J. Raxworthy, J.A. Campbell, B.L. Blotto, P. Moler, R.C. Drewes, R.A. Nussbaum, J.D. Lynch, D.M. Green, and W.C. Wheeler. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297:1-291.
- Gibbons JW (2003) Terrestrial habitat: A vital component for herpetofauna of isolated wetlands. *Wetlands* 23(3): 630-635.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183-190.
- Groffman PM, Gold AJ, Howard G (1995) Hydrologic tracer effects on soil microbial activities. *Soil Science Society of America Journal* 59(2): 478-481.
- Hudson F (2004) Standard Operating Procedure: sample preparation and calculations for dissolved gas analysis in water samples using a GC headspace equilibration technique. U. E. P. Agency. Washington, DC.
- Karraker NE and Gibbs JP (2011) Road deicing salt irreversibly disrupts osmoregulation of salamander egg clutches. *Environmental Pollution* 159:833-835.
- Karraker NE, Gibbs JP and Vonesh JR (2008) Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. *Ecological Applications* 18:724-734.
- Kaushal SS, Groffman PM, Likens GE, et al. (2005) Increased salinization of fresh water in the northeastern United States. *PNAS* 102(38) 12517-13520.
- LeConte, J. 1825. Remarks on the American species of the genera *Hyla* and *Rana*. *Annals of the Lyceum of Natural History of New York* 1:278-282.
- Morin, P.J. 1983. Predation, competition, and the composition of larval anuran guilds. *Ecological Monographs* 53:119-138.
- O'Malley C (1989) Guidelines for larval surveillance. *Proceedings of the Seventy-Sixth Annual Meeting of the New Jersey Mosquito Control Association, Inc.* pp 44-55.
- Parent, J., and J.C. Volin. 2014. Assessing the potential for leaf-off LiDAR data to model canopy closure in temperature deciduous forests. *ISPRS Journal of Photogrammetry and Remote Sensing* 95:134-145.
- Petranka JW and Doyle EJ (2010) Effects of road salts on the composition of seasonal pond communities: can the use of road salts enhance mosquito recruitment? *Aquatic Ecology* 44:155-166.
- Petranka JW and RA Francis (2013) Effects of Road Salts on Seasonal Wetlands: Poor Prey Performance May Compromise Growth of Predatory Salamanders. *Wetlands* 33:707-715.
- Scherer RD (2008) Detection of wood frog egg masses and implications for monitoring amphibian populations. *Copeia* 2008:669-672.

- Semlitsch RD and Bodie JR (1998) Are small, isolated wetlands expendable? *Conservation Biology* 12(5): 1129-1133.
- Tiner RW (2003) Geographically isolated wetlands of the United States. *Wetlands* 23(3): 494-516.
[http://dx.doi.org/10.1672/0277-5212\(2003\)023\[0494:GIWOTU\]2.0.CO;2](http://dx.doi.org/10.1672/0277-5212(2003)023[0494:GIWOTU]2.0.CO;2).
- Walsh CJ, Roy AH, Feminella JW, et al. (2005) The urban stream syndrome: current knowledge and the search for a cure. *Journal of the North American Benthological Society* 24(3): 706-723.
- Wharton, E.H., R.H. Widmann, C.L. Alerich, C.J. Barnett, A.J. Lister, T.W. Lister, D. Smith, and F. Borman. 2004. *The forest of Connecticut*. USDA Forest Service. Resource Bulletin NE-160.
- Willson JD and WA Hopkins (2013) Beyond the wetland: evaluating the effects of anthropogenic stressors on source-sink dynamics in pond-breeding amphibians. *Conservation Biology* 27:595-604.

Real-time in situ monitoring nutrient fate and hypoxia occurrence in natural water sources

Basic Information

Title:	Real-time in situ monitoring nutrient fate and hypoxia occurrence in natural water sources
Project Number:	2015CT291B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	CT-002
Research Category:	Water Quality
Focus Category:	Water Quality, Nutrients, Surface Water
Descriptors:	None
Principal Investigators:	Baikun Li, Yu Lei

Publications

1. Xu, Zhiheng, Qiuchen Dong, Brunah Otieno, Yucheng Liu, Isaiah Williams, Dingyi Cai, Yan Li, Yu Lei, Baikun Li. (in review). Milli-electrode array (MEA) for real-time in situ profiling of water quality. Environmental Science & Technology.
2. Xu, Zhiheng, Qiuchen Dong, Brunah Otieno, Yucheng Liu, Isaiah Williams, Dingyi Cai, Yan Li, Yu Lei, Baikun Li. (in preparation) Modeling water/sediment physio-chemical fates based on MEA profiles.
3. Xu, Zhiheng Milli-electrode array (MEA) for real-time in situ water quality monitoring. AEESP Annual Conference June 2015. Yale University (New Haven, CT).
4. Xu, Zhiheng. Fabrication of Milli-electrode array (MEA). New England Graduate Student Environmental Engineering Conference Sept. 2015. University of Massachusetts (Amherst, MA).

Proposal Title. Real-time *in situ* monitoring nutrient fate and hypoxia occurrence in natural water sources

Introduction/Research Objective

Water connects physical, geochemical, biological, and ecological processes occurring on the Earth's surface and in the sediment, and comprehensively links the natural environment with human activities at multiple scales of space and time ranging from small pond to open ocean. Steady modification of coastal watersheds through deforestation and urban development, dense population, and ignorant discharge/dumping of wastes and non-point pollutants have caused severe eutrophication and hypoxia in the large estuaries of in the State of Connecticut and Long Island Sound (LIS). Achieving “High Water Quality” and managing “Fate and Transport of Contaminants in Air, Water and Soils” is a prior initiative for Connecticut, and requires the innovative real-time *in situ* sensing technology. Moreover, understanding the biogeochemical cycle in natural water resources is critical for us to protect environment and manage water availability and biodiversity. Before any treatment process is designed, policy and regulation is developed, and protection and restoration strategy is executed, a comprehensive picture of ecosystems at physiochemical aspects should be acquired.

Currently, inorganic/organic contaminants (e.g. nitrogen, phosphate, organic carbon, and heavy metals) in water and sediment are measured using chromatography, atomic absorption (AA), induced coupled plasma (ICP), and fluorimetry. These techniques normally require samples to be collected and sent to the off-site laboratory for analysis. Problems were often encountered, such as pressure/temperature/oxygen changes for sediment samples and hydrothermal samples. These may result in systematic errors, and ultimately lead to misinterpretation of the results observed. With the development of microelectrodes (MEs), microprofiling becomes possible. MEs are micro-scale needle-shaped electrodes capable of penetrating into environmental samples (e.g. biofilms, activated sludge, and sediment) at different depths with the control of micromanipulators. The PI Li developed a ME setup to measure activated sludge flocs in wastewater treatment plants (Figure 1a). MEs technology can obtain the chemical micro-profiles without destroying samples. However, the needle-shaped MEs have three major problems hindering the real time measurement. **First**, most MEs can only measure a single parameter (e.g. oxygen, nitrogen, and pH) and only obtain one data point during each penetration into a sample, which makes it impossible for real-time measuring water-sediment environment. **Second**, these MEs require tedious fabrication procedures with low success rate and reproducibility. MEs are fragile and can not be reusable for multiple measurements. **Third**, the whole ME setup is complicated. The movement of MEs is controlled by a micromanipulator (Figure 1a and 1b), which makes it impossible to deploy for *in situ* measurement at harsh sites, such as deep-sea sediment.

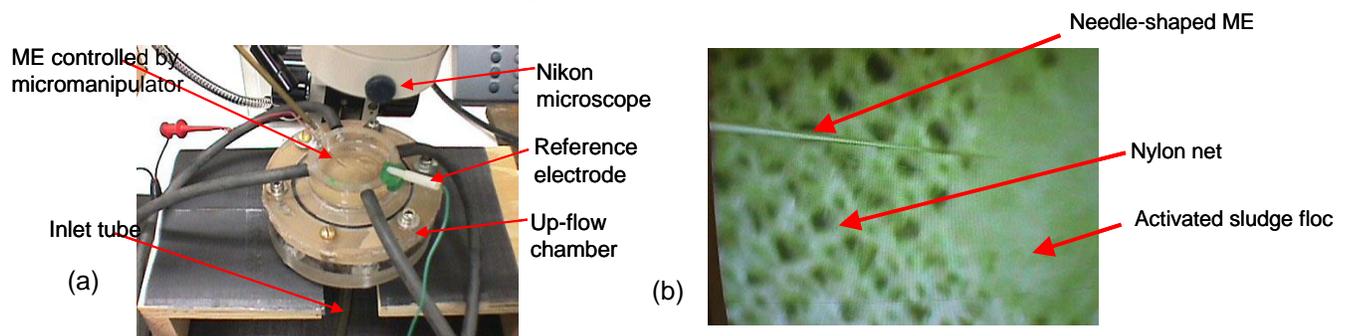


Figure 1. The experimental setup of the needle-shape microelectrode (ME) measurement of activated sludge flocs. (a) The whole ME system setup. (b) Penetration of a ME into activated sludge flocs .

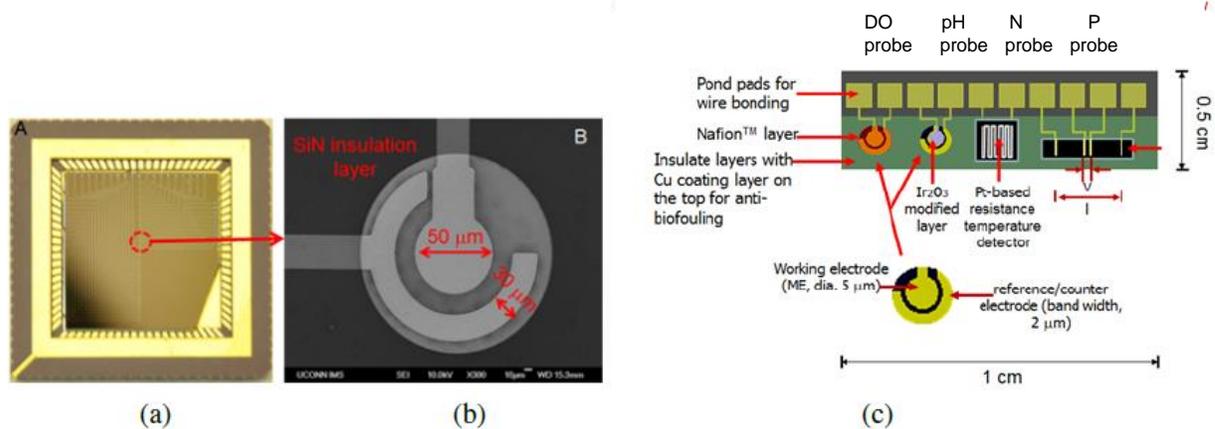


Figure 2. (a) Optical image of a microfabricated MEC device. (b) The SEM picture of one pair of microelectrodes (ME). (c) Schematic of the array of four types of MEs (not in scale).

To solve the ME problems for real-time *in situ* long-term monitoring, the research team led by the PIs has already fabricated a new generation micro-measurement device, all-in-one microelectrode chip (MEC) in a *NSF supported project (CBET 1336425)*. By precisely patterning multiple (e.g. 20 or 50) Cr-Au microelectrodes (MEs) on a single silicon wafer, a MEC is capable of measuring multiple parameters along the depth of samples (Figure 2a). The silicon dioxide layer is thermally grown on the silicon wafers for insulation. Each ME consists of a working electrode (diameter: 50 μm) and a circular strip reference/counter electrode (width: 30 μm) surrounding the working electrode (Figure 2b). Compared to the existing sensors in which reference/counter electrodes are separated far away with high-power consumption, the unique MEC pattern is more accurate and sensitive, and minimizes the power consumption. The MEC has four key advantages over traditional MEs: easy fabrication, sturdy configuration with long lifetime, simple measurement procedure without the need of micromanipulator, and measuring/profiling multiple parameters, which provides an excellent sensing platform to characterize the migration fate of contaminants in real-time format. The PIs have conducted oxygen detection in phosphate buffer solution and obtained high precision.

The PIs have developed a multi-module MEC for simultaneous measurement of four critical parameters related with water quality (dissolved oxygen (DO), pH, nitrogen, and phosphorous). Briefly, the MEs (each ME:20-nm Cr/100-nm Pt) were modified for specific parameter. An array of these four types of MEs were micro-fabricated and integrated on the same silicon chip (as a MEC) to minimize the sensor energy consumption for elongated operation time (Figure 2c). The MEs specific for each parameter were laid out in horizontal direction and extended to the vertical direction on the MEC. Nitrate, phosphate, and pH will be measured potentiometrically, and DO will be measured amperometrically. A copper (Cu) layer will be coated on the MEC surface as the anti-fouling solution for long-term monitoring [17]. The MECs overcome the problems of existing macro-electrodes and micro-electrodes, and can achieve a simultaneous measurement of multiple parameters at different depths of a given aquatic system. The PIs have developed a water-proof cartridge (1.5×1.5 cm) to hold the MEC (Figure 3) and connected with a data logging system through an external circuit. Each MEC chip has at least 50 MEs along the horizontal and vertical directions, which can be easily inserted into the test site (e.g. bulk water, sediment, and soil) to monitor multiple parameters at multiple depths.

The MEC is an excellent measurement tool for multiple parameters. Successful on-site application of this novel MEC technology can give insight to spatial physiochemical distributions in microenvironment, and will yield profound significance for modeling and designing a site of interest. This project targets the rapid sensing and characterization of natural water resources using MECs. The profiles will be integrated with kinetic models to elucidate the occurrence of eutrophication and hypoxia.

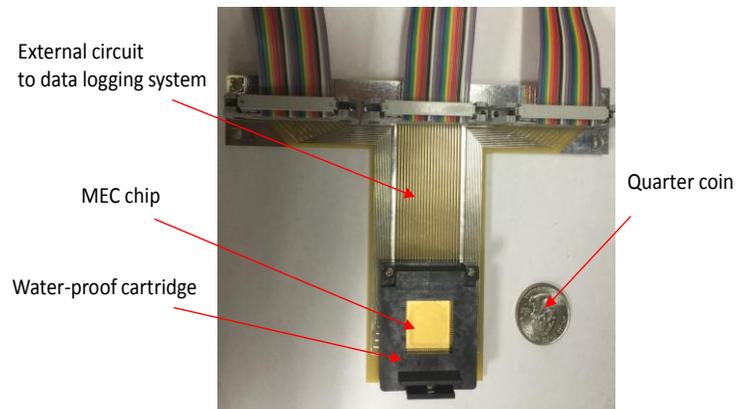


Figure 3. Water-proof MEC cartridge with each chip (1.5cm×1.5cm) holding at least 50 MEs in the horizontal and vertical direction.

This project features many innovations that have the potential to revolutionize the *in situ* real-time measurement of eutrophication and hypoxia in the State of Connecticut and the LIS. **First**, the lab tests and the field tests will testify the feasibility and accuracy of MECs for on-site monitoring, and make the *in situ* real-time profiling multiple parameters at multiple depths possible. *No any existing sensing technology has this unique feature.* The outcomes will lay a solid foundation for protecting, preserving and restoring a broad stratum of water-related systems (e.g. water/sediment, deep sea, boat hull, water supply networks, and water/wastewater treatment facilities) and solid-related systems (e.g. bioremediation sites and Brownfields). **Second**, by obtaining the real-time *in situ* profiles of nutrient and oxygen/pH in natural water resources, this study will quantify the dynamic variations and extrapolate nutrient flow patterns. This information will enable researchers and engineers to elucidate the contaminant fate across broad physical expanses and times and design efficient processes to minimize pollutant impacts. **Third**, the project will transform the way in which we observe the vulnerable aquatic environments and the fragile ecosystems they host, and provide the technical guidelines for regulators and policy makers to execute timely and efficient strategies. By developing high spatial/temporal resolution for substance migration, the project will have a significant contribution to nutrient reduction and hypoxia solution in the State of Connecticut, and bring us closer to the ultimate goal of U.S. long-term environmental sustainability.

The success of this multidisciplinary project will bridge the gap between state-of-the-art electrochemical sensing technology and rapid characterization of dynamic aquatic systems. The project is strongly correlated with the Connecticut Water Quality Protection and Preservation Initiative of the Connecticut Department of Energy Environmental Protection (CTDEEP), the Mission “resolve state and regional water related problems” of the Connecticut Institute of Water Resources (CTIWR), and Water Initiatives and Environmental Cleanup Initiatives at the University of Connecticut (UConn). Moreover, this project will strengthen the broad collaboration that the PIs have established at Departments of Electrical Engineering, Material Science, Chemistry, Microbiology, Marine Science, Nanoelectronics Center, Center for Environmental Science and Engineering (CESE), and Center for Clear Energy Engineering (C2E2). Several funding agencies (EPA, DOE, and DOD) have shown strong interests in the PIs’ vision for the application of MECs in engineered systems and natural resources. These

program directors have acknowledged that obtaining an accurate physicochemical spatial stratification in a given ecosystem is the very first step to develop effective protection and restoration strategy. The lab-scale and field tests of MECs in this project will build a foundation to apply these external funds.

Hypoxia is closely related with nutrient contents, and nutrient reduction could be reflected by the extents of hypoxia in natural water ecosystems. The objective of this multidisciplinary proposal is to achieve the real-time *in situ* profile of nutrient fate and oxygen content using the MECs that the PIs have developed, and apply models to predict eutrophication and hypoxia occurrence. The project was proposed in three steps: (1) To apply the micromanipulator-free and individually addressable MECs in the lab-scale system and measure hypoxia occurrence and water quality, (2) To apply the MECs to two real water resources (Swan Lake and Fenton River), determine the nutrient transfer along the depths of water bodies at different seasons, and correlate hypoxia and nutrient contents, and (3) To predict the long-term migration fate of nutrients and hypoxia at these two water bodies using a mass transfer model developed. *In this project, the interface of water and sediment will be selected as the target, which represents a broad spectrum of natural environments and biodegradation sites. The contaminants will be represented by nitrogen and phosphorous, which have caused severe eutrophication in Connecticut water resources.* The rationale of this research lies in the acquisition of the profiles of diverse parameters at multiple depths using MECs, so that the occurrence of eutrophication and hypoxia can be characterized as a whole set of picture and nutrient loads can be real-time *in situ* monitored. This project holds great promise to enhance our understanding of migration fates of contaminants in natural water resources.

Methods/ Procedures/Progress.

The variation of nutrient and oxygen concentrations in water resources was first examined in a lab-scale simulated aquatic system. The water and sediment was taken from the Swan Lake on the University of Connecticut (UConn) campus, and put in a continuous-flow tank (volume: 20 L) in the lab (Figure 4). The Swan Lake intakes the wastewaters from the UConn Wastewater Treatment Plant (UConn WWTP), which treats diverse wastewaters (e.g. office, dormitory, cafeteria, and labs) and contains diverse inorganic/organic compounds. Two critical environmental parameters (organic concentration and nitrogen concentration) have been tested. Chemical oxygen demand (COD), a commonly used parameter was selected to indicate organic contaminant concentrations on site. COD is related with bacterial activities and groundwater quality, and its variation will affect the removal of nitrogen and phosphate in water/wastewater systems and oxygen levels in water resources. High total nitrogen concentration (TN, organic nitrogen and inorganic nitrogen) of water bodies directly causes eutrophication and affects oxygen levels, pH, and microbial communities. These two variables were tested individually to simulate waste

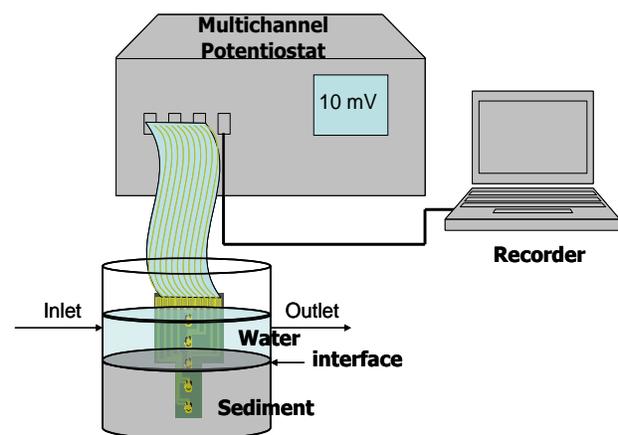


Figure 4. Schematic of the lab-scale setup for the profiling in the interface of water and sediment with a “T”-shaped MEC (not in scale).

discharge in natural water resources.

The system was operated at the COD of 50-100 mg/L, TN concentration of 15 mg/L, flow rate of 5L/hr, and temperature of 24 °C as the standard stable status. Four types of MECs (DO, pH, N, and P) developed in the PI's groups (as shown in [Figure 2c](#)) was inserted into the tank. To easily identify the position of water/sediment interface, the MEC will be designed as the "T"-shape, with the water/sediment interface being matched with the T"-shaped interface position marker ([Figure 4](#)). Actually, this is one of the unique features of the MEC, which avoids the usage of complicated micromanipulators to determine the MEs' positions on the monitoring site. Four profiles (DO, pH, N, and P) measured by each ME were collected real-time using a multichannel potentiostat and a data logging system, and saved in an MS EXCEL file ([Figure 4](#)). All these facilities are available at the PIs' labs. The main reason for conducting the tests in the lab-scale system first rather than in field is that a well controlled lab environment is crucial to verify the applicability of MECs.

After the lab-scale system is operated at the standard status for 3-4 weeks and the MECs reach the stable readings for all profiles, two variables (COD concentration and TN concentration) was introduced to the system at two levels (high and low) separately to simulate low concentration at raining season and high concentration after waste discharge: COD concentrations (20 and 500 mg/L) and TN concentration (2 and 40 mg/L). Different dosages of sodium acetate and ammonium chloride were individually added in the influent to achieve high/low COD and N concentrations. Each condition was examined for 3 weeks, during which the profiles of nutrient (N and P), oxygen, and pH will be simultaneously obtained in the water and sediment phases. The reproducibility of the MEC measurements was verified by conducting at last triplicate tests (each test duration: 3-4 days) under each condition.

Calibration and validation of MECs. The calibration and validation of MECs was performed before each measurement in the field tests. MECs were calibrated with the commercialized microelectrodes and macroelectrodes at different concentrations of dissolved oxygen, pH, NO_3^- , and PO_4^{3-} solutions. The accuracy and reliability of MECs was checked.

Results/Significance

The most significant result of the project was the 2D MEC development capable of monitoring multiple water-quality related parameters with high spatial and temporal resolution. Five types of MEC sensors (redox potential (ORP), pH, temperature, conductivity, and ammonium NH_4^+) specifically for water quality have been developed at the PIs' labs by precisely patterning multiple mm-sized electrodes using an inkjet printer ([Figure 5a](#)). Multiple rows vertically compose a MEC matrix capable of simultaneously measuring multiple parameters along depth (e.g. 4 cm (width) \times 30 cm (depth) hosting 60 electrodes) ([Figure 5b](#)). Each type electrode were modified for specific parameter using the reported procedures, with a bond pad on the top of the film for wire bonding, allowing the easy connection to external circuit ([Figure 6](#)). For ORP, pH and NH_4^+ sensors, three-electrode systems include same pattern gold-based working and counter electrodes, and silver-based reference electrodes ([Figure 6](#)). Then, the working electrode of pH sensor is modified through the deposition of Ir_2O_3 ^{74,75}, and the working electrode of NH_4^+ sensor is modified with a coating of polyaniline. Temperature sensor is made by zip-shape gold-based resistance and conductivity sensor consists of four gold-based electrodes as a capacitor ([Figure 6](#)).

The unique designed MECs are more accurate and sensitive, since the electrochemical signals can be immediately and fully collected by the adjacent counter electrode (Figure 6). This is very different from the existing electrodes which need a separated reference/counter electrode. The ink-jet printed MEA sensors are low cost (\$0.2 per sensor, mainly gold/silver ink cost) and easy to mass fabricate. The precise alignment of each electrode on the film makes the MEC individually addressable, which substantially eases the *in situ* measurement on the field. Electrochemical signals of MECs are recorded using a multiple-channel data-logging system. Specifically, NH_4^+ concentration is measured using current, pH and ORP using potential, conductivity using capacitance, and temperature using resistance.

MEC Calibration and Response to Variation of Parameters.

Each type sensors of MEAs are calibrated to establish an accurate correlation between electrochemical signals and the targeted parameters. The calibration has been conducted in water solutions that well represent typical water quality, with ORP of -350--+300 mV, pH of 2-10, temperature of 10-50°C, conductivity (indicating dissolved solid content) of 1500-3000 $\mu\text{S}/\text{cm}$ and NH_4^+ 20-100 mg/L. Each type sensor showed high accuracy between electrochemical signals and the targeted parameters with R^2 values higher than 99% (Figure 7a). Besides calibration, a shock test was conducted by rapidly changing water temperature and conductivity to simulate water system malfunction (e.g. temperature fluctuation and fatty acid accumulation in AD systems). MEA sensors showed the real-time signal change with the shock (Figure 7b. *only showing conductivity and temperature response due to the space limit*), indicating an excellent capacity of capturing the shock of impaired water quality. It should be noted that mm-sized electrodes on the MEC are not only limited to these five parameters. Each electrode can be easily modified using surface coating and electrochemical deposition to monitor wide range of parameters (e.g. toxic metal ions, pesticide, salt, and nutrients, etc.).

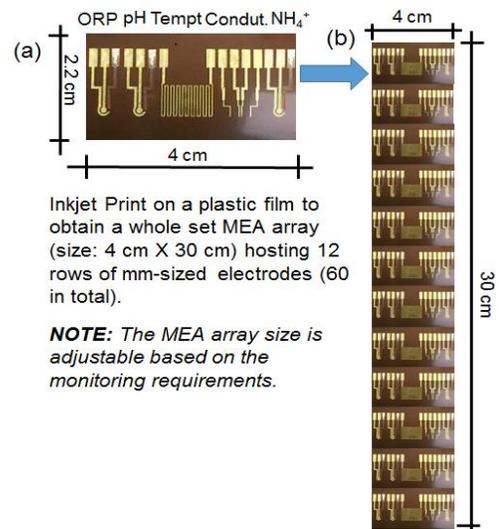


Figure 5. MEC ink-jet-printed on a film for real time *in situ* profiling multiple parameters.

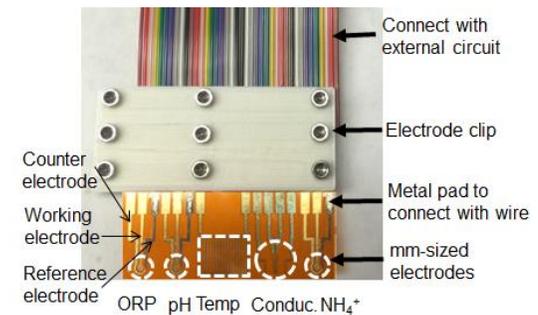


Figure 6. MEC setup with external circuit and data logging system.

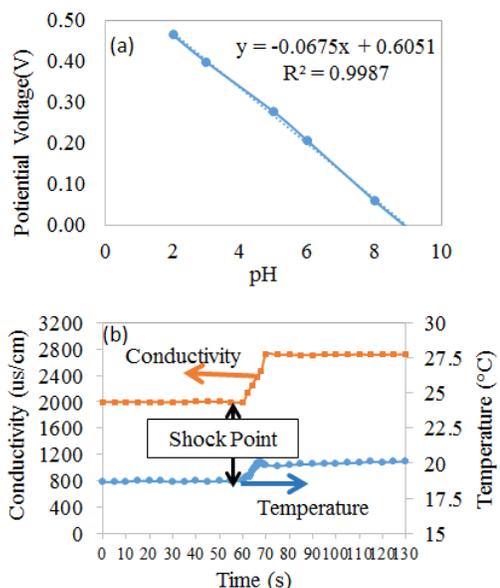


Figure 7. MEC calibration curve of pH (a) and MEA response to the changes of conductivity and temperature in water solution (b).

Applying the MEC profiles for understanding migration fates of nutrients and hypoxia occurrence. Based on the MEC profiles, the PIs have developed micro-environmental kinetic models that include mass transfer, substrate utilization by cell growth, substrate generation by cell lysis, and substrate consumption by endogenous respiration as shown below:

$$\frac{\partial S}{\partial t} = \frac{1}{L^2} \left(D_s \cdot L^2 \frac{\partial S}{\partial L} \right) - \frac{\mu_{\max}}{Y_s} \cdot \frac{S}{K_s + S} \cdot \frac{O}{K_o + O} \cdot X + K_{LYS} \cdot X - K_{RES} \cdot \frac{S}{K_s' + S} \cdot \frac{O}{K_{OD} + O} \cdot X$$

where dS/dt : the contaminant concentration (e.g. nitrogen, phosphorous in this project) change with time; L : the depth of the sample point; D_s : the diffusion constant; μ : the specific cell growth rate; O : oxygen concentration, X : the biomass concentration; K_{LYS} : the cell lysis constant; K_{RES} : the cell endogenous respiration constant; and K_{OD} : cell delay half-saturation constant. The MEC monitoring encompasses the horizontal/vertical profiles of various physical-geo-chemical parameters that are responsible for the migration of contaminants in water and sediment, which will substantially enhance the information required for model simulation.

In this project, the MEC profiles in the water and sediment phases was integrated with the models developed to predict the effects of environmental conditions on the migration fates of inorganic/organic contaminants, nutrients, and oxygen at the downstream of waste discharge points (e.g. high/low COD and nutrient concentrations). Unlike the traditional oxygen models that suffer the lack of spatial and temporal readings, the multiple MEs on the horizontal and vertical directions on MECs provide the sufficient resolution to enhance model accuracy. The extrapolated location and occurrence of eutrophication and hypoxia will provide the timely restoration solutions for site cleanup before the irreversible deterioration.

Based on the lab-scale MEC tests, profiling nutrient content and hypoxia occurrence in field tests will be planned in the future study such as along the depth in the Swan Lake and the Fenton River.

Project technology transformation.

Public dissemination of real-time monitoring in natural water resources using MEC technology is absolutely critical given the slow acceptance of new technologies that deliver commodities. Both the PI and Co-PI are part of a growing “Water Consortium” at UConn, which aims at supporting works on water and environmental sustainability. The team has disseminated this project through active publication in journals (two journal papers under preparation) and presentations at professional conferences (e.g. two New England Environmental Conferences) based on the MEC lab tests and model simulation.

The team has been dedicated to transforming the real-time *in situ* monitoring technology by working closely with the United Water Inc., a global leading environmental engineering company. Over fifty water quality related institutes/municipalities/government agencies/private owners have been interviewed regarding MEC technology and real-time profiling technology. The feedbacks are overwhelmingly positive. We have identified the urgent needs for MECs technology in water treatment/wastewater monitoring. The collaboration with industrial partners for field tests and pilot-scale tests will further validate MEC technology.

Reference

1. Cloern, J. E. (2001). *Mar. Ecol. Progr. Ser.* 210: 223-253.
2. Diaz, R. J., and Rosenberg, R. (2008). *Science* 321: 926-929
3. Doney, S. C., Fabry, V., Feely, R. A., and Kleypas, J. A. (2009). *Annual Rev. Mari. Sci.* 1: 169-192.

4. Caldeira, K., Wickett, M. E. (2005). *J. Geophysical Research-Oceans* 110:C09S4.
5. Williams, J. R., A.D. Nicks, J.G. Arnold (1985). *Journal of Hydraulic Engineering*, 111 (6): 970-986.
6. Wang, X., S. Shang, W. Yang, C. R. Clary, D. Yang (2010). *Ecological Engineering*. 36 (3): 328-344.
7. Broekaert, J. G. Tölg (1987). *Fresenius' Journal of Analytical Chemistry*. 326 (6): 495-509
8. Gómez-Ariza, J., T. Barrera, F. Lorenzo, V. Oliveira (2004). *Analytica Chimica Acta*. 524 (1-2):15-22.
9. Herdan, J., R. Feeney, S. P. Kounaves (1998). *Environ. Sci. Technol.*, 32 (1): 131–136.
10. Li, B. Bishop, P. (2004) *Water Research*, 38: 1248-1258.
11. Li, B., P.L. Bishop (2004). *Water Environment Research* 76 (5): 394-403.
12. De Beer, D., J.P.R.A. Swerts (1988). *Anal. Chim. Acta*. 219: 351-356.
13. Okabe, S., H. Satoh, Y. Watanabe (1999). *Appl. Environ. Microbiol* 65: 3182.
14. Revsbech, N.P., J. Sorensen, T.H. Blackburn, Y. Cohen (1983). *Limnol. Oceanogr*. 28: 1062-1074.
15. Luther III, G., P. Brendel, B. Lewis, D. Nuzzio (1998). *Limnol. Oceanogr*. 43 (2): 325-333.
16. Schramm, A., C. Santegoeds, D. de Beer (1999). *Appl. Environ. Microbiol*, 65 (9): 4189-4196.
17. Manov, DV., Chang, GC. Dickey, T. (2004). *J. Atmos. & Ocean.Tech.* 21 (6): 958-968.
18. Oghenerobor B., Akpor, B., N. B. Momba, J.Okonkwo (2008). *Biotech. J.I*, 3(8): 1083-1087.
19. Héry, M., Sanguin, H., Fabiel, S., Vogel, T., Paul, E., Alfenore, S. (2010). *Wat. Res.* 44: 6133-6143.
20. Hemond, H., Lin, K. (2010). *Wat. Res.* 44: 3645-3650.
21. Branco, B. F., T. Torgersen (2009). *Aquatic Sciences - Research Across Boundaries*. 71 (1): 65-79.
22. Wang, J., R. Bhada, J. Lu, D.MacDonald (1998). *Analytica Chimica Acta*, 361 (1/2): 85-91.
23. Li, B., P.L. Bishop (2003). *J. Environ. Engr.and Sci.* 2 (1): 27-37.
24. Wang, Y., La, A., Ding, Y., Liu, Y., Lei, Y. (2012). *Adv. Function. Matl.* 22(17), 3547-3555.
25. Parisi, J., Lei, Y (2013). *Lab on a Chip*. 13(8), 1501-1508.
26. X. Liu, X.C. Sun, Z.Q. Zhou, Yu Lei. (2014). 2014. *J. Mater. Chem A*. 2 (34), 14038-14047.

Mitigating Eurasian watermilfoil invasion success and ecosystem impact using native herbivores

Basic Information

Title:	Mitigating Eurasian watermilfoil invasion success and ecosystem impact using native herbivores
Project Number:	2015CT292B
Start Date:	3/1/2015
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	4
Research Category:	Biological Sciences
Focus Category:	Invasive Species, Ecology, Water Quality
Descriptors:	None
Principal Investigators:	LaTina Steele

Publications

1. Ray C, Steele L, Guidone M. In review. Potential of native amphipods and snails as biocontrol agents for Eurasian watermilfoil in New England. *Hydrobiologia*.
2. Steele L, Guidone M, Smith A, Mignogni M. In preparation. Eurasian watermilfoil invasion success and community impacts in New England.
3. Steele L, Guidone M, Zupetz S, Slack A. In preparation. Competition with native pondweeds may reduce Eurasian watermilfoil growth.
4. Steele L, Guidone M. In preparation. Timing of herbivory and chemical deterrent production in native vs. invasive aquatic plants.
5. *Smith A, *Mignogni E, Steele L. 2016. A comparison of aquatic community composition within plots of invasive *Myriophyllum spicatum* and native *Elodea canadensis*. Eastern Colleges Science Conference. Western New England University, Springfield, MA. Poster (* = student presenter).
6. *Zupetz S, *Slack A, Steele L. 2016. Competitive interactions between native *Elodea canadensis* and invasive *Myriophyllum spicatum* in Connecticut. Sacred Heart University Academic Festival. Fairfield, CT. Poster (* = student presenter).
7. Steele L, Guidone M. 2015. Eurasian watermilfoil (*Myriophyllum spicatum*) invasion success and community impacts in New England. Coastal & Estuarine Research Federation Meeting. Portland, OR. Oral.
8. Steele L, Guidone M. 2015. Using native herbivores to mitigate Eurasian watermilfoil invasion in New England. Benthic Ecology Meeting. Quebec City, Quebec (Canada). Oral.

Project Title: *Mitigating Eurasian Watermilfoil Invasion Success and Ecosystem Impact Using Native Herbivores*

Final Report, FY 2015-2016

LaTina Steele, Sacred Heart University, 5151 Park Ave., Fairfield, CT 06825

Introduction & Research Objectives

Eurasian watermilfoil has invaded lakes across the state of Connecticut, often becoming dominant within these submerged aquatic vegetation communities. Factors contributing to milfoil's invasion success are poorly understood and are limited mainly to nutrient conditions and the broad assertion that invasion is less likely in lakes with an established submerged macrophyte community (Smith and Barko 1990, Madsen 1998). However, evidence suggests that allelopathic interactions between milfoil and epiphytic algae may contribute to milfoil's establishment (Gross et al. 1996). These allelopathic phenolic compounds produced by *M. spicatum* are also well-known feeding deterrents in terrestrial, aquatic, and marine plants (Constabel 1999). Thus, it seems reasonable that chemical interactions may reduce milfoil herbivory and play a role in its invasion success. Increased understanding of factors leading to successful milfoil invasions is critical for effective management and prevention of milfoil invasion, highlighting the importance of studies like the one proposed here. Managers, policy-makers, and those who use our state's lakes for recreational purposes will all benefit from this study.

Common techniques for eradicating nuisance milfoil involve costly and harmful chemical application and physical removal of milfoil. Such measures often need to be repeated in order to be effective and inherently affect other members of the lake community (e.g., DeLong and Mundahl 1996). Furthermore, physical removal of milfoil could increase its spread to other areas, since it is propagated via fragmentation (Maezo et al. 2010). Mitigation of *M. spicatum* using native herbivores is a much more palatable alternative to many common eradication measures.

Many studies have investigated the potential of a North American weevil to mitigate Eurasian watermilfoil impacts (e.g., Sheldon and Creed 1995). However, few have considered additional herbivores native to particular regions or the impacts of community composition (i.e., the identity and abundance of herbivores, predators, and algal species) that can also influence invasion success. For example, herbivorous snails may either directly or indirectly affect milfoil populations, as some gastropods feed on *M. spicatum* (Boland et al. 2008), while others positively impact milfoil growth by limiting the growth of algal competitors (Chase and Knight 2006). Predator identity and abundance is also vital to our understanding of milfoil success. In lakes where predators are abundant, herbivore populations may be suppressed to levels that inhibit their control of milfoil growth (Ward and Newman 2006). This last point is particularly important for making informed mitigation choices, as there are a number of predatory fish species that are commonly stocked for recreational fishing.

Most studies proposing herbivory as a milfoil control measure have been conducted in the Midwest or the southeast United States. Few have been conducted in New England, and none of those have considered the use of multiple native herbivores to mitigate milfoil impacts. Nor have

those studies considered the role of chemical deterrents in determining when and where milfoil will invade, despite evidence that *M. spicatum* produces many allelopathic chemicals (Gross et al. 1996, Spencer and Ksander 1999), which commonly contribute to plant invasion success (Callaway and Ridenour 2004). Connecticut lakes are home to many potential herbivores, including crustaceans (amphipods and crayfish), insect larvae, gastropods, and herbivorous fishes. Previous studies in other regions suggest that crayfish (Parker and Hay 2005, Maezo et al. 2010) and insect larvae (Johnson et al. 1998) are milfoil consumers, with some insects leading to shifts in community structure from milfoil-dominated systems to dominance by *Elodea canadensis* (Gross et al. 2001).

The objectives of the second year of this project were to 1) investigate the role of competitive interactions between plants in determining milfoil invasion success, 2) measure the effects of milfoil invasion on the lake community by a) comparing community composition and diversity between Eurasian watermilfoil and a native aquatic plant and b) assessing milfoil's effects on predator foraging efficiency.

Methods & Progress

Competition Experiment

A laboratory experiment using varying plant fragment densities (1, 6, or 12 plant fragments) in monoculture and polyculture was conducted to determine how competition with a native plant affects milfoil growth. The experiment included five replicates each of eight treatments. The six monoculture treatments included 1, 6, or 12 milfoil or *Elodea canadensis* fragments, and the two polyculture treatments comprised 3 milfoil and 3 *E. canadensis* fragments or 6 milfoil and 6 *E. canadensis* fragments. Each 10-cm fragment had at least three visible roots. Fragments were planted in 1-gallon aquaria containing ~1 inch of play sand fortified with 1 g of Osmocote fertilizer. Fragments were blotted with paper towels and weighed before being placed into experimental tanks. All tanks were kept in a temperature-controlled greenhouse for two weeks under ambient light, and water in each tank was replenished daily. Plants were blotted and reweighed after two weeks; change in wet weight was then calculated. One 50-ml water sample was collected from each tank at the beginning and end of the experiment and analyzed for nitrate and phosphate using Hach kits (NitraVer 5 and PhosVer 3). After plant fragments were weighed at the end of the experiment, they were frozen for chemical analysis. A colorimetric assay (cf. Steele et al. 2005) was used to quantify condensed tannins in frozen milfoil and *E. canadensis* tissue from the competition experiment. HPLC is being used to quantify gallic acid and 3,4-dihydroxybenzoic acid concentrations in the frozen plant tissues; these analyses are currently underway. Two separate two-way ANOVAs with factors of species and density were used to determine if there were differences in the growth and condensed tannin content of milfoil and *Elodea* among densities in monoculture and polyculture. Post-hoc Tukey tests were used to identify which treatments differed from each other. Results were considered significant at $p < 0.05$.

Field Sampling

During June 2015, five throw trap samples were collected from each of three areas: 1) milfoil-dominated areas of Osbourndale Pond (Derby, CT), 2) *Elodea*-dominated areas of Osbourndale Pond, and 3) Colony Pond (Ansonia, CT), where milfoil is not present. To confirm that areas visually identified as milfoil or *Elodea*-dominated were actually dominated by those species, all plants within throw traps were identified and the wet weight was recorded. Animals from these trap samples were preserved in 10% formalin for two weeks, then rinsed and stored in 70% isopropyl alcohol. Rose Bengal stain was added to each sample to facilitate identification and counting of all animals within the sample. Primer-E software was used to conduct Analysis of Similarity (ANOSIM) on a Bray-Curtis similarity matrix constructed using abundance of each taxon per m² to determine if community composition differed between 1) plant species in Osbourndale Pond (Derby) and 2) *Elodea*-dominated areas of Osbourndale Pond (Derby) and Colony Pond (Ansonia). Results were considered significant at $p < 0.025$ to account for multiple tests on the data from Derby. A Similarity Percentage (SIMPER) analysis was conducted to determine which taxa drove differences in community composition between sites.

Predation Experiments

Experiments conducted during year 1 of this project suggested that amphipods (*Hyaella azteca*) could suppress milfoil growth and reduce its biomass. Moreover, amphipods are among the most abundant potential prey species and invertebrate predators are very abundant within plant patches at our study sites. Thus, predation experiments were performed to assess the potential effects of invasive milfoil on foraging efficiency of invertebrate predators (damsselfly and dragonfly nymphs), with amphipods (*H. azteca*) as prey. Two separate experiments were conducted using the two most abundant invertebrate predators, damsselfly (*Enallagma* sp.) and dragonfly (*Gomphus* sp.) nymphs (separate experiments for each predator). All predators were starved for 24 hours prior to use in feeding trials.

Methods for the two experiments were identical and comprised ten replicates each of three plant treatments (two milfoil fragments, two *Elodea* fragments, 1 milfoil and 1 *Elodea* fragment). Ten amphipods were placed into each experimental container with the plant fragments and allowed to acclimate for 30 minutes. One damsselfly or dragonfly nymph was then placed into five replicates of each plant treatment. The other five replicates of each treatment acted as predator-free controls to account for amphipod mortality that was not due to predation. Amphipods in each replicate were counted every 24 hours until ~50% of amphipods had been consumed in at least one treatment (4 days in the damsselfly experiment, 2 days in the dragonfly experiment). Mean amphipod mortality was calculated in each of the three predator-free control treatments, and this value was subtracted from each replicate within the corresponding predator treatment; this corrected mortality value was used in statistical analyses. Repeated measures ANOVAs with factors of Day and Treatment were used to determine if the number of amphipods consumed differed among plant treatments. Separate one-way ANOVA's were conducted on each day when there was a significant Day*Treatment interaction. Post-hoc Tukey tests were used when appropriate to determine which treatments differed from each other. Results were considered significant at $p < 0.05$.

Results and Significance

The most abundant native aquatic plant in southern Connecticut appears to be an inferior competitor compared to invasive milfoil. In the competition experiment, milfoil's growth was much higher than that of the native *E. canadensis* (Figure 1). This was especially apparent in polyculture, where milfoil's growth more than doubled that of *E. canadensis* (Figure 1). At the highest fragment density, growth of *E. canadensis* was clearly suppressed in the presence of milfoil (Figure 1). Although fragment density did not have a significant effect on plant condensed tannin content, milfoil consistently contained more than twice the condensed tannins found in the native species (Figure 2). These results suggest that the presence of a native plant competitor will not prevent milfoil from overgrowing a waterway. Furthermore, low densities of native plants may actually stimulate milfoil growth (Figure 1). This may be due, in part, to the higher levels of allelopathic compounds in milfoil (Figure 2; Gross et al. 1996). Future work should examine competitive interactions between invasive milfoil and native plants that contain higher levels of chemical deterrents.

Based on throw trap surveys, milfoil has not had a strong negative effect on the animal community within local ponds (Figure 3). A similar suite of animals seems to take up shelter in both milfoil and the native *Elodea*, which are the two dominant aquatic plants in Osbourndale Pond (Derby, CT). In areas such as this where milfoil has not caused a noticeable effect on the consumer community, costly and ecologically harmful removal methods for the invasive plant may not be necessary. Likewise, the particular herbivore community found at this site may be acting to prevent milfoil from overgrowing the area.

In contrast to the results within Derby, ANOSIM showed a significant difference in community composition within *Elodea* patches in Derby and Ansonia (Figure 3), driven primarily by higher oligochaete and isopod abundances in Derby and higher chironomid, amphipod, and nematode abundances in Ansonia (Table 1). Although it is possible that the difference is related to presence of milfoil in Derby, it is likely that the difference is simply due to variation among sites. Additional sampling in multiple ponds with and without milfoil would be required to fully address this possibility.

Milfoil seems to have a positive effect on invertebrate predator foraging efficiency. Damsely predation was more successful in invasive milfoil than in native *Elodea* (Figure 4). Likewise, dragonfly nymphs consumed significantly more amphipods when milfoil was present than when it was not (Figure 5). This suggests that amphipods may be less able to control milfoil growth when invertebrate predators are abundant. However, our throw trap surveys found no evidence that amphipods avoid invasive milfoil, where they are more likely to be consumed. It is possible that amphipod abundances at our study sites are high enough to overcome losses to predation. Future work should determine if invertebrate predator abundances in the field are high enough to hinder amphipod consumption of milfoil.

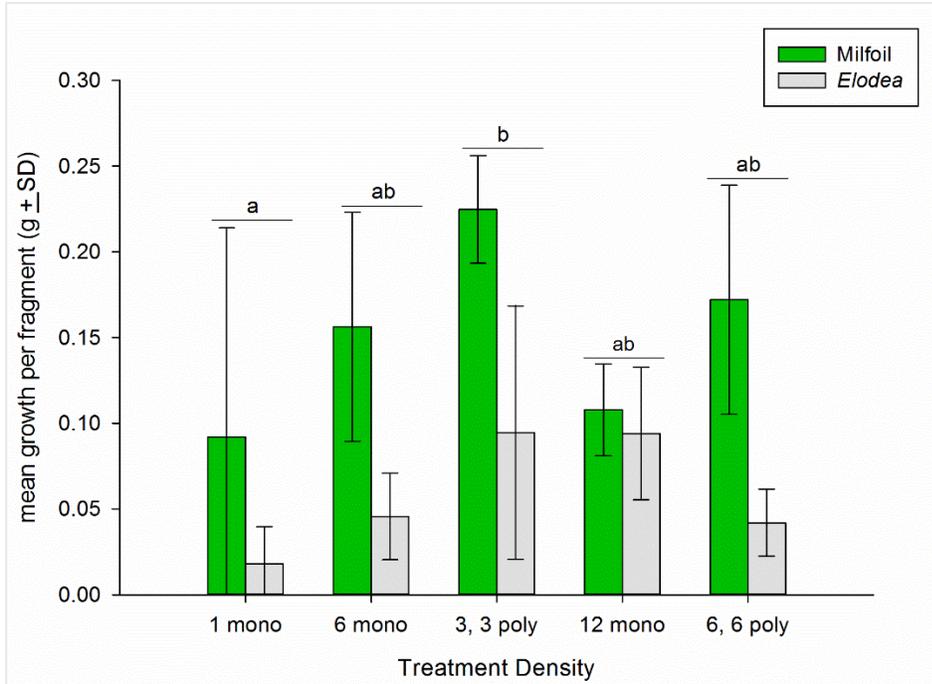


Figure 1. Mean change in weight (g of wet mass \pm 1 SD) of *Myriophyllum spicatum* (milfoil) and *Elodea canadensis* grown at densities of 1, 6, or 12 plants in monoculture or polyculture (2 way ANOVA: Density $F_{4,40} = 4.069$, $p = 0.007$; Species $F_{1,40} = 31.907$, $p < 0.001$).

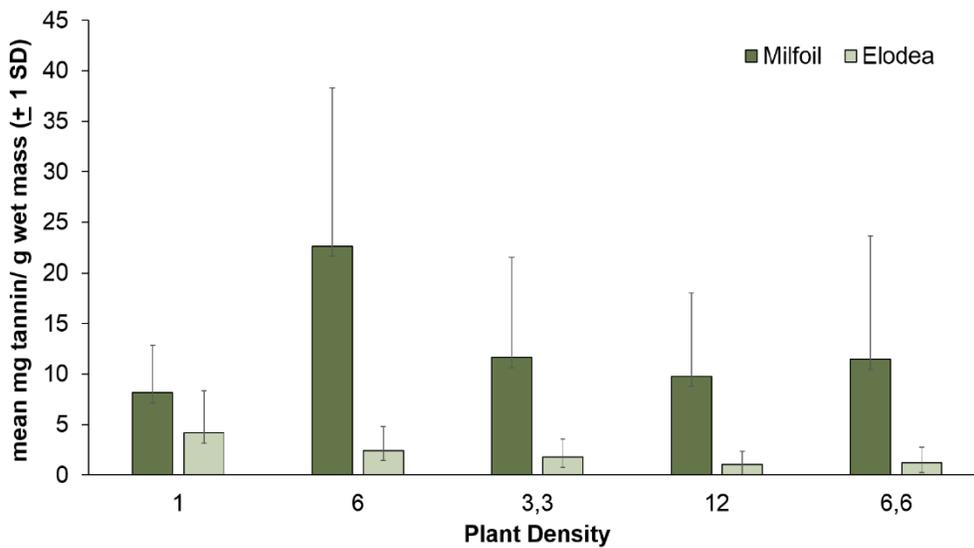


Figure 2. Condensed tannin content (mean mg/g \pm 1 SD) of *Myriophyllum spicatum* (milfoil) and *Elodea canadensis* grown at densities of 1, 6, or 12 plants in monoculture or polyculture (2-way ANOVA: Species $F_{1,35} = 26.523$, $p < 0.001$; Density $F_{5,35} = 0.568$, $p = 0.724$; Species* Density $F_{4,35} = 0.808$, $p = 0.529$).

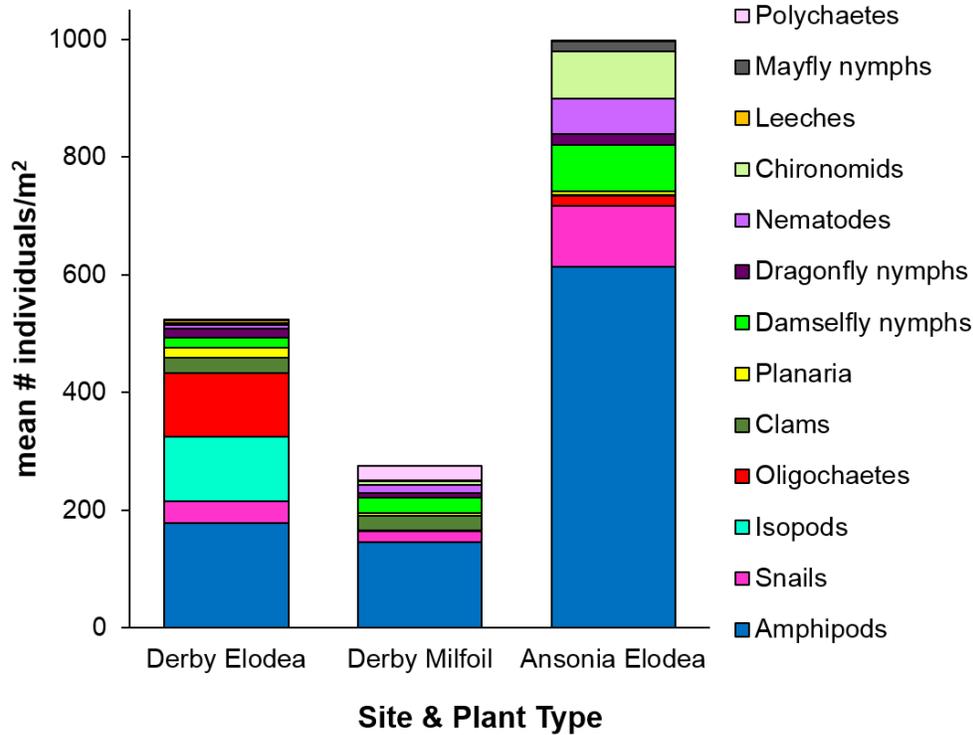


Figure 3. Most abundant taxa in throw trap samples (# individuals/m²) collected in *Elodea*- and milfoil-dominated areas of Osbourndale pond (Derby, CT) and Colony Pond (Ansonia, CT). Analysis of similarity (ANOSIM) detected no significant difference in community composition between *Elodea* and milfoil dominated habitats in Derby (Global R = 0.076, p = 0.262). ANOSIM showed a significant difference in community composition between *Elodea*-dominated patches in Ansonia and Derby (Global R = 0.388, p = 0.024).

Table 1. Similarity percentage (SIMPER) analysis of animal abundance in *Elodea* patches from Derby (Obsourndale Pond; contains milfoil) and Ansonia (Colony Pond; milfoil-free reference pond). Rows highlighted in **blue** show higher oligochaete and isopod abundances in Derby, while rows highlighted in **red** show higher chironomid, amphipod and nematode abundances in Ansonia.

	Group Derby	Group Ansonia				
Taxa	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Oligochaeta	2.57	0.61	5.47	1.59	11.72	11.72
Chironomid	0.94	2.44	4.32	1.11	9.27	20.99
Amphipods	3.38	4.72	3.94	1.25	8.45	29.43
Nematode	0.48	1.61	3.81	1.14	8.18	37.61
Isopod	1.75	0	3.64	0.99	7.8	45.41
Mayfly larvae	0.37	1.62	3.63	1.38	7.79	53.2
Clam	1.77	0.57	3.61	1.43	7.74	60.94
Damselfly	1.64	2.91	3.6	1.09	7.71	68.65
Snail	1.91	2.59	3.36	1.15	7.2	75.85
Planaria	1.39	0.74	3.01	1.11	6.45	82.3
Moth larvae	0.57	0.34	1.57	0.85	3.37	85.67
Leech	0.68	0	1.42	0.79	3.04	88.71

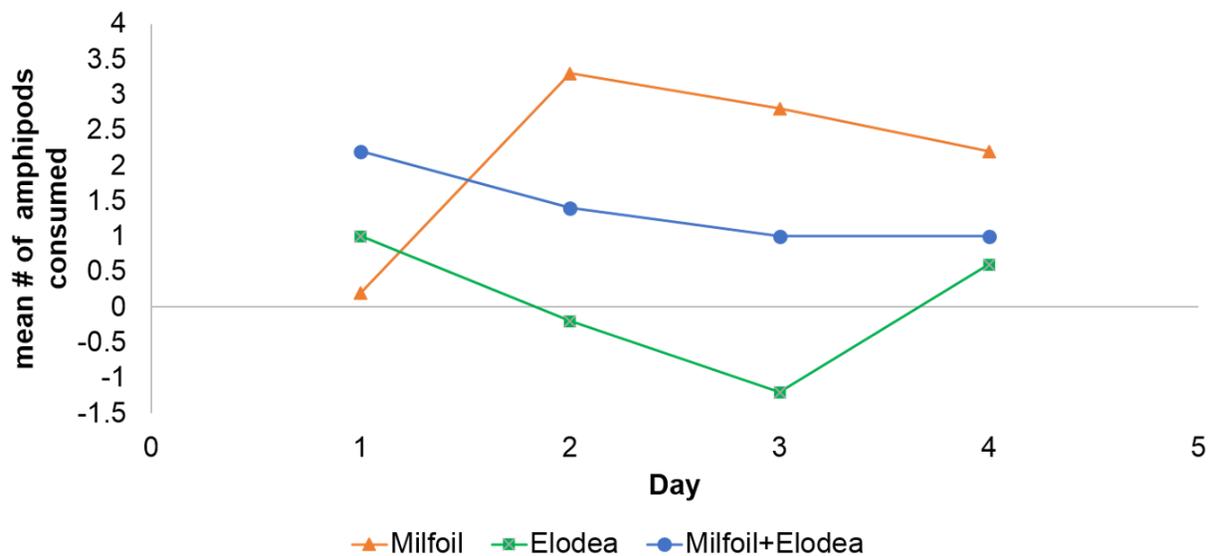


Figure 4. Mean number of amphipods (*Hyalella azteca*) consumed by damselfly nymphs (*Enallagma* sp.) over four days in treatments containing two invasive milfoil (*Myriophyllum spicatum*) fragments, two native *Elodea canadensis* fragments, or one milfoil and one *E. canadensis* fragment (RM ANOVA: Day $p = 0.009$, Treatment $p = 0.012$, Day*Treatment $p = 0.001$).

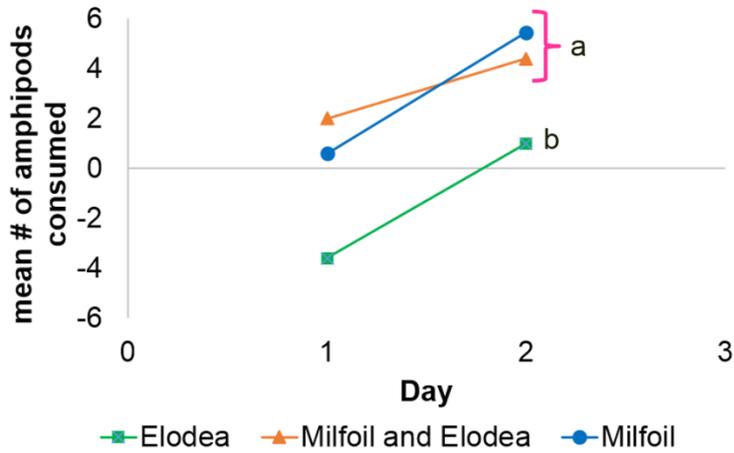


Figure 5. Mean number of amphipods (*Hyalella azteca*) consumed by dragonfly nymphs (*Gomphus* sp.) over two days in treatments containing two invasive milfoil (*Myriophyllum spicatum*) fragments, two native *Elodea canadensis* fragments, or one milfoil and one *E. canadensis* fragment (RM ANOVA: Day $p = 0.001$, Treatment $p < 0.001$, Day*Treatment $p = 0.491$).

Literature Cited

- Boland BB, Meerhoff M, Fosalba C, Mazzeo N, Barnes MA, and Burks RL. 2008. Juvenile snails, adult appetites: contrasting resource consumption between two species of applesnails (*Pomacea*). *J Molluscan Stud* 74: 47-54
- Callaway RM and Ridenour WM. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Front Ecol Environ* 2: 436-443
- Chase JM and Knight TM. 2006. Effects of eutrophication and snails on Eurasian watermilfoil (*Myriophyllum spicatum*) invasion. *Biol Invasions* 8: 1643-1649
- Constabel CP. 1999. A survey of herbivore-inducible defensive proteins and phytochemicals. In: AA Agrawal, S Tuzun, and E Bent (eds). *Induced Plant Defenses Against Pathogens and Herbivores*. APS Press, St. Paul, MN, pp 137-166
- Delong MD and Mundahl ND. 1996. Secondary effects of fluoridone treatment on invertebrate community structure in lake ecosystems. Winona State University Biology Dept., Report to the MN DNR-St. Paul, Winona, MN
- Gross EM, Johnson RL, and Hairston NG. 2001. Experimental evidence for changes in submersed macrophyte species composition cause by the herbivore *Acentria ephemerella* (Lepidoptera). *Oecologia* 127: 105-114
- Gross EM, Meyer H, and Schilling G. 1996. Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. *Phytochemistry* 41: 133-138
- Johnson R L, Gross EM and Hairston NG. 1998. Decline of the invasive submersed macrophyte *Myriophyllum spicatum* (Haloragaceae) associated with herbivory by larvae of *Acentria ephemerella* (Lepidoptera). *Aquat Ecol* 31: 273-282
- Madsen JD. 1998. Predicting Invasion Success of Eurasian Watermilfoil. *J Aquat Plant Manage* 36: 28-32.
- Maezo MJ, Fournier H, and Beisner BE. 2010. Potential and realized interactions between two aquatic invasive species: Eurasian watermilfoil (*Myriophyllum spicatum*) and rusty crayfish (*Orconectes rusticus*). *Can J Fish Aquat Sci* 67: 684-700
- Parker JD and Hay ME. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecol Lett* 8: 959-967
- Sheldon SP and Creed RP Jr. 1995. Use of a native insect as a biological control for an introduced weed. *Ecol Appl* 5: 1122-1132
- Smith CS and Barko JW. 1990. Ecology of Eurasian Watermilfoil. *J Aquat Plant Manage* 28: 55-64
- Spencer DF and Ksander GG. 1999. Seasonal changes in chemical composition of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and water temperature at two sites in northern California: implications for herbivory. *J Aquat Plant Manage* 37: 61-66
- Steele L, Caldwell M, Boettcher A, and Arnold T. 2005. Seagrass-pathogen interactions: 'pseudo-induction' of turtlegrass phenolics near wasting disease lesions. *Mar Ecol Prog Ser* 303: 123-131
- Ward DM and Newman RM. 2006. Fish predation on Eurasian watermilfoil (*Myriophyllum spicatum*) herbivores and indirect effects on macrophytes. *Can J Fish Aquat Sci* 63: 1049-1057

Laboratory evaluation of materials to treat chloride in stormwater

Basic Information

Title:	Laboratory evaluation of materials to treat chloride in stormwater
Project Number:	2015CT293B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	2nd
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Surface Water, Water Quality
Descriptors:	None
Principal Investigators:	Michael Dietz, John Campbell Clausen

Publications

There are no publications.

Laboratory Evaluation of Materials to Treat Chloride in Stormwater

FINAL REPORT

to

Connecticut Institute of Water Resources

United States Geological Survey

FY 2014-2015 104 B Program

Award# G11AP20069

March 1, 2016

Michael Dietz, UConn Extension

John Clausen, UConn Department of Natural Resources and the Environment

Table of Contents

Introduction/Research Objective	1
Materials/Procedures/Progress.....	2
Results/Significance.....	3
Chloride.....	3
pH.....	7
Color.....	7
Conclusions.....	9
Literature Cited	10

List of Figures

Figure 1. Experimental setup in laboratory.....	2
Figure 2. Chloride concentrations for ten successive leachings for different salt treatments with no resin. Error bars represent 95% confidence intervals.	4
Figure 3. Chloride concentrations for 10 successive leachings for different salt and resin treatments. Error bars represent 95% confidence intervals.	4
Figure 4. Crystals forming around base of columns with resin present, presumed to be NaOH....	5
Figure 5. Influent and effluent chloride concentrations for the 10% resin/bioretention media blend. Error bars represent ± 1 standard deviation.	6
Figure 6. Influent and effluent chloride concentrations for 100% resin. Error bars represent ± 1 standard deviation. Dates not used on this Figure because there were sometimes multiple leachings on one day.....	6
Figure 7. Leachate pH in 10 successive leachings. Error bars represent 95% confidence intervals.	7
Figure 8. Color differences in leachate from different treatments.....	8
Figure 9. Leachate color of selected treatments.....	9

Introduction/Research Objective

Road salt application rates have increased steadily since the 1940s, with dramatic increases in the last 30 years. State departments of transportation as well as municipal public works departments are primarily concerned with public safety during winter events. Deicing salts such as sodium chloride or magnesium chloride lower the freezing point of water. Once dissolved, the sodium and chloride ions dissociate in solution. If this meltwater runs into the stormwater system, in many cases it is discharged directly into a local stream or river. If the meltwater runs onto a pervious area, it enters the soil and percolates through the unsaturated zone and travels readily through the soil, due to its negative charge and the negative charge on most soil surfaces. Practices have been implemented in many states to reduce salt loss from storage areas (Fitch, et al., 2005), however these methods are not practical on the expansive network of roads where deicing salts are applied. The ecological impacts of road salting are becoming clear (Brady, 2012; Findlay & Kelly, 2011).

Chloride concentrations in ground and surface waters in northern climates have been increasing for decades (Cassanelli & Robbins, 2013; Corsi, et al., 2015; Kaushal, et al., 2005; Mullaney, et al., 2009), with the most drastic increases found in urban watersheds, where deicing salt loading is highest. Although some mechanisms for chloride retention in soils exist (Bastviken, et al., 2006; Öberg, 2000), to date, no technique has been identified to prevent chlorides from getting into ground or surface waters. Reducing the mass of salt applied by using more efficient application methods seems to be the only practice to date aimed at minimizing the ecological impact of deicing salts.

Although a low percentage of drinking water wells have had chloride concentrations above the maximum contaminant level of 250 mg/L set by the U.S. EPA (Mullaney et al., 2009), much higher concentrations of chloride have been found in shallow groundwater and small streams (Kaushal, et al., 2005). High chloride levels in these ecosystems have negative effects on aquatic life (Brady, 2012; Findlay & Kelly, 2011). Connecticut is particularly vulnerable to the effects of road salt applications due to the large amount of urban land in our state. Clearly, this problem is going to get worse before it gets better, as no reasonably cost-effective alternative to road salt exists that can be used on a broad scale.

This project was a laboratory experiment designed to test the potential of two materials to reduce chloride concentrations in water. The objectives of the project were to determine the effect of an anion exchange resin and bioretention media on Cl retention. The broader goal was to build on the results of this laboratory research to reduce the impacts of road salt on aquatic systems in areas where salt is heavily applied. If successful, the results of this research could lead to a material and method to reduce chloride concentrations in stormwater runoff. This would benefit the aquatic life in Connecticut's streams (or potentially waters in other cold regions), and could reduce the potential for contamination of drinking water supplies as well.

Materials/Procedures/Progress

This project was performed in the Hydrology Laboratory in the Klinck Building at the University of Connecticut. A completely randomized factorial design was used. The factors included road salt concentration (zero, low and high), and ratio of resin:bioretention media (three levels from 0 to 10% resin by weight). There were three replicates of each factor, for a total of 27 treatments (3 salt levels X 3 anion resin percentages X 3 replicates = 27).

The anion exchange resin (A464, purchased from Evoqua Water Technologies LLC) was mixed with bioretention media (approximately 60% coarse sand, 20% leaf compost, and 20% topsoil) and installed in pvc soil columns (10.2 cm diameter x 30.5 cm long). Two layers of fiberglass window screen were installed on the bottom of the columns to contain the media. Column location on the bench was chosen at random (Figure 1).



Figure 1. Experimental setup in laboratory.

Road salt (95-99% sodium chloride, obtained from the facilities department at the University of Connecticut) was dissolved in deionized water to levels approximating those expected in melt water from winter deicing. Approximately once per week a measured quantity of salt solution (500 mL, or 2.7 cm, which is roughly equivalent to weekly precipitation in Connecticut) was applied to the top of the column, and collected after it passed through the media/resin blend. Three different influent chloride concentrations were applied: zero, low

(average 1,074 mg/L Cl), and high (4,645 mg/L). The columns were allowed to drain freely in between leachings, to simulate unsaturated soil conditions. The chloride concentration in leachate was measured with an ion-specific probe using method 9212 (US EPA 1996), and pH was also measured using an Orion pH meter. Although not part of the original proposal, the color of the leachate was also measured using method 2120 (APHA 2012), as distinct color differences were noted for different treatments. The experiments were conducted from June through November 2015. A total of 22 leaching events were performed.

Results/Significance

In the preliminary laboratory investigation that led to this project, conductivity measurements were used to get an estimate of how well the resin and the resin combined with bioretention media would reduce salt levels. Initial findings showed lower conductivity measurements from bioretention media combined with resin, whereas the resin alone did not change the conductivity in influent water.

Chloride

The current laboratory experiment measured chloride concentrations in influent and effluent water. The bioretention media alone with no resin added reduced influent chloride concentrations slightly, however by the fourth leaching (both high and low salt), effluent chloride concentrations were at or above influent levels (Figure 2). These findings indicate some storage of chloride in the media, presumably in the organic matter present (compost is a component of the media). Interestingly, the media with no resin and no salt showed measurable concentrations of chloride for the first two leachings, but concentrations dropped to near zero after that (Figure 2). It is not clear where this chloride came from.

In the treatments with resin included, the 10% resin/bioretention media blend was able to completely remove low concentrations of chloride, however breakthrough occurred after 5 leachings in the high salt application (Figure 3). The 100% resin treatment removed all of the chloride applied, even in the high salt application. This finding was in contrast to our preliminary investigation on the resin alone, where conductivity was measured instead of chloride. The likely explanation for this finding is due to the retention mechanism in the resin: the resin has hydroxyl (OH⁻) anions on exchange sites that are displaced by chloride (Cl⁻) anions. Therefore, when Cl is removed from water in contact with the resin, OH concentrations in that water increase proportionally. This is likely why the conductivity remained essentially the same in preliminary findings. When only Cl is examined, it is clear that the resin retains it quite readily, even at high concentrations. Interestingly, some white crystals began to form around the base of the columns with resin present after several leachings (Figure 4). It is presumed that this is sodium hydroxide, formed when dissociated sodium (Na) in influent water combined with free OH to form NaOH.

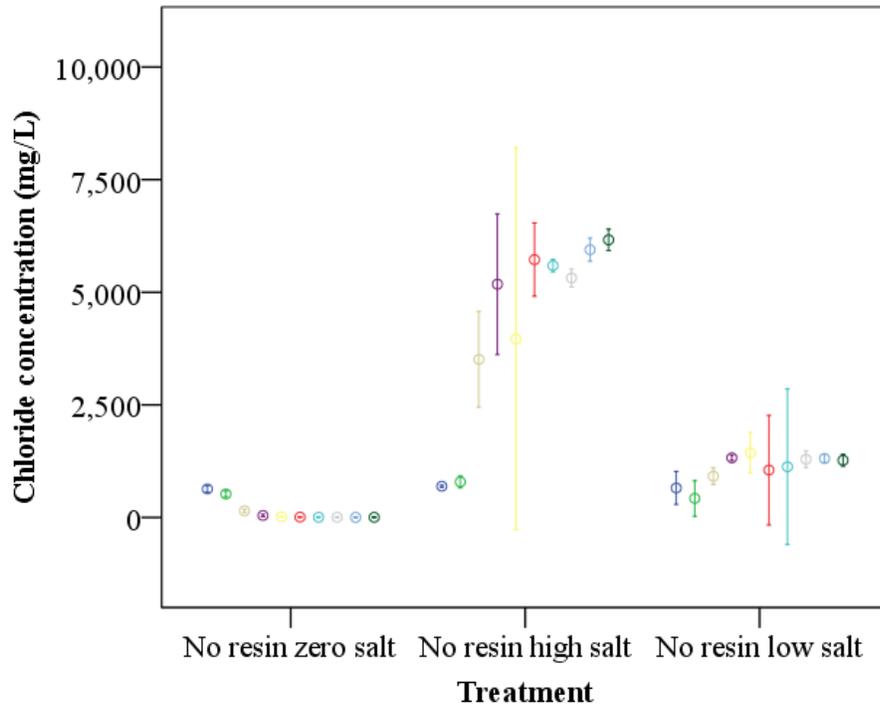


Figure 2. Chloride concentrations for ten successive leachings for different salt treatments with no resin. Error bars represent 95% confidence intervals.

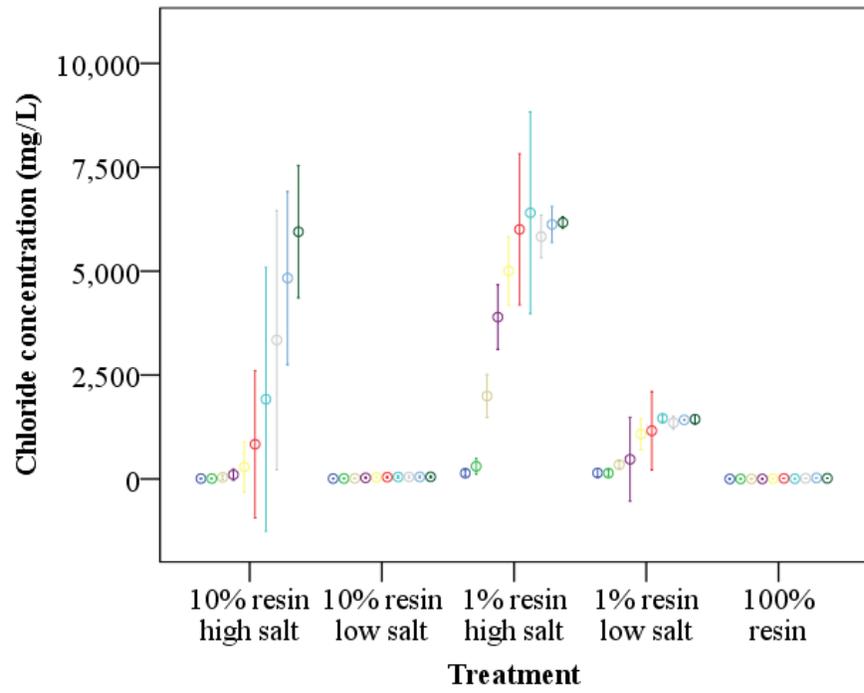


Figure 3. Chloride concentrations for 10 successive leachings for different salt and resin treatments. Error bars represent 95% confidence intervals.



Figure 4. Crystals forming around base of columns with resin present, presumed to be NaOH.

Due to the finding that the 1% resin treatment did not appear to have much impact, it was dropped from further analysis. Additional leachings were performed on the 10% resin until breakthrough occurred (Figure 5). After a number of leachings, an “extreme” salt solution (average 6,746 mg/L Cl) was applied to the 100% resin (Figure 6). The 100% resin retained all of the applied Cl through the duration of the study; additional leachings were performed with very high (~15,000 mg/L Cl) to force breakthrough. The total mass of Cl applied to the 100% resin treatments was 68.9 g Cl; the mass of the resin was 1,150 which results in roughly 6% retention by weight. However, breakthrough was observed in the 10% resin treatments after 4 leachings. In the 10% resin treatments, 205 g of resin was included in the mixture, and 9.2 g of

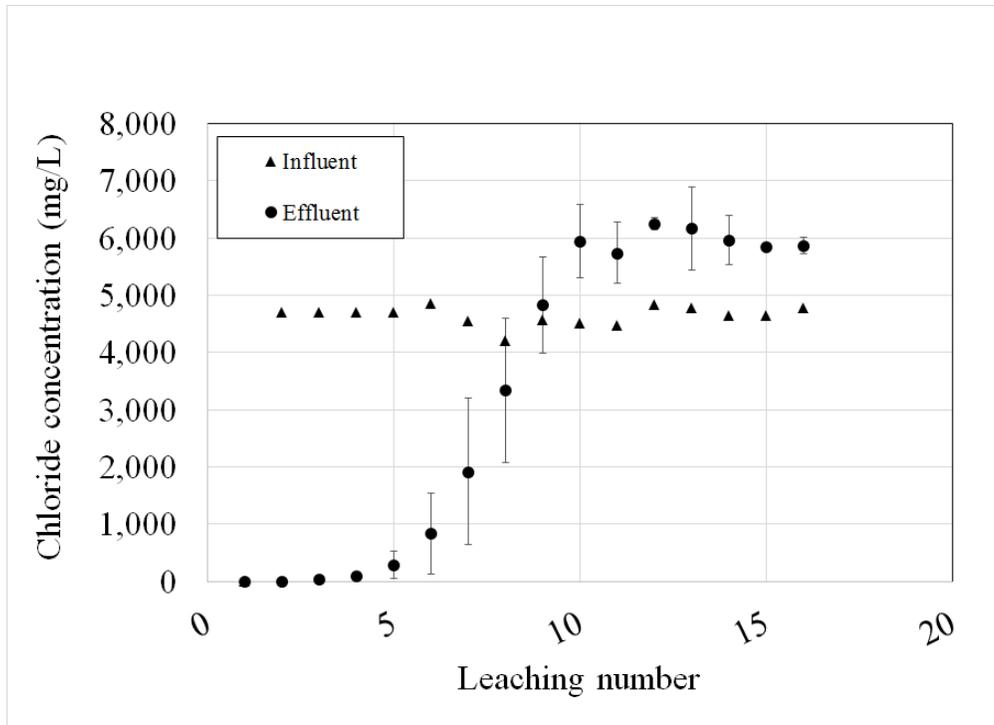


Figure 5. Influent and effluent chloride concentrations for the 10% resin/bioretention media blend. Error bars represent ± 1 standard deviation.

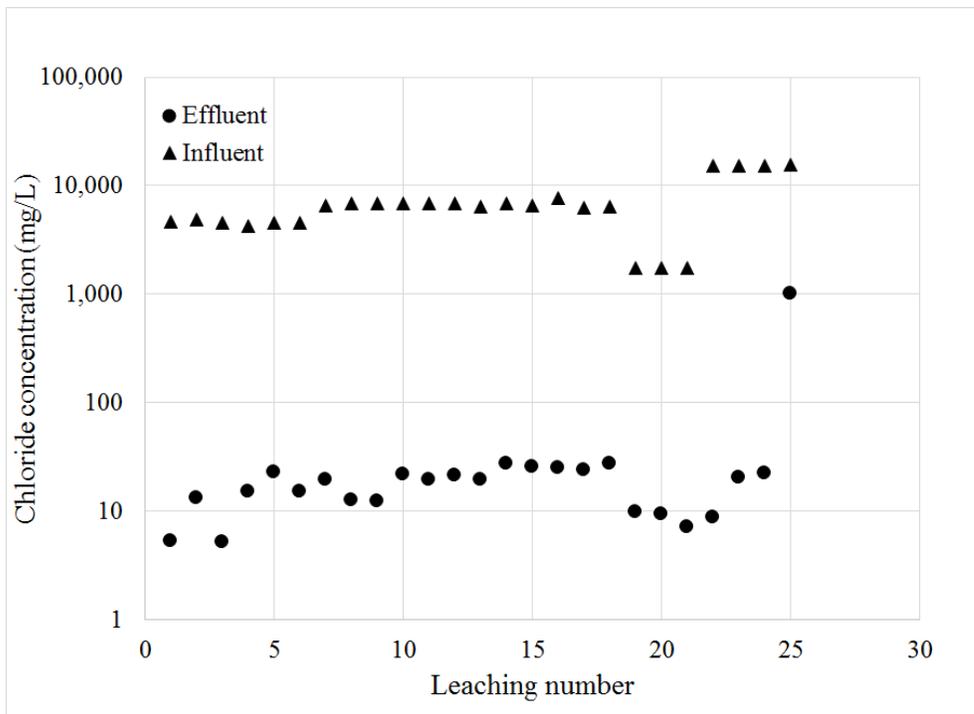


Figure 6. Influent and effluent chloride concentrations for 100% resin.

Cl was fully retained. Therefore, 9.2 g of Cl was retained per 205 g of resin, or roughly 4.4%. It is not clear why breakthrough in the 100% resin was 6% instead of 4.4%; perhaps there is some confounding effect from the bioretention media.

pH

pH of leachate from columns with zero resin/zero salt treatments averaged between 7 – 8, whereas treatments with resin and salt application were much higher (Figure 7). Leachate from the zero resin control was significantly lower than the 10% resin high salt ($p < 0.01$) and the 10% resin low salt ($p < 0.05$) treatments. As discussed earlier, the excess OH anions displaced from the resin would raise the pH of the leachate, so these findings are not surprising. Leachate from the 100% resin treatment (not shown on graph) was consistently above pH 12, and was significantly higher ($p < 0.001$) than any other treatment.

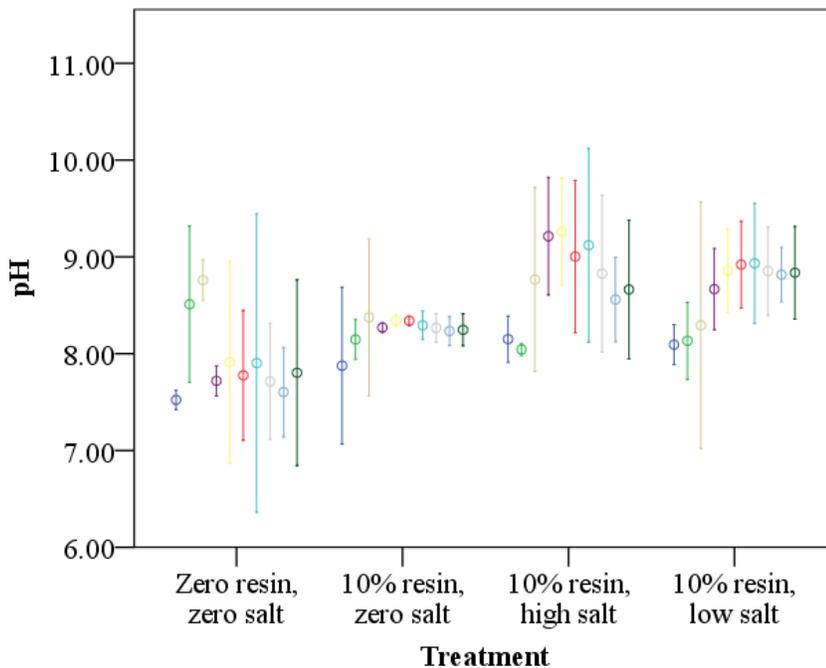


Figure 7. Leachate pH in 10 successive leachings. Error bars represent 95% confidence intervals.

Color

During the experiment, substantial differences in the color of the leachate were noted (Figure 8). As noted in the methods, color analysis was not part of the original proposal, however when the extreme color differences in leachate were noted, color analysis was performed. Some coloration was noted in the columns with no salt applied (both no resin and 10% resin treatments) initially, however color lightened over the course six analyses (Figure 9). Very dark color was noted on the 10% resin/high salt columns, and moderately dark color was noted on the

10% resin/low salt columns (Figure 9). It is suspected that the high pH generated by the OH anions caused leaching of humic molecules from organic matter in the media. Leaching of humic and fulvic acids has been noted in waters with high pH (Christman & Ghassemi, 1966). The effect moderated after six leachings, presumably after the organic matter pool became exhausted.



Figure 8. Color differences in leachate from different treatments.

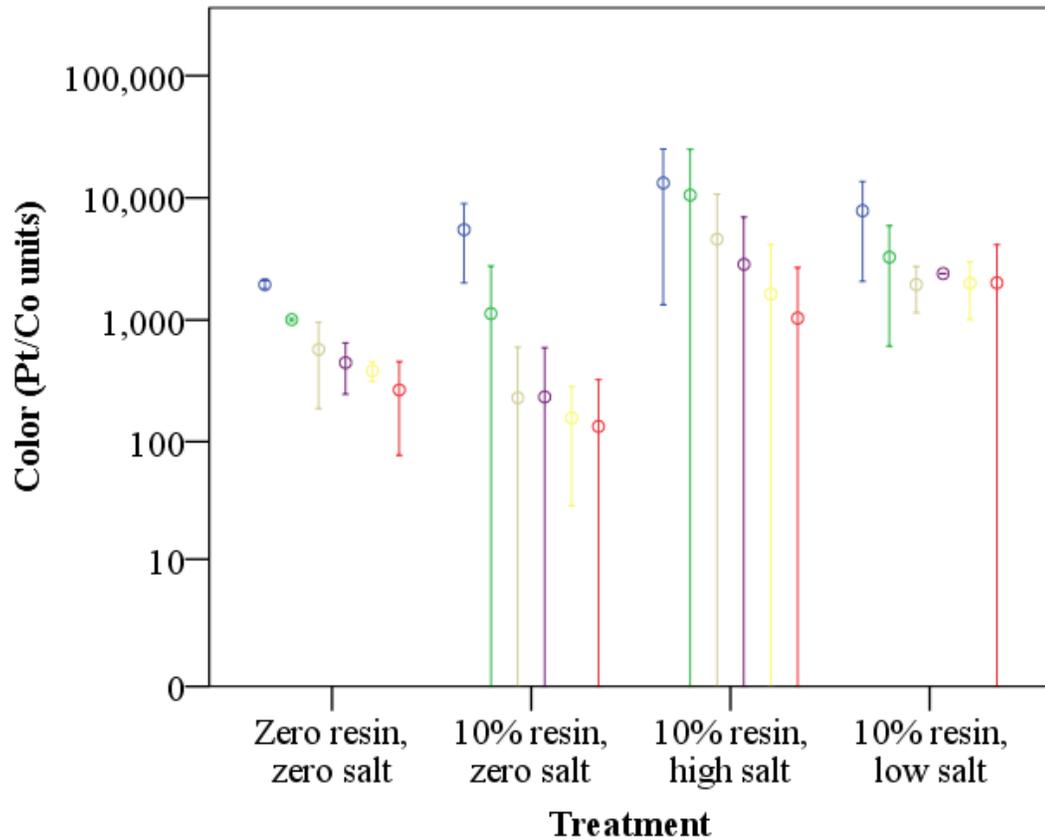


Figure 9. Leachate color of selected treatments.

Conclusions

Bioretention media and an anion exchange resin were leached with different concentrations of road salt. Although preliminary investigations prior to this study indicated an interaction effect between the resin and the bioretention media, the laboratory research associated with this study has indicated that the resin is the dominant chloride capture mechanism. The resin alone was able to retain very high (up to ~15,000 mg/L) concentrations of chloride, and 6% of applied chloride was retained before breakthrough occurred. The pH of water passing through the resin treatments was increased due to the release of OH ions from the resin. Unexpected changes in the color of leachate among treatments was also documented; it is surmised that the high pH increased the loss of humic and fulvic acids from the organic matter in the treatments which had bioretention media.

The anion exchange resin investigated in this study performed unexpectedly well in retaining high concentrations of chloride. The resin could be a promising tool in addressing the ongoing issue of excess road salt loading to surface and ground waters. Testing of the resin in a field application is the next step in determining if this is a viable solution.

Literature Cited

- APHA. 2012. Standard methods for the examination of water and wastewater. American Public Health Association; American Water Works Association; Water Environment Federation. Eds. Rice, E.W., Baird, R.B., Eaton, A.D., and Clesceri, L.S. Port City Press, Baltimore, MD.
- Bastviken, D., Sandén, P., Svensson, T., Ståhlberg, C., Magounakis, M., and Öberg, G. 2006. Chloride retention and release in a boreal forest soil: effects of soil water residence time and nitrogen on chloride loads. *Environmental Science and Technology*. Vol. 40(9), pp. 2977-2982.
- Brady, S.P. 2012. Road to evolution? Local adaptation to road adjacency in an amphibian (*Ambystoma maculatum*). *Scientific Reports*. Vol. 2, pp. 1-5.
- Cassanelli, J.P. and Robbins, G.A. 2013. Effects of road salt on Connecticut's groundwater: a statewide centennial perspective. *Journal of Environmental Quality*. Vol. 42, pp. 737-748.
- Christman, R.F., and Ghassemi, M. 1966. Chemical nature of organic color in water. *American Water Works Association*, Vol. 58(6), pp. 723-741.
- Corsi, S.R., DeCicco, L.A., Lutz, M.A., Hirsch, R.M. 2015. River chloride trends in snow-affected urban watersheds: increasing concentrations outpace urban growth rate and are common among all seasons. *Science of the Total Environment*, Vol. 508, pp. 488-497.
- Findlay, S.E.G. and Kelly, V.R. 2011. Emerging indirect and long-term road salts effects on ecosystems. *Annals of the New York Academy of Sciences*. Vol. 1223, pp. 58-68.
- Fitch, G.M., Bartelt-Hunt, S., and Smith, J.A. 2005. Characterization and environmental management of storm water runoff from road salt storage facilities. *Transportation Research Record: Journal of the Transportation Research Board*. No. 1911, pp. 125-132.
- Kaushal, S.S., Groffman, P.M. Likens, G.E., Belt, K.T., Stack, W.P., Kelly, V.R., Band, L.E., and Fisher, G.T. 2005. Increased salinization of fresh water in the northeast United

States. *Proceedings of the National Academy of Sciences*, Vol. 102(38), pp. 13517-13520.

Mullaney, J.R., Lorenz, D.L., and Arntson, A.D. 2009. Chloride in groundwater and surface water areas underlain by the glacial aquifer system, northern United States. United States Geological Survey Scientific Investigations Report 2009-5086. U.S. Department of the Interior and U.S. Geological Survey.

Öberg, G.M. 2003. The Handbook of Environmental Chemistry Vol. 3, Part P: 43–62. DOI 10.1007/b 10447

US EPA. (1996) *Test Methods for Evaluating Solid Wastes Physical/Chemical Methods*. EPA SW-846 Third Edition, United States Environmental Protection Agency Office of Solid Waste: Economic, Methods, and Risk Analysis Division. Washington, D.C.

Internally-Calibrated Passive Samplers for Water Quality Assessment of Pharmaceuticals and Other Organic Compounds of Wastewater Origin

Basic Information

Title:	Internally-Calibrated Passive Samplers for Water Quality Assessment of Pharmaceuticals and Other Organic Compounds of Wastewater Origin
Project Number:	2015CT294B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	CT 2nd
Research Category:	Water Quality
Focus Category:	Water Quality, Methods, Solute Transport
Descriptors:	None
Principal Investigators:	Allison Mackay

Publications

There are no publications.

Internally-Calibrated Passive Samplers for Water Quality Assessment of Pharmaceuticals and Other Organic Compounds of Wastewater Origin

Allison MacKay, PhD
University of Connecticut
Department of Civil and Environmental Engineering

May 19, 2016

Introduction

Assessments of the fate, and ultimate biological and human exposures, of pharmaceuticals and other organic compounds that originate from municipal or agricultural wastewater discharges are limited by the expense of sample analyses. Many municipal wastewater treatment plants in Connecticut discharge to small streams and rivers such that treated wastewater constitutes a significant fraction (10 – 30 %) of total flow. Nationwide, fractional contributions of greater than 10 percent treated effluent in river systems are characteristic of about one quarter of the discharge points under average flow conditions and up to two thirds under low flow conditions (*e.g.* 7Q10) (Brooks *et al.*, 2006). Pharmaceuticals and other compounds of wastewater origin are of concern because of their potential biological activity, including endocrine disruption; however, few studies have documented their subsequent degradation rates or phase transfer processes following release to aquatic systems. Most environmental reports of pharmaceuticals and other organic compounds of wastewater origin are point observations obtained through grab samples. More detailed evaluations of compound loss mechanisms require multi-observation time series that are not feasible given the \$200-\$800 price per sample for compound analyses. Thus, there is a need to develop robust, yet cost-effective, sampling methodologies that provide insights into the environmental system dynamics of pharmaceuticals and other organic compounds of wastewater origin.

Passive samplers offer an alternative to grab samples that overcome some of the limitations of grab samples, including detection limits, episodic concentration changes and cost for long time-series sampling (Morin *et al.*, 2012). Passive samplers are deployed for an extended period of time (days to weeks) to take up analytes of interest from the contacting water. Mass uptake in the sampler is quantified through a less-labor intensive extraction step than utilized in conventional grab samples. The mass uptake (M , ng) is then converted to an effective aqueous concentration by normalizing to a compound-specific mass uptake rate (k , L h⁻¹) multiplied by the deployment time (t , h), yielding a time-weighted average concentration (C_w , ng L⁻¹):

$$C_w = M \cdot k^{-1} \cdot t^{-1} \quad (1)$$

Time-averaged concentrations are considered more representative of endpoint biological exposures than point observations of CWO concentrations (Morin *et al.*, 2012). Passive sampler deployment can also improve upon detection limits, thereby reducing costs associated with analyzing samples that ultimately are below the detection limit. A larger sample mass uptake can be obtained through a longer deployment period than for a single 500 – 1000 mL water sample. Monitoring of time-weighted concentrations smooths episodic changes in CWO concentrations that could result in missing ‘peaks’ with grab sampling (Morin *et al.*, 2012).

The most widely applied passive samplers for CWO are Polar Organic Compound Integrative Samplers (POCIS) developed by Alvarez *et al* (2004). POCIS contain a layer of high-polarity sorbent resin (as used for conventional water extraction) sandwiched between two membrane filters (Fig. 1 (a)). The specialized resin allows for the uptake of very polar and/or charged CWO; however, the mass uptake rates must be derived empirically from extensive laboratory calibrations that are compound-specific and quite sensitive to flow conditions (Fig. 1(a)).

Jones and collaborators recently proposed an alternative to the POCIS sampler design that integrates a fundamental parameter (diffusion coefficient) into the mass uptake rate (Chen *et al.*, 2012). The thin film diffusive gradient (DGT) passive sampler for CWO contains a layer of high polarity sorbent resin overlaid with a porous gel layer (Fig. 1(b)). Because the limiting mass transfer step is diffusion across the porous gel layer (Fig. 1(b)), the mass uptake rate is constant for a particular compound:

$$k_{DGT} = D_{DGT} \cdot A \cdot \Delta x^{-1} \cdot 10^{-3} \quad (2)$$

where D_{DGT} ($\text{cm}^2 \text{h}^{-1}$) is the compound-specific diffusion coefficient in the gel layer, A (cm^2) is the exposed area of the sampler, Δx (cm) is the thickness of the gel layer, and 10^{-3} (L cm^3) is a unit conversion. As a result, the DGT sampler design has very low sensitivity to variations in flow conditions during deployment (Chen *et al.*, 2013). Importantly, compound-specific D_{DGT} values for a large suite of CWO can be derived from a small number of experimentally measured values by applying a molecular weight scaling model (Zhang and Davison, 1999):

$$D_{DGT} = 3 \times 10^{-5} \cdot \Theta^2 \cdot MW^{-1/3}$$

(3)

where Θ is the gel porosity, MW is the compound molecular weight. Initial field deployments showed good agreement between DGT estimates of time-weighted CWO concentrations in water samples and observations from 24-hour composite sampling (Chen *et al.*, 2013).

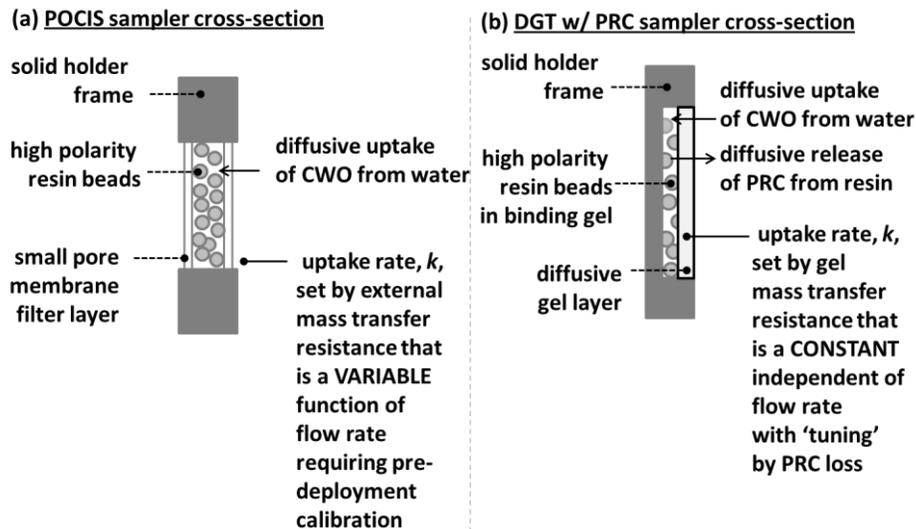


Figure 1. Schematic cross-section profiles of passive samplers noting the location of limiting mass transfer steps and the consequent influence on the sampler uptake rate, k , that is critical to calculating time-averaged aqueous concentrations (Eq. 1). Sampler length 5-8 cm.

Rc

The objective of this project is to develop a passive sampling device that will provide an integrated assessment of pharmaceuticals and other compounds of wastewater origin in water bodies so that low concentration samples can be quantified with lesser effective expense.

Specific tasks to develop such a sampling device include:

- i. Assess the use of internal standards to calibrate for flow variations across sampling device deployments.
- ii. Evaluate the sensitivity of sampling devices to temporal variations in input mass fluxes of compounds.
- iii. Measure environmental loss rates of compounds in streams receiving modest inputs of treated municipal wastewater.

At the time of report preparation, progress has only been made toward completion of Task (i). Other tasks will be completed during summer 2016.

Methods

Experiments were performed to characterize the components of the DGT sampler by measuring diffusion coefficients in the agarose material from which the gel layer was cast and binding coefficients for the sorbent resin. Target compounds were chosen to be complementary to parallel NSF-funded research examining photodegradation of pharmaceutical compounds, downstream of wastewater discharge locations (Bodhipaksha *et al.* 2016). Compounds are commonly observed in treated municipal wastewater discharges and each has a unique degradation mechanism when exposed to sunlight – sulfamethoxazole (SMX) and sulfadimethoxine (SDM) both degrade by direct photolysis, cimetidine (CMT) reacts with sunlight-produced singlet oxygen, and caffeine (CAF) reacts with sunlight-produced hydroxyl radicals. Additionally, these compounds all exhibited different speciation characteristics under the circumneutral pH conditions expected for many river systems. Sulfamethoxazole and sulfadimethoxine are both zwitterionic compounds with both positive and negative charge, cimetidine is positively charged and caffeine is neutral.

Chemicals. Sulfamethoxazole, sulfadimethoxine, caffeine and cimetidine (all $\geq 99\%$ purity) were purchased from Sigma-Aldrich (US). Agarose powder (3:1) was obtained from Nusieve. Acetonitrile (HPLC grade) was from Acros Organics. High purity water (18 M Ω -cm) was used for all experiments and obtained from a Milli-Q system (Waters). HLB Max anion exchange resin was obtained from Waters.

Gel preparation. Agarose gel was prepared by adding an appropriate mass of agarose powder to pre-heated high purity water to a final concentration of 1.5 % agarose by weight. The solution was mixed in a boiling water bath until the solution was clear. The solution was then poured onto a glass plate. Spacers of thickness 0.5 mm were placed on the corners of the glass plate and the gel mixture was compressed to 0.5 mm thickness by placing a second glass plate overtop of the spacers. The gel was allowed to firm and then stored in a high purity water bath to keep it hydrated and to avoid any tearing.

Diffusion cell. A diffusion cell was used to measure the transport of compounds through the gel material. The diffusion cell consisted of two glass reservoirs that contacted opposite sides of a holder containing a sample of the gel (Fig. 1). The effective diameter of the gel material contacting the two sides was 1.5 cm. A circular piece of agarose gel was cut from a gel sheet using a custom-designed punch. The gel section was then placed in a holder that was clamped between the two reservoirs. One of the reservoirs was filled with 100 mL of solution containing a 1×10^{-4}

M concentration of the compound of interest. The other reservoir was filled with 100 mL of high purity water. Solutions were adjusted to a pH of 7 ± 0.3 and held at room temperature (22 ± 2 °C). The system was held in darkness to avoid compound photodegradation. Subsamples were collected every 3 hours for a period of 3 to 5 days, depending on time to equilibrium.

Analysis. Pharmaceutical compound concentrations were quantified using high performance liquid chromatography (HPLC) (HP 1050, Agilent) with a C₁₈ reverse phase column (Licosphere) and an acetonitrile (30%)-water (70%) eluent. Compounds were detected at a wavelength of 225 nm and concentrations were quantified with an external calibration curve.

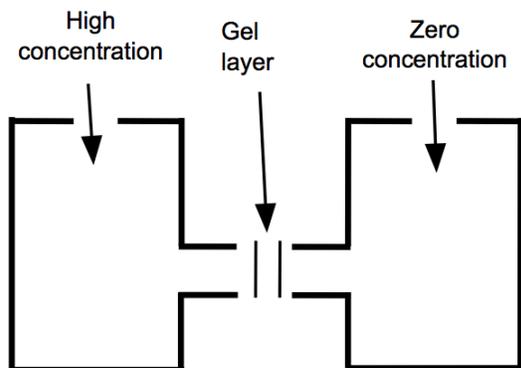


Fig. 1 Diagram of diffusion cell used to obtain data for compound diffusion through the agarose gel material. Compounds moved from left to right in response to a diffusion gradient from high concentration (1×10^{-4} M) to zero concentration (high purity water).

Diffusion coefficient calculations. Diffusion coefficients for compound transport across the agarose gel were obtained by fitting compound concentrations as a function of time (Crank, 1956):

$$C(t) = C(\infty) + (C(0) - C(\infty))e^{(-\frac{D \cdot A \cdot t}{V \cdot \Delta g})} \quad (1)$$

where C (M) is the concentration on the ‘high concentration’ side as defined in Fig. 1, $C(\infty)$ (M) is the concentration at equilibrium (equal to one half of the initial concentration), $C(0)$ (M) is the initial concentration at time zero, D (cm^2/s) is the diffusion coefficient, A (cm^2) is the exposed gel area between the two reservoirs, t (s) is the elapsed time at subsample collection, V (cm^3) is the volume of the ‘zero concentration’ reservoir as defined by Fig. 1m and Δg (cm) is the thickness of gel layer.

Sorbent Resin Binding. Compound sorption to HLB Max resin was quantified using a column chromatography method (Jolin *et al.* 2016) with a high performance chromatography system. A small dimension (30-mm length, 2.1-mm diameter, Restek) column was packed with a mixture of resin and the inert solid silicon carbide. A second column was packed with silicon carbide to confirm that compounds had no interactions with this material. Uracil was used as a non-interacting tracer to measure transport times through the column system. Flow of a 5 mM CaCl₂ solution (to mimic river background ionic strength) was initiated at a rate of 100 $\mu\text{L}/\text{min}$.

Results and Discussion

Diffusion Coefficients. The mass transport of the neutral compound, caffeine, across the agar gel was first examined for comparison to previously published results. Experimental runs were monitored seven days until equilibrium was reached between both of the reservoirs, as evaluated by final concentrations in both of the reservoirs being half of the starting concentration in the initial ‘high concentration’ reservoir (Fig. 1). The resultant diffusion coefficient was $1.5 \times$

$10^{-5} \text{ cm}^2/\text{s}$ and similar to a previously observed value of $4 \times 10^{-5} \text{ cm}^2/\text{s}$ for caffeine diffusion through agar gel (McCabe 1972). A lower measured than reported caffeine diffusion coefficient could indicate that the gel layer was somewhat thicker than the targeted thickness of 0.5 mm from the spacers used herein, or because caffeine interacts with the agarose gel material as it diffuses through the gel layer. The latter explanation was excluded after conducting batch sorption experiments in which the concentration of caffeine in a solution was contacted with pieces of agarose gel material in well-mixed test tubes and found not to change over a four-day period.

The reproducibility of the gel casting method was evaluated by examining the sulfamethoxazole diffusion across gel material subsamples. First, differences in gel thickness across the same cast were examined by comparing data for SMX-1 and SMX-2 in Fig. 2. Although the trends in concentration with time look similar between the two trials, fits of the data with Eq. 1 indicated the diffusion coefficients to vary by 30% (Tab. 1). These differences indicate variations in the thickness of the gel layers, as a constant value of Δg was assumed in calculating the diffusion coefficients. A second gel cast (SMX-3) showed the range of diffusion coefficients to be similar between replicate gel sheets (Fig. 2, Tab. 1). Together these results highlight the importance of a performance reference compound to be incorporated into the sampler to account for differences in gel layer thickness between subsamples that may be up to 30 percent.

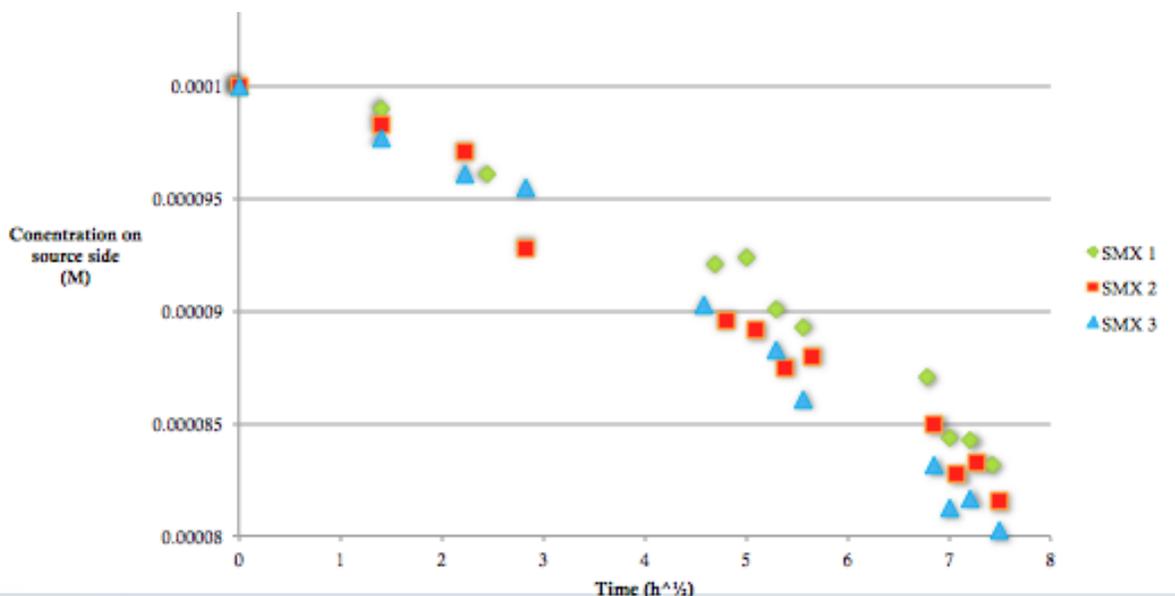


Fig. 2. Sulfamethoxazole transport from the ‘high concentration’ reservoir for duplicate subsamples of a single gel sheet (SMX-1 green diamonds, SMX-2 red squares) and from a duplicate gel sheet (SMX-3).

Tab. 1. Compound diffusion coefficients obtained by fitting Eq. 1 to the changing compound concentrations in the ‘high concentration’ reservoir (Fig. 1) over time.

Compound	Mixture	Diffusion Coefficient (cm ² /s)
Sulfamethoxazole	No	SMX-1: 3.4×10^{-5} SMX-2: 4.5×10^{-5} SMX-3: 4.9×10^{-5}
	Yes	3.6×10^{-5}
Cimetidine	No	4.9×10^{-5}
	Yes	7.0×10^{-5}
Sulfadimethoxine	Yes	4.1×10^{-5}

The presence of multiple compounds was also confirmed to have no effect on the diffusion of a single compound through the gel layer. The change in cimetidine concentration over time showed little effect from the presence of the other compounds (CMT Mix, Fig. 3), compared to cimetidine alone (CMT, Fig. 3). Fitted diffusion coefficients were within the variation observed for replicate gel sections (Tab. 1). Overall, diffusion coefficients through the

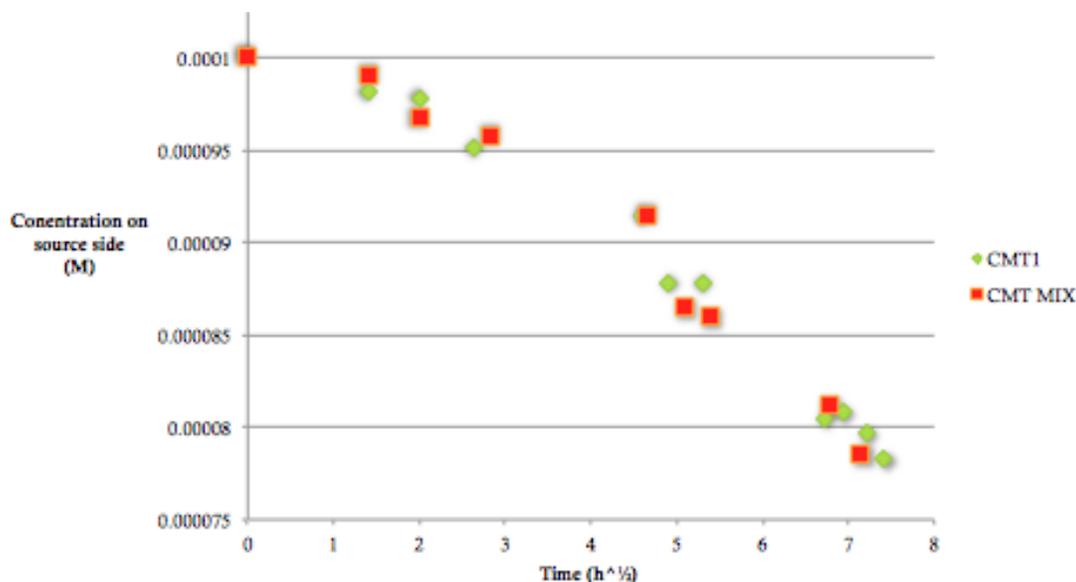


Fig. 3. Comparison of concentration changes for cimetidine diffusion through the gel media as a single solute (green diamonds) and in the presence of a mixture of cimetidine, sulfamethoxazole, sulfadimethoxine, all present initially at 1×10^{-4} M (red squares).

Column Chromatography to Measure Compound Binding to Sorbent Resin. Initial evaluations were performed to assess whether column chromatography can be used as a high throughput approach for measuring binding coefficients of compounds to HLB Max anion exchange resin. First, silicon carbide was determined to be an inert diluent for mixing with sorbent resin beads. The retention time of sulfamethoxazole transport through a column packed with silicon carbide was only slightly greater than for the compound uracil which is known not to interact with sorbent media (Tab. 2). Sulfamethoxazole was retained on a column containing HBL Max anion exchange resin, compared to uracil.

Tab. 2. Compound retention times on chromatography columns.

Column	Compound	Time to center of peak
Silicon Carbide	Uracil	2.24
Silicon Carbide	Sulfamethoxazole	2.91
HLB Max resin	Uracil	2.37
HLB Max resin	Sulfamethoxazole	11.25

Work is continuing to replicate results in Tab. 2 and to obtain retention times for the other compounds of interest so that their binding to sorbent resin is well-characterized before sampler construction.

References

- Alvarez, D. A.; Petty, J. D.; Huckins, J. N.; Jones-Lepp, T. L.; Getting, J. P., Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. *Environ. Toxicol. Chem.* 2004, 23, 1640-1648.
- Bodhipaksha, L.C., Sharpless, C.M., Chin, Y. P., MacKay, A.A., Role of effluent organic matter in the photodegradation of compounds of wastewater origin, *Water Research*, 2016 *in review*.
- Brooks, B. W.; Riley, T. M.; Taylor, R. D., Water quality of effluent-dominated ecosystems: ecotoxicological, hydrological, and management considerations. *Hydrobiologia* 2006, 556, 365-379.
- Chen, C.-E.; Zhang, H.; Jones, K. C., A novel passive sampler for in situ sampling of antibiotics. *J. Environ. Monit.* 2012, 14, 1523-1530.
- Chen, C.-E.; Zhang, H.; Ying, G.-G.; Jones, K. C., Evidence and recommendations to support the use of a novel passive water sampler to quantify antibiotics in wastewaters. *Environmental Science and Technology* 2013, 47, 13587-13593.
- Crank, J. *The Mathematics of Diffusion*, Oxford Science Publications, Oxford, UK, 1956.
- Jolin, W.C.; Sullivan, J.; Vasudevan, D.; MacKay, A.A., Column chromatography to obtain organic cation sorption isotherms. *Environ Sci Technol*, 2016 *in review*.
- McCabe, M., The diffusion coefficient of caffeine through agar gels containing a hyaluronic acid–protein complex. A model system for the study of the permeability of connective tissues. *Biochemical Journal*, 1972, 127, 249-253.

Morin, N.; Miede, C.; Randon, J.; Coquery, M., Chemical calibration, performance, validation and applications of the polar organic chemical integrative sampler (POCIS) in aquatic environments. *Trends in Analytical Chemistry* 2012, 36, 144-175.

Zhang, H.; Davison, W., Diffusional characteristics of hydrogels used in DGT and DET techniques. *Analytica Chimica Acta* 1999, 398, 329-340.

Information Transfer Program Introduction

The general purpose of the Connecticut Institute of Water Resources information transfer program is to support a number of ongoing educational efforts regarding water resources. These efforts include a seminar series, conferences, educational information and web site development and maintenance; as well as specifically funded special projects and publications implemented as the need arises. Although during this past reporting period the Institute did not request funds specifically for information transfer purposes, we continued to work on improving our information transfer capabilities and develop new information.

Web Site: Our Institute maintains the CTIWR web site, which we update as needed. It includes information about the WRI program, our Institute and its Advisory Board members, a listing of the current year's seminars, a list of sponsored projects and publications, and access to electronic copies of our "Special Reports" series. We also use the web to announce special events and our RFP in addition to secure access to grant proposals and information for the Advisory Board's review. We continue to cooperate with the University of Connecticut's digital archives department, which maintains our electronic reports as a part of its "Digital Commons @ University of Connecticut" project. This past year we have worked on making available through the Publications area of the CTIWR website the results of previously funded research projects in digital format. We have scanned into searchable PDF format documents nearly 100 reports that were stored in the Institute archives that date back to the 1960s. We are working on getting these online so they are easily available to the public.

Digital Media Applications. We have started development of storyboards related to infiltration mechanism as impacted by land use and land treatment. The storyboards include the infiltration and runoff for various rainfall rates as calculated by the Green-Ampt infiltration equation. The changes in infiltration, runoff, soil water content and depth to water table change are graphically illustrated as a storm progresses. The next steps include animation of the storyboards that will serve as an educational tool to help the public understand basic hydrologic concepts and the role that land use and treatment play in runoff.

Conferences. The Institute co-sponsored the annual Connecticut Conference on Natural Resources (CCNR) held each March during spring break recess at the University of Connecticut. CTIWR contributes \$500 to support the conference.

Service and Liaison Work. Currently, the Director actively serves on the following water related panels, committees or workgroups: • Participant, CT Water Planning Council Advisory Group (WPCAG). • Member, CT WPCAG, Drought Plan Workgroup • Member, CT Water Planning Council (WPC), Other State's Water Plans Workgroup • Member, Scientific and Technical Subcommittee of the Steering Committee, CT State Water Plan

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	12	0	0	0	12
Masters	3	0	0	0	3
Ph.D.	3	0	0	0	3
Post-Doc.	0	0	0	0	0
Total	18	0	0	0	18

Notable Awards and Achievements