Water Resources Center
Annual Technical Report
FY 2008

Introduction

The Minnesota WRRI program is a component of the University of Minnesota's Water Resources Center (WRC). The WRC is a collaborative enterprise involving several colleges across the University, including the College of Food, Agriculture and Natural Resource Sciences (CFANS), the University of Minnesota Extension, Minnesota Agricultural Experiment Station (MAES) and the University of Minnesota Graduate School. The WRC reports to the Dean of CFANS. In addition to its research and outreach programs, the WRC is also home to the Water Resources Sciences graduate major. The WRC has two co-directors, Professor Deborah Swackhamer and Faye Sleeper, who share the activities and responsibilities of administering its programs.

Research Program Introduction

The WRC funds 3-4 research projects each year, and the summaries of the current projects are found in the rest of this report.

Application of Wireless and Sensor Technologies for Urban Water Quality Management

Basic Information

Title:	Application of Wireless and Sensor Technologies for Urban Water Quality Management
Project Number:	2006MN187G
Start Date:	9/1/2006
End Date:	8/31/2009
Funding Source:	104G
Congressional District:	MN 5
Research Category:	Water Quality
Focus Category:	Nutrients, Surface Water, Non Point Pollution
Descriptors:	
Principal Investigators:	William Alan Arnold, Miki Hondzo, Raymond M Hozalski, Paige J Novak

Publication

1. Jazdzewski, J. 2007. Stream Water Quality Monitoring using Wireless Embedded Sensor Networks. M.S. Dissertation. Department of Civil Engineering, University of Minnesota – Twin Cities, MN. 44 pp.

Application of Wireless and Sensor Technologies for Urban Water Quality Management

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ABSTRACT

The water quality of streams draining watersheds has been degraded by increasing urbanization. The general symptoms of this degradation include more frequent large flow events, reduction in channel complexity, reduced retention of natural organic matter, and elevated concentrations of nutrients. Newly emerging urban water quality threats, including insecticides, herbicides, pharmaceuticals, and estrogens, are known or suspected to damage the health of humans and ecosystems. The restoration and management of streams have traditionally attempted to improve the hydrological and water quality conditions in-stream or in riparian zones. Recent studies have indicated the portion of a watershed covered by impervious surfaces and connected to the stream by stormwater drainage is the primary degrading process of stream ecology and health. These findings suggest that the sustainable restoration and management of stream water quality require quantification of hydrological, chemical, biological, and geomorphological processes, and that these processes must be assessed across a range of scales. Furthermore, interactions among biogeochemical processes across watersheds are either non-linear processes or linear processes dependent on non-linear drivers. The monitoring of such a system inherently requires a change in traditional field sampling strategies. We propose to transform traditional and very limited (in terms of spatial and temporal resolution) field measurements through the integration of multi-scale, spatially-dense, high frequency, real-time, and event-driven observations by a wireless network with embedded networked sensing.

The goals of the proposed research were to assess the benefits of stormwater best management practices in mitigating the pollutant loads from urban and peri-urban sources, to evaluate the effectiveness of traditional grab sampling in calculating pollutant loads, and to develop correlations to predict the concentrations of non-sensed chemical or biological pollutants. These goals were achieved by establishing a wireless sensor network capable of monitoring fundamental water quality parameters at high spatial and temporal resolution. It was hypothesized that sensed fundamental water quality parameters can be used for predicting the presence of emerging chemical contaminants in urban streams. It was also hypothesized that the water quality in streams draining similar impervious urban areas is controlled by the mean and variance of effective stormwater residence time. The mean and variance of water residence time, the time it takes urban runoff to travel between the impervious urban land and a receiving aquatic

body, was characterized by radio frequency identification technology (RFID), which augmented the proposed wireless network. Ultimately, data generated from such a monitoring network will enable mechanistically-based scaling and forecasting of water quality in urban streams and rivers. This will transform urban planning practices and management of water quality in streams draining urban land.

PROGRESS REPORT

In the spring through summer of 2008, the network was deployed in Shingle Creek to allow continuous monitoring of water quality and BMP performance. An additional six-week deployment in Minnehaha creek was done in the fall. The network consists of 5 stations, each equipped with a datalogger and water quality sensors. The sensors measure temperature, depth (used to calculate flow), pH, specific conductance, dissolved oxygen, and turbidity every minute. Nitrate is measured at either 30 minute or 2-hour intervals. All stations are equipped with radios and antennae. One station (the base station) also has a wireless cellular modem, which is used to communicate remotely with the entire network. The data collected is uploaded via the cell phone modem to our server every night. Data is available using the Hydrologic Information System (HIS) at http://his.safl.umn.edu/dash3. Grab samples for the target pesticides and fecal coliforms were also taken during dry periods and during rainfall events.

At the Shingle Creek site, we investigated the performance of ponds used as stormwater on the removal of nitrate and total suspended solids (TSS). We have found that the ponds effectively removed nitrate and TSS during rain events and during dry periods. The percentage removals were higher during a rainfall event (~90%) versus a dry period (~50%). The mechanism of removal appears to be denitrification in the sediments. A comparison of real-time data collection versus limited grab sampling was also made for the rainfall event and the dry period. The percentage removals calculated for limited grab sampling were within 8% of the removal calculated for continuous data collection. The actual mass loading, however, was twice as high for a grab sample collected during peak discharge versus the continuous data. During the dry period, the different sample collection times produced similar removal efficiencies and mass loading.

Sediment cores were taken from the stormwater pond, and sediment oxygen demand tests were performed on the cores. Oxygen-saturated pond water was applied to the sediment core, and dissolved oxygen levels were recorded continuously (every second). The sediment oxygen demand tests showed that the pond sediments consumed most of the oxygen in the overlying water within 1-3 minutes (Figure 1). On average, 67% of the oxygen was depleted within the first minute.

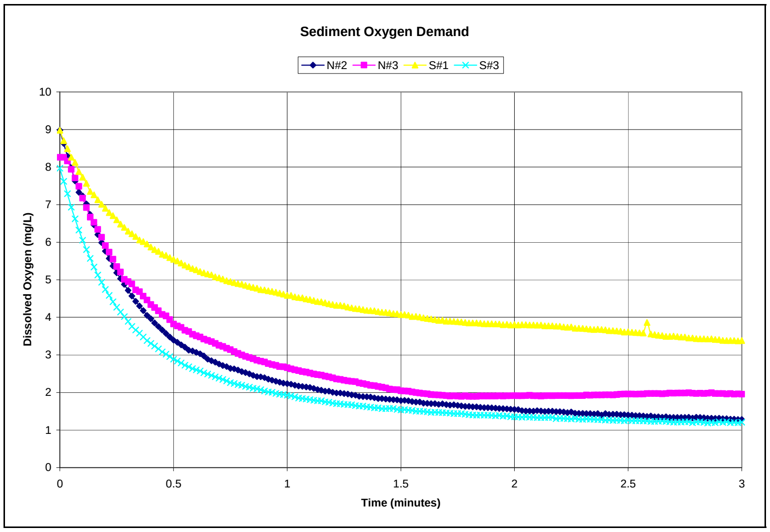


Figure 1: Sediment oxygen demand of stormwater pond sediment.

We have also used the continuous sensor data to evaluate the practice of using grab samples to determine total maximum daily loads of pollutants. It was concluded that the most effective way to determine pollutant-loading rates is through continuous monitoring. Alternative methods either are too labor intensive (daily sampling) or result in significant (> 50%) error (monthly and bi-weekly sampling) relative to continuous monitoring. As a partial result of urbanization and increased watershed imperviousness, pollutant fluxes within urban streams are highly variable. The majority (>90%) of pollutant loading rate occurs during the minority of the time (<20%). Additionally, over half of the 28-day load was discharged within a 4-day period beginning on August 28th. It is therefore probable that discrete sampling will under predict annual fluxes.

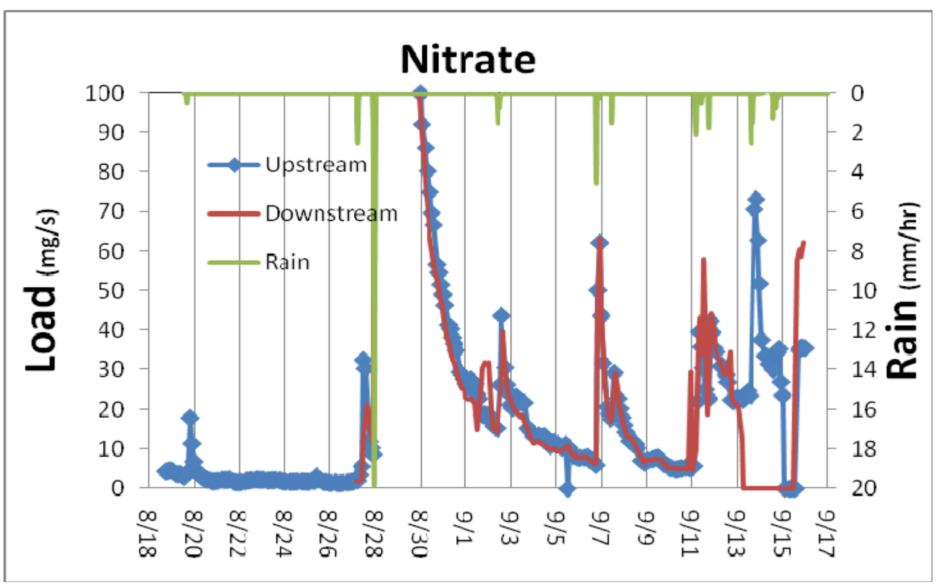
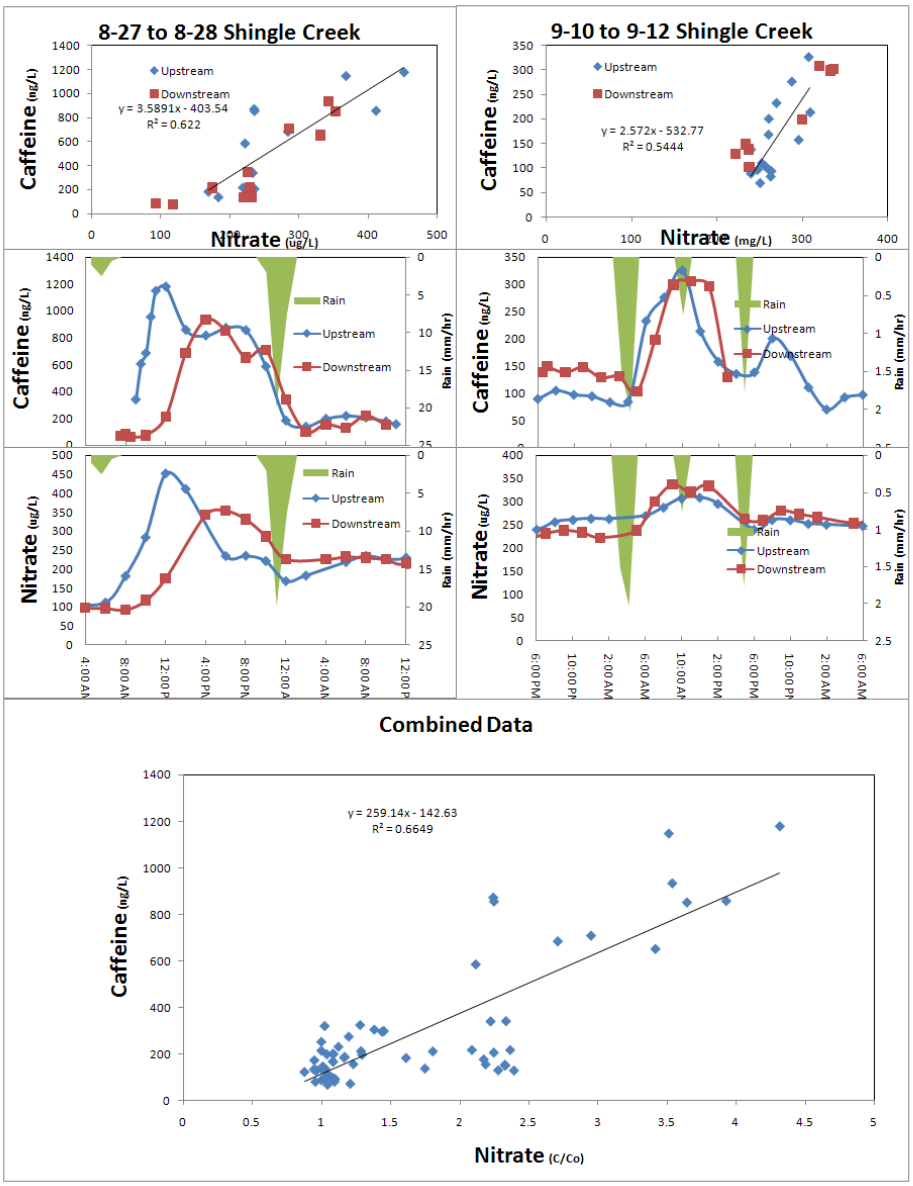


Figure 2: Nitrate loading results within Shingle Creek in Brooklyn Center, MN. Although, the range of load data fluctuated as high as 800-mg/s the displayed axis was limited to 100-mg/s. Upstream and downstream stations are relative to a stormwater input discharged from a series of retention ponds.

It was hypothesized that consistent relationships exist between easy-to-measure fundamental water quality parameters and difficult-to-measure organic and bacterial pollutants within urban streams. This was tested by collecting and analyzing grab samples for four-target analytes (atrazine, prometon, caffeine, and fecal coliforms) concurrently with seven-high frequency, near-real-time sensed water quality parameters. Within Shingle Creek, surrogate relationships were developed between nitrate and caffeine (C.D. = 0.68), turbidity and prometon (C.D. =0.91), and discharge and prometon (C.D. =0.92) and at the Minnehaha Creek location a relationship between caffeine and specific conductivity (C.D. =0.64) was developed. In turn, observations of bacterial and organic pollutants concentrations in near real-time can forecast potential human or ecological health hazards, give suppliers and municipalities timely information to adjust water treatment strategies, and can enhance pollutant loading calculations.



We have also had productive meetings with local watershed district and USGS personnel which have been helpful in interpreting data and planning future deployments.

PUBLICATIONS

Jazdzewski, J. 2007. Stream Water Quality Monitoring using Wireless Embedded Sensor Networks. M.S. Dissertation. Department of Civil Engineering, University of Minnesota – Twin Cities, MN. 44 pp.

Two M.S. theses are being prepared and will be defended in early June 2009. Two publications will be submitted to peer-reviewed journals by July 1, 2009.

PRESENTATIONS

Conference Presentations

Henjum, M.B., Wennen, C.R., Hondzo, M., Hozalski, R.M., Novak, P.J., Arnold, W.A. 2009. Linking Near Real-Time Water Quality Measurements to Fecal Coliforms and Trace Organic Pollutants in Urban Streams. Oral Presentation. 2009 Joint Assembly (AGU), Toronto, CA, 2009.

Kang, J.M., S. Shekhar, M. Henjum, P. Novak, W.A. Arnold. 2009. Discovering teleconnected flow anomalies: a relationship analysis of dynamic neighborhoods (RAD) approach. 11th International Symposium on Spatial and Temporal Databases, Aalborg, Denmark accepted. (**peer-reviewed)**

Kang, J.M., S.Shekhar, Wennen, C., Novak, P. 2008. Discovering Flow Anomalies: A SWEET Approach. In: IEEE International Conference on Data Mining. (2008) 851–856. (**peer-reviewed)**

Novak, P.J. 2009. Sensor Networks for Urban Water Quality Monitoring. Oral Presentation. Environmental Sciences: Water Gordon Research Conference, Plymouth, NH.

Wennen, C.R., Henjum, M.B, Hozalski, R.M., Novak, P.J., Arnold, W.A. 2008. Application of Wireless and Sensor Technologies for Urban Water Quality Management: Pollutant Loading in Stormwater Ponds. Oral Presentation. 2008 Minnesota Water Conference, St. Paul, MN.

Henjum, M., Wennen, C., Hondzo, M., Hozalski, R.M., Novak, P.J., Arnold, W.A. 2008. Application of Wireless and Sensor Technologies for Urban Water Quality Management: Pollutant Detection in Urban Streams. Oral Presentation. 2008 Minnesota Water Conference, St. Paul, MN.

Novak, P.J., J. Jazdzewski, S. Kim, W.A. Arnold, R.M. Hozalski, and M. Hondzo. 2007. Wireless Technologies and Embedded Networked Sensing for Urban Water Quality Management. Oral Presentation. Association of Environmental Engineering and Science Professors Education and Research Conference, Blacksburg, Virginia, July 2007.

Hozalski, R.M., Kim, S., Jazdzewski, J., Hondzo, M., Novak, P.J., and Arnold, W.A. 2007. Wireless Technologies and Embedded Networked Sensing: Application to Integrated Urban Water Quality Management. Oral Presentation. World Environmental and Water Resources Congress 2007, May 15-18, Tampa, FL.

Kim, S.-C.; Hondzo, M.; Hozalski, R.M.; Novak, P.; Arnold, W.; Jazdzewski, J.D.; Jindal, N.; Capel, P.D. 2006. Integrated urban water quality management: wireless technologies and embedded networked sensing. Poster Presentation. American Geophysical Union National Meeting, San Francisco, CA. December 2006.

Jazdzewski, J.D.; Hondzo M.; Arnold, W.A. 2006. Stream water quality monitoring using wireless embedded sensor networks. Poster Presentation. Minnesota Water 2006 and Annual Water Resources Joint Conference, Brooklyn Center, MN, October 24-25, 2006.

Other Presentations

Arnold, W.A. 2009. The WATERs Project: Wireless Sensor Technologies for Urban Water Quality Management. Oral Presentation. Urban Ecosystems Seminar Series, University of Minnesota, St. Paul, MN.

Hondzo, M., Arnold, W.A., Hozalski, R.M., Novak, P.J., Capel, P.D. 2006. Wireless Technologies and Embedded Network Sensing: Options for Environmental Field Facilities. Oral Presentation. International Research and Education Planning Visit: Cyberinfrastructure based water research: towards the next generation of environmental observatories. August 31- September 1 Delft, The Netherlands and September 2-3, Newcastle upon Tyne (UK).

Arnold, W.A., Hozalski, R.M., Hondzo, M., Novak, P.J., Capel, P.D. 2006. Wireless Technologies and Embedded Network Sensing: Options for Environmental Field Facilities. Oral Presentation. CLEANER Planning Grant PI meeting, March 2006, Arlington, VA.

STUDENT SUPPORT

Name: Michael Henjum
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AWARDS

None

ADDITIONAL FUNDING
Funding has been obtained for related or complimentary projects from the National Science Foundation/Consortium of University for the Advancement of Hydrologic Sciences Inc. (as part of the test-bedding activities for the WATERS Network; www.watersnet.org) and from the Mississippi Water Management Organization/Minnehaha Creek Watershed District.

Triclosan and Triclosan-derived Dioxins in the Mississippi River Sediment Record

Basic Information

Title:	Triclosan and Triclosan-derived Dioxins in the Mississippi River Sediment Record
Project Number:	2007MN203B
Start Date:	3/1/2007
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	MN 05
Research Category:	Water Quality
Focus Category:	Toxic Substances, Sediments, Acid Deposition
Descriptors:	
Principal Investigators:	Kristopher McNeill, William Alan Arnold

Publication

1. Arnold, W.A., K. McNeill. 2007. "Transformation of Pharmaceuticals in the Environment: Photolysis and Other Processes" In M. Petrovic and D. Barcelo (eds). A Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle. Elsevier Science, Amsterdam, The Netherlands. Pages 361 – 383.
2. Steen, P.O., M. Grandbois, K. McNeill, W.A. Arnold. 2009. Photochemical formation of halogenated dioxins from hydroxylated polybrominated diphenyl ethers (OH-PBDEs) and chlorinated derivatives (OH-PBCDEs). Environ. Sci. Technol. accepted.
3. Buth, J.M., M. Grandbois, P.J. Vikesland, K. McNeill, W.A. Arnold. 2009. Aquatic photochemistry of chlorinated triclosan derivatives: potential source of polychlorodibenzo-p-dioxins. Environ. Toxicol. Chem., accepted.

Triclosan and Triclosan-derived Dioxins in the Mississippi River Sediment Record

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William A. Arnold, Associate Professor, Department of Civil Engineering, University of Minnesota

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Charles V. Sueper, Technical Director, Pace Analytical Services, Inc.

Research Assistants
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Matthew L. Grandbois, Department of Chemistry, University of Minnesota
Peter O. Steen, Water Resources Science, University of Minnesota

ABSTRACT

This project focused on establishing whether triclosan was and continues to be a source of dioxins to the aquatic environment. It was hypothesized that triclosan, a widely used antimicrobial found in consumer products, is transformed into toxic dioxin compounds through chlorination of triclosan-containing wastewater and sunlight exposure in rivers that receive chlorinated wastewater. We further hypothesized that triclosan and its products will associate with the sediment downstream of point of discharge and their release to the environment thus will be recorded in the sediment record. To determine the historical inputs of triclosan and its products in to the Upper Mississippi river from Minnesota's largest wastewater treatment plant, the Metro Plant, in St. Paul, sediment cores from Lake Pepin were be analyzed. It was expected that triclosan and its products would not be found in pre-1960 sediment, that it would be at low levels between 1960 and 1990 when its use was limited, and at the highest levels after 1990 following its widespread use in liquid handsoap and toothpaste. The results of this study will further our understanding of micropollutants in wastewater and will provide specific information about the appropriateness of chlorination disinfection for triclosan-containing waters.

PROGRESS REPORT

Methods

Triclosan analytical method development

A liquid chromatography (LC) method with triple quadrupole mass spectrometry (MS-Q^3) detection was developed to determine triclosan and its chlorinated derivatives in extracts of sediment and wastewater samples. An 1100 Series Agilent capillary HPLC coupled with a Finnigan TSQ Quantum Discovery MAX triple quadrupole mass spectrometer was used for this analysis. Electrospray ionization (ESI) was carried out in negative mode. Wastewater samples were collected in pre-washed glass bottles, filtered through 0.2 μm filters to remove particulate matter, adjusted to pH 2 for preservation, and stored at 4 °C in the dark until analysis. 250 mL wastewater samples were solid-phase extracted using Oasis HLB cartridges followed by a washing step with 50:50 water:methanol to remove interfering dissolved organic matter (DOM). The cartridges were eluted with methanol/methyl-*t*-butyl ether. The extracts were concentrated under nitrogen to a minimal volume (~ 100 µL) and analyzed by LC/MS.

Synthesis of chlorinated derivatives of triclosan

The synthetic approach was inspired by the route taken by Marsh et al. (*1*) Starting with either commercially available or readily synthesized chlorinated phenols, *ortho*-directed formylation followed by phase-transfer catalyzed methylation of the aromatic hydroxyl group yielded 2-methoxy-chlorinated benzaldehydes. Baeyer-Villager oxidation of the resulting aldehydes yielded the respective phenols. The chlorinated triclosan precursors underwent basic couping with 2,2',4,4'-tetrachlorodiphenyliodonium iodide to form the diphenylether backbone, which was then deprotected to yield the target triclosan derivatives. The synthetic scheme for the chlorinated triclosans is shown in Figure 1.

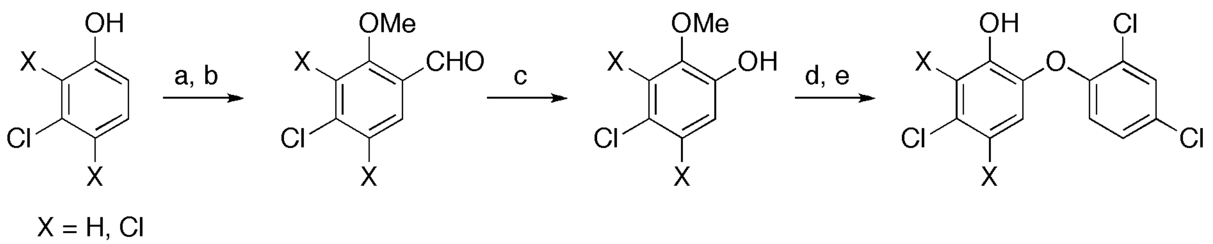

Figure 1. Synthesis of Chlorinated Triclosan Derivatives. Reaction conditions: a) $MgCl_2$, paraformaldehyde, TEA, CH_3CN; b) $(C_4H_9)_4NOH$, NaOH, MeI, CH_2Cl_2, H_2O; c) 1. H_2O_2, $(CF_3CO)_2O$, KH_2PO_4, CH_2Cl_2, 2. MeOH, HCl; d) K_2CO_3, 18-crown-6, 2,2',4,4'-tetrachlorodiphenyliodonium iodide, DMAC; e) BBr_3, CH_2Cl_2.

Results to date

Triclosan analytical method development

A liquid chromatographic method has been developed to effectively separate the triclosan and its chlorinated derivatives, including two mono-chlorinated isomers. The injection volume and mass spectrometer parameters have been optimized to maximize sensitivity while maintaining satisfactory chromatographic resolution. A method detection limit on the order of 1 ng/L has been realized. The recovery of the solid-phase extraction pre-concentration step was maximized by optimizing the cartridge type, sample pH, washing step, and elution volume. The Oasis HLB cartridge was found to provide the greatest recovery. The amount of interfering dissolved organic matter (DOM) was reduced by increasing the pH of the sample. However, to keep the analytes fully protonated to aid in their extraction efficiency, a pH of 4 was chosen to extract the samples. Washing the cartridge with 3-5 mL aliquots of 50:50 methanol:water solution prior to elution was found reduce the amount of interfering DOM in the extract by approximately an order of magnitude. Elution with sequential 5 mL aliquots of methanol and 90:10 methyl-*t*-butyl ether:methanol provided maximum recovery. Greater than 75% recovery was obtained for every analyte. Wastewater samples have been collected quarterly from the Metropolitan Plant in St. Paul, MN for 1.5 years both prior to chlorination and after the chlorination step to determine the extent of formation of chlorinated triclosan derivatives during chlorine disinfection.

Synthesis of chlorinated derivatives of triclosan and HO-BDE-47
The synthesis of 5,6-dichloro-2-(2,4-dichlorophenoxy)phenol was completed in 5 steps to yield 1.670 g as a white solid. The overall synthetic yield for this derivative was 26%. The synthesis of 4,5-dichloro-2-(2,4-dichlorophenoxy)phenol did not follow the general scheme presented above, due to difficulty purifying several intermediates. An alternative approach utilizing regioselective chlorination of guaiacol followed by basic coupling/deprotection mentioned above yielded 1.051 g as a white solid over 3 steps. The overall synthetic yield for this derivative was 18%. The synthesis of 4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol required an additional synthetic step not mentioned in the above scheme. 2,3,4-Trichloroaniline was diazotized and then subjected to water to synthesize the corresponding phenol. This was then subjected to the above-mentioned synthetic scheme to yield 0.107 g as a white solid over 6 steps. The overall synthetic yield for this derivative was 5%.

All synthesized compounds have been characterized and found to match by nuclear magnetic resonance (^{1}H and ^{13}C), mass spectrometry, and melting point when literature values have already been reported.

Sediment coring and analysis
Sediment cores were collected in June 2008. The cores were dated via magnetic profiling and then sectioned. The sections were extracted into methanol and are undergoing sample cleanup and analysis for triclosan and chlorinated triclosan derivatives by LC/MS.

Reference

Marsh, G.; Stenutz, R.; Bergman, A. Synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers - natural products and potential polybrominated diphenyl ether metabolites. European Journal of Organic Chemistry 2003, 2566-2576.

PUBLICATIONS

Peer Reviewed Publications

Steen, P.O., M. Grandbois, K. McNeill, W.A. Arnold. 2009. Photochemical formation of halogenated dioxins from hydroxylated polybrominated diphenyl ethers (OH-PBDEs) and chlorinated derivatives (OH-PBCDEs). *Environ. Sci. Technol.* accepted.

Buth, J.M., M. Grandbois, P.J. Vikesland, K. McNeill, W.A. Arnold. 2009. Aquatic photochemistry of chlorinated triclosan derivatives: potential source of polychlorodibenzo-p-dioxins. *Environ. Toxicol. Chem.,* accepted.

Book Chapters

Arnold, W.A., K. McNeill. 2007. "Transformation of Pharmaceuticals in the Environment: Photolysis and Other Processes" *In* M. Petrovic and D. Barcelo (eds). Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle. Elsevier Science, Amsterdam, The Netherlands. Pages 361 – 383.

PRESENTATIONS

Invited Presentations

Arnold, W.A., 2007. Solar photochemistry of pharmaceutical compounds. American Water Works Association Water Quality Technology Conference, Advanced Oxidation Technologies in Water Treatment: Fundamentals and Applications Workshop, November 4, 2007.

Arnold, W.A., 2008. *Pharmaceutical Photolysis and Impacts: Tetracycline and Triclosan* ETH-Zurich, Institute of Biogeochemistry and Pollutant Dynamics, Zurich, Switzerland.

McNeill, K., 2009. Incineration or liquid handsoap: Which is the larger source of dioxins to the aquatic environment? College of St. Catherine, St. Paul, MN.

McNeill, K., 2009. Incineration or liquid handsoap: Which is the larger source of dioxins to the aquatic environment? Gustavus Adolphus College, St. Peter, MN.

Conference Presentations

Buth, J.M., Arnold, W.A., McNeill, K. 2008. Photochemical Fate of Chlorinated Triclosan Derivatives. Poster Presentation. Gordon Research Conference, Environmental Sciences: Water, June 22 – 28, 2008, Holderness, NH.

Steen, P.O., M. Grandbois, W.A. Arnold, K. McNeill. 2008. Hydroxylated polybrominated diphenyl ether photolysis quantum yields and product identification. *Environ. Chem. Div., ACS National Meeting,* Philadelphia, PA, 48(2), 608-611.

Steen, P.O., Grandbois, M., Arnold, W.A., McNeill, K. 2008. Hydroxylated Polybrominated Diphenyl Ether Photolysis: Quantum Yields and Product Identification. *Minnesota Water Conference*, October 27-28, 2008, St. Paul, MN.

Steen, P.O., Grandbois, M., McNeill, K., Arnold, W.A. 2009. Photolysis of Hydroxylated Polybrominated Diphenyl Ethers. *Micropol & Ecohazard 2009. 6th IWA/GRA Specialized Conference on Assessment and Control of Micropollutants/Hazardous Substances in Water*, June 8-10, 2009, San Francisco, CA.

Buth, J.M., Arnold, W.A., K. McNeill. 2009. Formation and Occurrence of Chlorinated Triclosan Derivatives (CTDs) and their Dioxin Photoproducts. *Micropol & Ecohazard 2009. 6th IWA/GRA Specialized Conference on Assessment and Control of Micropollutants/Hazardous Substances in Water*, June 8-10, 2009, San Francisco, CA.

STUDENT SUPPORT

Name: Jeffrey M. Buth
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Degree being sought: Ph.D.

Name: Matthew L. Grandbois
Program: Chemistry, University of Minnesota
Degree being sought: Ph.D.

Name: Peter O. Steen
Program: Civil Engineering, University of Minnesota
Degree earned: M.S. (2009)

AWARDS

Jeffrey M. Buth, EPA STAR Fellowship
Jeffrey M. Buth, 2008 ACS Graduate Student Award in Environmental Chemistry

ADDITIONAL FUNDING

This project is complemented by a project from the National Science Foundation (2006-2009) to study the photolysis of triclosan and polybrominated diphenyl ethers in both the laboratory and in the field.

The Role of Local Stakeholders in Water Resource Management: Characterization and Diffusion of Minnesota Lake Improvement Districts

Basic Information

Title:	The Role of Local Stakeholders in Water Resource Management: Characterization and Diffusion of Minnesota Lake Improvement Districts
Project Number:	2007MN204B
Start Date:	3/1/2007
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	4
Research Category:	Social Sciences
Focus Category:	Law, Institutions, and Policy, Water Quality, Management and Planning
Descriptors:	
Principal Investigators:	Dennis R. R Becker

Publication

1. Steiger-Meister, K. The drama of the commons and its impact on adaptive management, conference proceeding paper, American Water Resource Association Specialty Conference: Adaptive Management of Water Resources II, Snowbird, UT. (6/09) In review
2. Steiger-Meister, K., D. R. Becker. Connecting environmental policy with citizen engagement: A comparative study between Minnesota's Lake Improvement Districts and Wisconsin's Lake Districts. Manuscript in preparation for Journal of the American Water Resources Association.
3. Steiger-Meister, K., D. R. Becker. Citizen stewardship of water resources: A look at how water policy can create and coordinate citizen action in Minnesota for environmental change. Manuscript in preparation for Water Policy.

The Role of Local Stakeholders in Water Resource Management: Characterization and Diffusion of Minnesota Lake Improvement Districts

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Research Assistant
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ABSTRACT

According to the Minnesota Pollution Control Agency, as of 2006 there were 1,013 lakes listed as impaired waters, up from 920 in 2004. A large number are impacted by human development, recreation, and pollution leading to unsafe conditions for swimming or fishing, excessive algal blooms, and high levels of mercury transferred throughout the food chain. Despite progress since passage of the Clean Water Act in 1972, more Minnesota lakes are contaminated than at any time in history. Because they are an integral part of community economies and the lifestyle of Minnesota citizens, alternative management solutions are required that capitalize on existing planning efforts and initiatives. The development of policy tools to enable and facilitate management actions at the local level is paramount. The research sought to assess the effectiveness of existing Minnesota programs that empower citizens to affect water quality solutions in the places they live. In particular, the research assessed use and diffusion of Lake Improvement Districts (LID), which are local units of government organized to enhance water quality by securing grants and taxing landowners to support mitigation activities within a lake district. Diffusion of the LID program has been slow in MN. Only 24 have been created since 1976, compared to more than 200 in Wisconsin. The research charactelized existing LIDs including funds secured, staff resources, partnerships formed, and accomplishments relative to state priorities. We identified barriers to diffusion and effective utilization of similar forms of stakeholder engagement to affect water quality. Finally, we explored the role of stakeholder engagement in resource management activities focusing on how state programs can better facilitate on-the-ground accomplishments. Recommendations for incorporating stakeholder initiatives into statewide management activities and policy development will be provided. The cumulative impact of such could dramatically decrease the number of impaired waters in Minnesota.

PROGRESS REPORT

The research investigates the development of policy tools to enable and coordinate water quality management actions at the community level. It assesses the effectiveness of existing Minnesota programs empowering citizens to affect water quality solutions. In particular, the research assesses use and diffusion of Lake Improvement Districts (LIDs), where local units of government organize to enhance water quality by securing grants and taxing landowners to support mitigation activities within a lake district.

In order to characterize existing LIDs including funds secured, staff resources, partnerships formed, and accomplishments relative to state priorities, all 24 LIDs in the state were contacted, and of those in-depth semi-structured interviews were conducted with representatives from 14

LIDs during the summer and fall of 2007. Interviews were subsequently coded to identify barriers to the diffusion of the LID program.

Researchers also met with the LID coordinator from Minnesota's Department of Natural Resources to learn about the agency's role in the program and collect education and outreach materials focused on LIDs. The research assistant is currently in the process of interviewing county water planners, select a subset of applicable case study locations for further analysis, and conducting a legal analysis of the LID legislation. The research assistant also attended the annual meetings of three LIDs and conducted participant observation for how the meetings operated. Follow-up thank you letters were sent to all involved stakeholders in the winter 2007.

During the summer of 2008, the research assistant carried out a comparative study between Minnesota's LIDs and Wisconsin's Lake Districts. She is currently completing the analysis of the institutional arrangements surrounding Lake Districts in Wisconsin, as well how they operate at the local level to accomplish water quality objectives. The research assistant successfully completed her dissertation written and oral exams in September of 2008 and is now in the beginning stages of writing her dissertation on this topic. A final report of the findings of this funded research is forthcoming June 2009. Using separate funds leveraged with this project, the research assistant is continuing this research and this summer will be conducting three county level case studies in Isanti, Morrison and Wright Counties to examine the importance and impact of institutional arrangements on citizen-based water stewardship activities.

PUBLICATIONS

The following papers are in preparation or review:

Steiger-Meister, K. The drama of the commons and its impact on adaptive management, conference proceeding paper, American Water Resource Association Specialty Conference: Adaptive Management of Water Resources II, Snowbird, UT. (6/09) *In review*

Steiger-Meister, K. and D. R. Becker. Connecting environmental policy with citizen engagement: A comparative study between Minnesota's Lake Improvement Districts and Wisconsin's Lake Districts. Manuscript in preparation for *Journal of the American Water Resources Association.*

Steiger-Meister, K. and D. R. Becker. Citizen stewardship of water resources: A look at how water policy can create and coordinate citizen action in Minnesota for environmental change. Manuscript in preparation for *Water Policy*.

PRESENTATIONS

The following papers have been presented, accepted or in review:

Steiger-Meister, K. 2008. When ripples become waves: Building synergy among local stakeholders to affect top-down water policy. Presented at the 14th International Symposium on Society and Resource Management (ISSRM) on June 13, 2008, University of Vermont in Burlington, VT.

Steiger-Meister, K. 2009. Minnesota's Lake Improvement Districts. Panelist at the Lakes and Rivers Conference hosted by Minnesota Waters, Rochester, MN. (5/2009) *Abstract accepted*

Steiger-Meister, K. 2009. The Drama of the commons and its impact on adaptive management. American Water Resource Association Specialty Conference: Adaptive Management of Water Resources II, Snowbird, UT. (6/2009) *Abstract accepted.*

Steiger-Meister, K. 2009. Connecting environmental policy with citizen engagement: A comparative study between Minnesota's Lake Improvement Districts and Wisconsin's Lake Districts. Minnesota Water Resources Conference, University of Minnesota in Saint Paul, MN. (10/2009) *Abstract in review.*

STUDENT SUPPORT

Name: Kaitlin Steiger-Meister
Program: Natural Resource Science and Management (NRSM), University of Minnesota
Degree being sought: Ph.D.

AWARDS

See "Additional Funding" section

ADDITIONAL FUNDING

Upon recommendation from the review panel for this project, the study was expanded to include a comparative analysis of Minnesota's Lake Improvement Districts with Wisconsin's Lake Districts. Steiger-Meister successfully secured competitive funding in the amount of $5,654 through the 2008 University of Minnesota Consortium on Law and Values in Health, Environment & the Life Sciences (project title: *Building clean water communities: Understanding how environmental policies can promote and coordinate community participation in the long-term management of local freshwater resources*). The research will supplement the project by examining Wisconsin Lake Districts and the Wisconsin Lakes Partnership.

Additional funding in the amount of $100 was provided by the International Association for Society and Resource Management for Steiger-Meister to present a paper on the project at the 14th International Symposium on Society and Resource Management.

Steiger-Meister was also awarded a Graduate School Block Grant Fellowship in the amount of $1,184 from the University of Minnesota to support her while she continues analysis and final write-up of research findings for publication.

The Influence of Drainage on Biogeochemical Cycling of Carbon in Agricultural Ecosystems

Basic Information

Title:	The Influence of Drainage on Biogeochemical Cycling of Carbon in Agricultural Ecosystems
Project Number:	2007MN205B
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Congressional District:	4
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Focus Category:	Hydrogeochemistry, Water Quality, Geochemical Processes
Descriptors:	None
Principal Investigators:	Jennifer Y King, Brent james Dalzell, Jacques C. Finlay, David J. Mulla, Gary Sands

Publication

The Influence of Drainage on Biogeochemical Cycling of Carbon in Agricultural Ecosystems

Principal Investigators

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ABSTRACT

In the Upper Midwest region of the United States, annual water yield from many rivers has been increasing over recent decades. In addition to increases in annual precipitation, greater water yield in agricultural landscapes is also attributable to more efficient landscape drainage due to surface ditches and subsurface tile drainage systems. The influence of modified drainage on export of dissolved organic carbon (DOC) and carbon cycling in agricultural landscapes is poorly understood. To that end, we conducted a field-scale study of dissolved carbon export in subsurface drainage water from agricultural fields located at the University of Minnesota Southern Research and Outreach Center in south-central Minnesota. In fields that have been modified with tile drainage, overland flow through organic-rich soils is uncommon and the majority of water yield occurs via subsurface flow. The goal of this research was to quantify and characterize the influence of these drainage systems on carbon export.

Results from two field seasons showed that dissolved organic carbon (DOC) concentrations are not positively correlated to drainage flow. This is contrary to results from watershed-scale studies and suggests that different mechanisms appear to be responsible for DOC flux at the field- and watershed-scales. Experimental plots have varied depth and spacing of subsurface tile drainage designed to represent common (13mm day^{-1}) and more intense (51mm day^{-1}) practices. Plots with intense drainage tended to have slightly higher DOC concentrations during periods of low flow; these low flow periods make minimal contributions to total annual loads. In contrast, there are no differences in DOC concentrations between field plots that have differing drainage intensities during periods of high flow. Drainage discharge is strongly correlated with drainage intensity, however, and variations in drainage intensity have a strong influence on annual DOC loads. Based on DOC relationships measured here and water yield differences from 8 years of flow data, plots with more intense drainage treatments can export from 60 to 100% more DOC annually than plots with standard drainage treatments. In contrast to effects on DOC concentrations, the intensity of tile drainage did influence concentrations of dissolved inorganic carbon (DIC) from the field plots. Plots with more intense drainage practices average about 10% greater DIC concentrations than plots with conventional drainage. The mechanisms responsible for this may be related to drainage effects on soil respiration or weathering of soil carbonates.

These results may provide interesting insight into broader long-term trends in DIC that have been observed in larger river basins.

Molecular weight distributions of dissolved organic matter (DOM) from field plots were compared to DOM samples from soil and downstream ditch and river sites throughout the 2008 sampling season. Results showed that DOM is characterized by two main groups: one group of more heterogeneous DOM characterized by relatively larger molecular weight and one group of lower molecular weight DOM. The relative abundance of the homogenous, low molecular weight DOM is greatest in samples collected from the field plots, and the high molecular weight DOM becomes increasingly dominant with distance downstream. These results suggest that either: (1) low molecular weight DOM is being preferentially removed in the ditch and river sites; or (2) high molecular weight DOM is being preferentially added to downstream sites via additional terrestrial sources and/or via in-stream primary productivity. The relative source and microbial availability of DOM at these sites is currently being assessed through additional lab-based experiments.

Results from this study demonstrated that subsurface drainage is capable of producing changes in biogeochemical cycling and export of both organic and inorganic dissolved carbon. These changes appear to be the result of drainage influences on water yield as well as actual C export from the fields. In addition to quantitative differences in C exported from the field plots, DOM quality is also different (when compared to downstream sites) with subsurface tile drainage being a source of low molecular weight DOM to streams and rivers. Additional samples have been collected to assess microbial availability of the DOM from these sites and are awaiting final results and analysis.

ACCOMPLISHMENTS – FINDINGS AND SCIENTIFIC PRODUCTS

Introduction and Overview

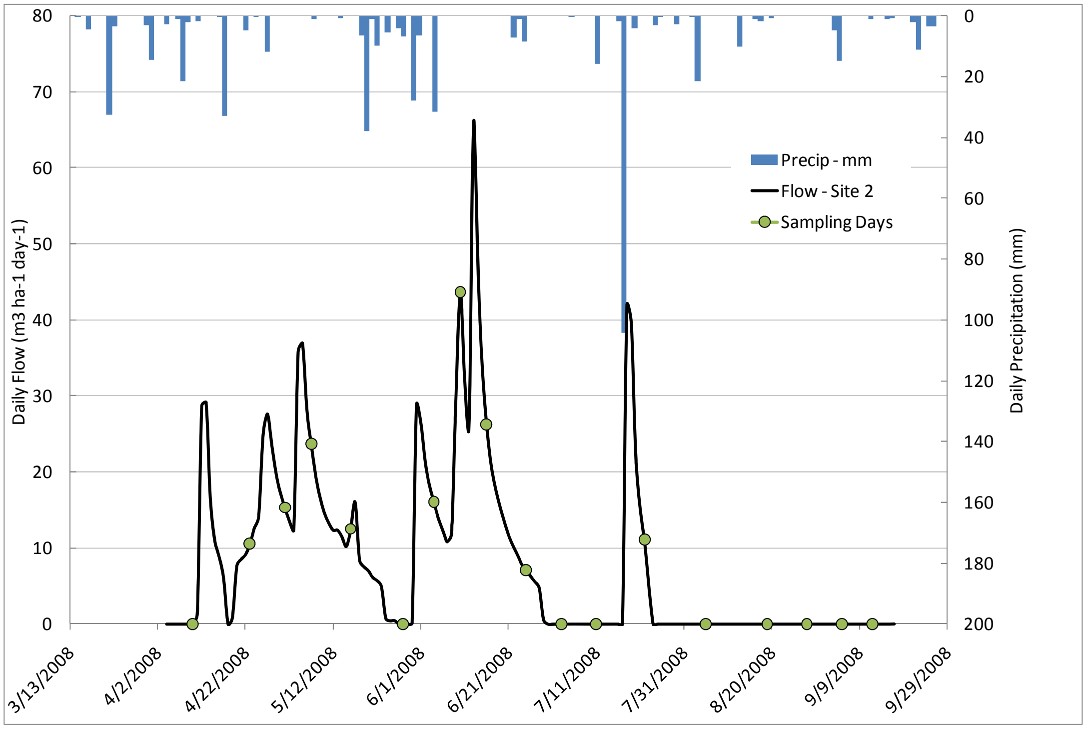
Carbon cycling in agricultural ecosystems has received much attention in recent years due to the loss of carbon (C) that resulted from land use conversion from native vegetation to intensive agriculture. This loss in soil carbon represents the potential for rebuilding soil carbon pools by C sequestration through implementation of alternative agricultural management practices such as reduced tillage in row-cropping systems common in the midwestern United States (Lal et al., 1999; see however, Baker et al., 2007). Studies that focus on C cycling in agricultural ecosystems are often centered on measurements of changes in soil carbon pools as well as carbon dioxide (CO_2) fluxes from the landscape (Reicosky et al., 2002). One aspect of C cycling that has not received much attention, however, is the export of dissolved carbon from agricultural landscapes in the forms of dissolved organic carbon and dissolved inorganic carbon, DOC and DIC, respectively. Subsurface tile drainage systems are a common practice in row-crop producing regions of the midwest, and subsurface flow accounts for a significant proportion of water yield from fields modified with drainage (Jin and Sands, 2003). The goal of this study was to quantify and characterize dissolved C export in subsurface tile drainage flow from experimental field plots in an effort to develop a more complete understanding of all aspects of C cycling in agricultural ecosystems.

To address the impacts of subsurface drainage systems on dissolved carbon cycling, we are quantifying and characterizing dissolved carbon export from experimental drainage plots located at the University of Minnesota Southern Research and Outreach Center (SROC) located at Waseca, MN. A series of 12 experimental plots (all roughly 1 hectare) have been modified with subsurface tile drainage systems of varying depth and spacing in order to achieve drainage coefficients of either 13mm day^{-1} (conventional drainage) or 51 mm day^{-1} (intense drainage). All plots were monitored continuously for flow and sampled periodically for dissolved C quantification and characterization. Overland flow from these experimental plots is negligible due to the presence of the subsurface drainage. This is a 2-year project that extended from spring 2007 through early 2009. A summary of research efforts and presentation of results is provided below.

Summary of work completed for the reporting period from March 2008 through May 2009.

Field Sampling - Flow monitoring from the subsurface tile drainage plots occurred nearly continuously throughout the growing seasons (there is no subsurface drainage flow in the winter when soils are frozen). This monitoring resulted in a large database of sub-daily flow data from 12 plots which will be used with DOC and DIC data to calculate annual C export. Data from the current study will also be used in conjunction with additional flow data (initiated in 2001) in order to assess the role of interannual hydrologic variability in overall C export. Problems with monitoring equipment and data storage occurred in 3 of the plots, resulting in some brief periods of incomplete flow data; if possible, these data gaps will be filled based on correlation with the remaining plots. Water samples were collected at roughly weekly intervals (when flow was present) from April through October 2008. In addition to sample collection from field plots, water samples were also collected from 2 downstream ditch sites as well as the LeSueur River in order to allow for comparison of sample characteristics over different watershed areas. In all, 248 water samples were collected from 12 research plots, 1 wetland, and 3 downstream ditch and river sites. These samples represent a range of flow conditions representing early season drainage from snowmelt and spring rains as well as late summer storm flow (Fig 1). All samples were analyzed for concentrations of DOC and DIC on a Shimadzu TOC Vcpn carbon analyzer and related to corresponding flow data in order to determine annual dissolved carbon flux from the study sites.

Figure 1. Summary of tile drainage and sampling dates for 2008. Precipitation events are shown along the top of the figure (with units on the secondary y-axis). Drainage is shown for one plot from the study area for illustration purposes. The amount of flow varied with drainage intensity; however, the overall shape of the hydrograph is similar between treatments.

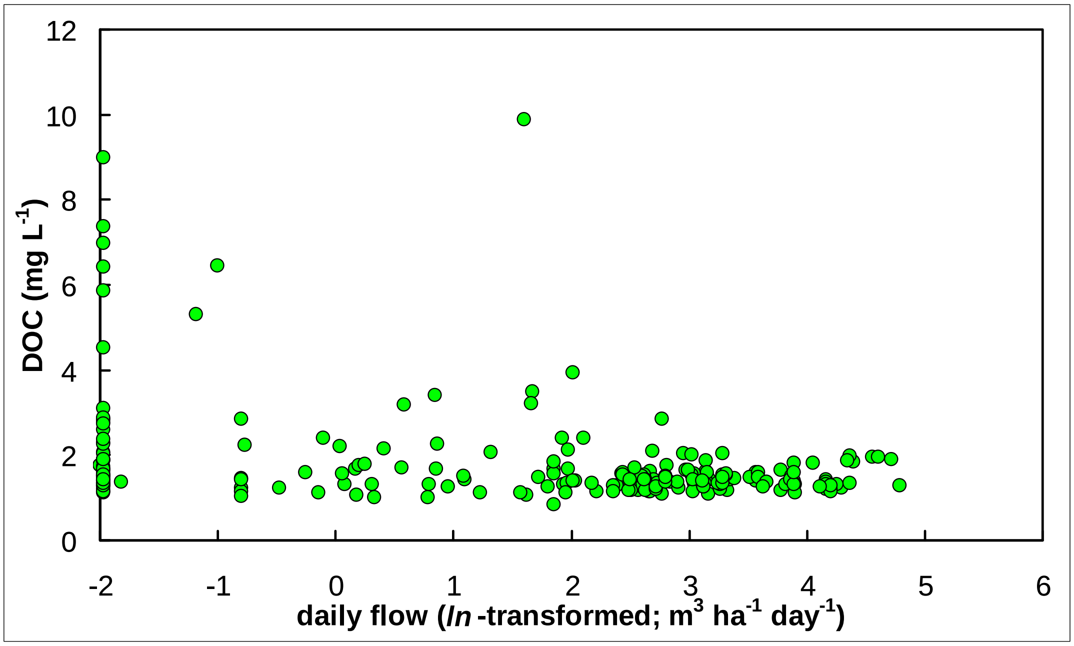


Results

Dissolved Organic Carbon – While there was considerable variability in DOC concentrations among treatments, the plots with intense drainage had, on average, greater DOC concentrations than the plots with standard drainage. Differences in DOC concentrations between treatments occurred primarily during low flow conditions and these differences became negligible during periods of high flow. These results are consistent with field data collected during 2007 and reinforce the conclusion that annual DOC yield from these plots is dictated primarily by water yield, which is greater in plots with more intense drainage treatments.

The concentration-discharge graph of DOC data from this field-scale study (Fig 2) stands in contrast to results from watershed-scale studies (e.g., Dalzell et al., 2007; Royer and David, 2005) which show a positive correlation between stream flow and DOC concentrations. This difference suggests that different mechanisms are responsible for DOC export at different spatial scales and highlights the potential importance of alternative C sources such as in-channel algal productivity and/or additional terrestrial sources that contribute to riverine C budgets including surface runoff from non-drained areas.

Figure 2. Relationship between dissolved organic carbon concentrations and flow from experimental drainage plots at the Agricultural Ecology Research Farm.



Based on average DOC concentrations from 2007 field work and annual water yields, total annual DOC yields from the field plots at the SROC ranged from approximately 1 to 5 kg ha^{-1} yr^{-1}. These values are near the low end of the range of annual DOC yields that would be expected based on the situation of the study area near the interface of grassland and deciduous forest ecosystems (Aitkenhead and McDowell, 2000). It is important to note that plots with more intense drainage treatments export from 60 to 100% more DOC annually than plots with standard drainage. If this observation is applicable at the scale of larger watersheds, then these results indicate that annual DOC export from agricultural watersheds in the Midwestern United States (where subsurface drainage is common) is influenced by varying land management practices. This is an important consideration for future work that considers biogeochemical carbon cycling in waters draining landscapes that have been influenced by anthropogenic activities.

Dissolved Inorganic Carbon – Similar to results from sampling conducted in 2007, DIC concentrations were greater in plots with more intense subsurface tile drainage compared to conventional drainage treatments. These results provide evidence for one mechanism by which perturbations in agricultural soils (either through drainage or, perhaps, through root and soil respiration) can contribute to the observed trends of increasing DIC export in the Mississippi River Basin (Raymond and Cole, 2003; Raymond et al., 2008). Results from this study are important in that they show that drainage practices do have a significant impact on DIC export, and annual fluxes from more intensely-drained plots have greater DIC yields through both (1) increased concentrations and (2) increased total water yield. DIC concentrations (and, subsequently, annual DIC yields) are roughly an order of magnitude greater than DOC concentrations in drainage water sampled in this study.

Figure 3. High performance size exclusion chromatography chromatograms of DOM from field, ditch, and river sites. A chromatogram of water-extractable soil organic matter is shown for reference. The relative importance of low molecular weight DOM decreases with increasing distance downstream.

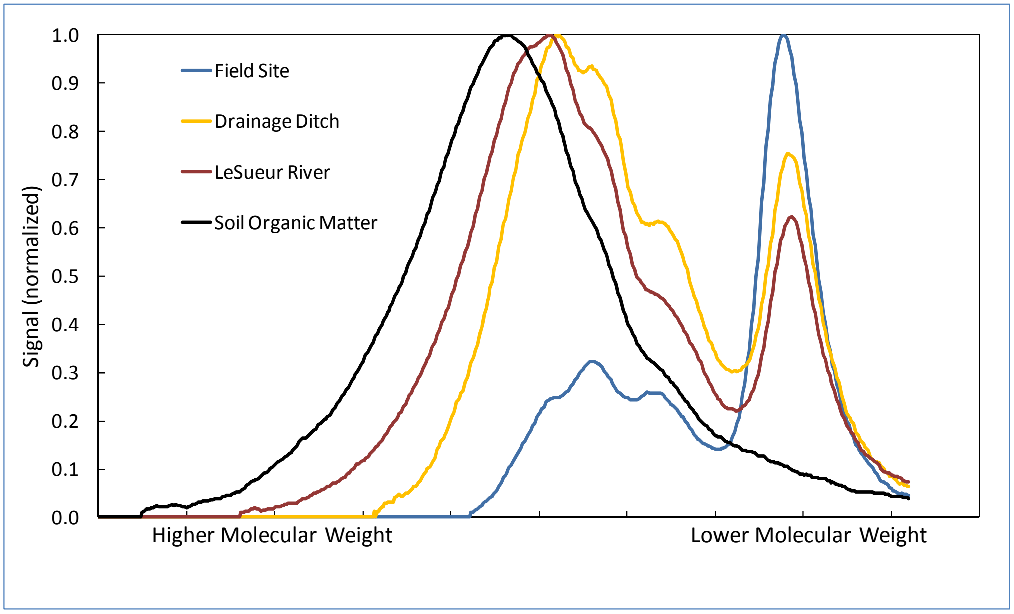
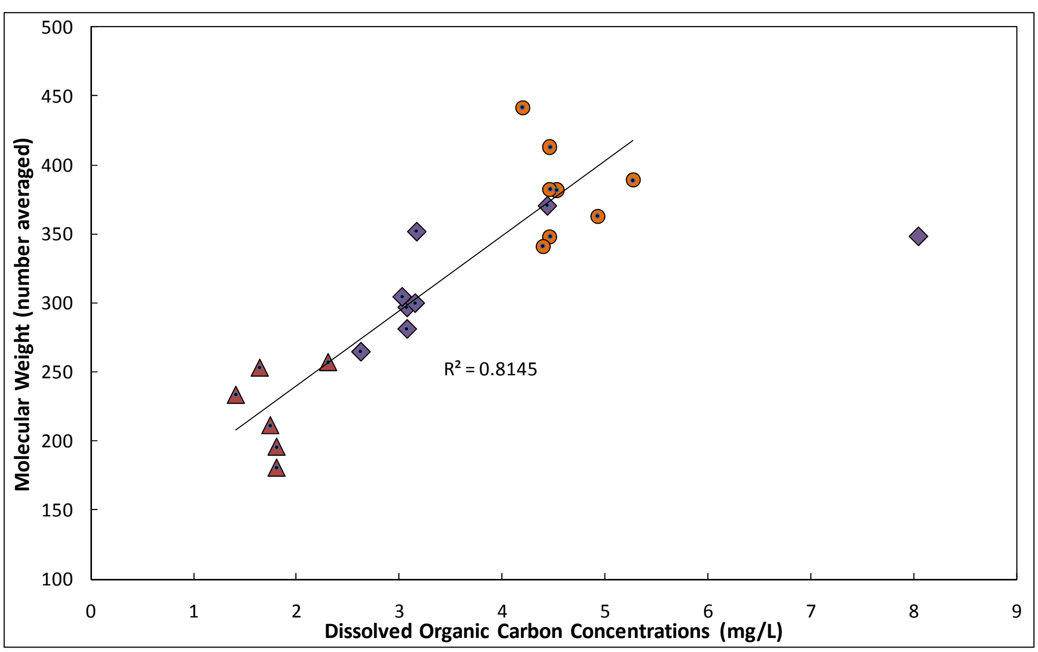


Figure 4. Relationship between molecular weight and DOC concentrations from sites. Increases in DOC concentrations are correlated with increases in molecular weight, suggesting preferential addition of high molecular weight DOM in downstream sites. Data are from sampling dates ranging from April to October 2008. The outlier from the Ditch 2 site (not included in the regression) was collected in late August during a period of very low flow when high autotrophic productivity was observed in the ditches.



Molecular Weight Distribution – Several samples were collected from field, ditch, and river sites during 2008 for molecular weight characterization via high-performance size exclusion chromatography. Samples from the study area are generally characterized by two distinct groups of dissolved organic matter: one group of higher molecular weight (HMW) DOM that appears to be heterogeneous material and a group of low molecular weight (LMW), more homogenous DOM (Fig 3). The relative abundance of the two DOM groups changes with increasing catchment area , with the HMW DOM becoming a more dominant component of the overall pool in the ditch and river sites (Fig 4). This pattern suggests that either: (1) LMW DOM is being preferentially removed, or (2) HMW DOM is being preferentially added with distance downstream. The latter explanation is more plausible in light of downstream increases in DOC concentrations, and suggests an additional source (most likely surface runoff and/or in-stream primary productivity). Additional work is being performed to characterize different sources of DOM from varying samples. These results are important because they will help to identify the extent to which perturbations in upland carbon cycling are persistent in receiving water bodies. This has implications for a variety of important issues ranging from water quality impairments due to turbidity to continental scale biogeochemical carbon cycling.

Final analyses, paper writing, and future work.

Microbial Availability Experiments and DOM characterization – Experiments were conducted in April/May 2009 to assess how qualitative differences in DOM (as determined via molecular weight) may relate to aquatic ecosystem function via microbial degradation. DOM samples were collected from 4 different sites (from field to river) and subjected to microbial degradation in laboratory incubations over a period of two weeks. In addition to assessing microbial availability via changing DOC concentrations, the DOM pool will be characterized for source differences via optical characterization (ultraviolet and visible light absorption, as well as excitation-emission fluorescence spectroscopy) and changes in molecular weight distributions over the course of the incubation. This experiment has been completed, and samples are awaiting final instrumental analysis. Results from this experiment will indicate the relative biodegradability of DOM collected across the landscape and will provide an ecological context to these field-scale studies of C cycling.

Manuscript Preparation – Final analysis of data and interpretation of results are ongoing in conjunction with preparation of manuscripts related to this work. Currently, two peer-reviewed manuscripts are anticipated from this work; tentative titles are presented below:

1. The role of subsurface tile drainage systems in influencing the annual carbon flux and dissolved organic matter quality from agricultural landscapes.
2. The role of landscape drainage and changing precipitation on export of dissolved carbon from agricultural ecosystems.

Future work – Data collected from this study provide important information necessary to understand how biogeochemical carbon cycling may change in landscapes that have been converted to agricultural use. Future research building on these results will likely be centered upon more detailed assessment of DOM sources and processing over varying scales in agricultural landscapes as well as comparisons of DOM among landscapes with varying degrees of agricultural and hydrologic modification. It may also be important to study the light scattering properties of these different size fractions.

The role of subsurface drainage on increasing DIC export from agricultural landscapes is a topic of high current interest due to recent high-profile research on this topic that has identified a long-term increase in DIC export in the Mississippi River Basin (Raymond and Cole, 2003; Raymond et al., 2008). This trend represents a sink for atmospheric carbon (through mineral weathering). The increases have been strongest in basins with agricultural land use, although the actual mechanisms responsible have not been unequivocally identified. Results from the research presented here will be helpful in generating ideas to approach this topic through additional research projects.

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Reicosky, D.C., Evans, S.D., Cambardella, C.A., Allmaras, R.R., Wilts, A.R., and Huggins, D.R. 2002. Continuous corn with moldboard tillage: Residue and fertility effects on soil carbon. Journal of Soil and Water Conservation. 5, 277-284.

Royer, T.V., and David, M.B. 2005. Export of dissolved organic carbon from agricultural streams in Illinois, USA. Aquatic Sciences. 67, 465-471.

PUBLICATIONS
None

PRESENTATIONS
Dalzell, B.J. **INVITED** – 2008. Climate change as a factor in export of dissolved organic matter from agricultural watersheds. Oral presentation in symposium titled "Global Climate Change and Agriculture: Interactions, Land-Use Patterns, and Educational Connections" at the 93rd annual meeting of the Ecological Society of America. August 3-8, 2008. Milwaukee, WI.

Dalzell, B. J., J. Y. King, D. J. Mulla, J. C. Finlay, and G. R. Sands. 2008. The Influence of Landscape Drainage on Biogeochemical Cycling of Carbon in Agricultural Ecosystems. Oral presentation given at the annual fall meeting of the American Geophysical Union. December 2008. San Francisco, CA.

Dalzell, B. J. 2008. Effects of Landscape Drainage on Dissolved Carbon Export. Presentation given at the Minnesota/Iowa Drainage Research Forum. December 2008. Owatonna, MN.

Results from this research were also incorporated into lecture materials on global carbon cycling and impacts of land use change for a class on "Biogeochemical Processes" (EEB 4611) University of Minnesota – Spring Semester, 2008.

Preliminary results from this research have also been presented in smaller group discussion sessions in the Departments of Soil, Water, and Climate and Ecology, Evolution, and Behavior at the University of Minnesota.

EDUCATION, OUTREACH, AND PROFESSIONAL DEVELOPMENT

There are no students directly receiving financial support from this project. However, undergraduate students from the lab of Dr. Jennifer King have been involved with this project. In particular, undergraduate Katrina Hill (an African American and Veteran) has been heavily involved in much of the field sampling and laboratory aspects of this research. Katrina is a senior in the College of Food, Agricultural and Natural Resource Sciences and has worked closely alongside Dr. Brent Dalzell as part of her requirements towards her major in Environmental Sciences, Policy and Management. Katrina has been involved in data analysis and interpretation aspects of this project and presented her research results during a lab group research symposium in August 2008. Additionally, Katrina gave a presentation about this research to her peers during fall semester 2008 in an undergraduate class in the Environmental Sciences, Policy and Management program (ESPM 4096 Professional Experience Program: Internship). Further, Katrina's involvement in this project was used to leverage additional funding from internal University of Minnesota sources to support her research.
This work has been led by Dr. Brent Dalzell who is a postdoctoral research associate in the Department of Soil, Water, and Climate. The project has provided an opportunity for Brent to lead this highly interdisciplinary research and to gain further experience in collaborative work. This project has also given Brent an opportunity to mentor undergraduate research.

Determination of Appropriate Metric(s) for Sediment-related Total Maximum Daily Loads (TMDLs)

Basic Information

Title:	Determination of Appropriate Metric(s) for Sediment-related Total Maximum Daily Loads (TMDLs)
Project Number:	2008MN231B
Start Date:	3/1/2008
End Date:	2/28/2009
Funding Source:	104B
Congressional District:	5
Research Category:	Water Quality
Focus Category:	Sediments, Surface Water, Methods
Descriptors:	
Principal Investigators:	Anne Lightbody, Patrick Belmont, Jeff Marr, Cailin Orr, Christopher Paola

Publication

Determination of Appropriate Metric(s) for Sediment-related Total Maximum Daily Loads (TMDLs)

Principal Investigators
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Patrick Belmont, Post Doctoral Associate, St Anthony Falls Laboratory, University of Minnesota
Jeffrey Marr, Research Fellow, St Anthony Falls Laboratory, University of Minnesota
Cailin Huyck Orr, Post Doctoral Associate, National Center for Earth-Surface Dynamics, University of Minnesota
Chris Paola, Professor, Department of Geology and Geophysics, University of Minnesota

ABSTRACT
The most common cause of impaired rivers and streams in the United States is sediment pollution. High levels of suspended sediment impact aquatic communities in different ways depending on the type and texture of the sediment. However, many state regulatory programs use a water quality criterion based on turbidity alone, not taking into account sediment type or the potential underestimate of total suspended sediment. To measure the differential impact of sediment types on physical habitat metrics, benthic invertebrates, and warm-water fishes, we introduced water with clay, fine sand, agricultural soil and an inorganic mixture in a series of controlled floods in an experimental sand-bed stream fed by Mississippi River water within the Outdoor StreamLab (OSL) at St. Anthony Falls Laboratory in Minneapolis, MN. By providing a nearly natural stream setting while maintaining similar environmental conditions between upstream-control and downstream-treatment environments, the OSL enabled us to isolate the effects of suspended sediment. Resulting observations support previous sediment-dose-fish-response models for small sediment grain sizes and contribute new information on the effects of larger-sized sediment particles on mortality and selected health indices for white sucker and smallmouth bass. Observations also suggest that a macroinvertebrate community suited to an urban or agricultural site is able to tolerate short-term sediment pulses. An improved understanding of suspended sediment effects on aquatic organisms will enhance our abilities to develop numeric water quality criteria, to determine factors contributing to biotic impairments, and thus to effectively manage water quality of streams and rivers.

INTRODUCTION
The most common cause of impaired rivers and streams in the United States is sediment pollution, and significant provisions in the federal Clean Water Act have been created to address this pollutant to protect water quality and ensure ecological integrity of the nation's waters (USEPA 2002; Asmus et al. 2009). These provisions include development of water quality standards, assessment and listing of all waters that do not meet standards, and subsequent methods to reduce sediment pollution, often through implementation of Total Maximum Daily Load (TMDL) assessments (Vondracek et al. 2003; MPCA 2007a; 2007b). Effects of non-point source pollutants, including suspended sediment and turbidity, on aquatic organisms are less well understood than point-source pollutants (Waters 1995).

Several recent studies have clearly documented the detrimental effects of sediment loading on stream communities. For example, in an opportunistic study after a coal spill in a small stream in New York, Harper and Peckarsky (2005) documented immediate declines in benthic invertebrate abundance and richness relative to untouched upstream sites. Connolly and Pearson (2007) measured the benthic community response in artificial in-situ channels consisting of split PVC pipes installed within a natural first-order stream in Australia. Observations showed that the impulse introduction of fine clay sediment reduced the numbers of all taxa examined. Suttle et al. (2004) introduced fine bed sediment into in-stream enclosures in a northern California river and observed that juvenile salmonid growth and survival were negatively correlated with increasing levels of sediment deposition. Suspended sediment effects are often categorized as either direct mortality or as sub-lethal effects (Waters 1995; Newcombe and Jensen 1996). Sub-lethal effects are further categorized as behavioral changes (e.g., avoidance), reduced feeding and growth, respiratory impairment, and general physiological stress often resulting in reduced tolerance to disease and toxicants (Waters 1995; Newcombe and Jensen 1996).

Newcombe and Jensen (1996) provide the most comprehensive review of suspended sediment effects on fishes to date. They conducted a meta-analysis of existing studies and derived six models that predict the level of lethal or sub-lethal effect that various combinations of suspended sediment concentration and duration of exposure will have on selected fishes. Although they included 48 fish species or taxa in their meta-analysis and models, only 11 species would be considered common to Midwestern warmwater rivers and streams; these species did not include the two species white sucker *Catostomus commersoni* and smallmouth bass *Micropterus dolomieu*. Further, they found little empirical data for short-term (i.e., 3–7 hr duration) effects of sediment on freshwater nonsalmonids or for coarse sands with grain sizes larger than 0.075 mm. As a result, Newcombe and Jensen (1996) recommended that future research examine the effects of different sediment particle sizes and particle angularity (Newcombe and Jensen 1996). Other studies have considered effects of grain size and shape. For example, Lake and Hinch (1999) found that larger more angular sediment particles resulted in greater physiological stress responses in juvenile coho salmon *Oncorhynchus kisutch* in a laboratory study. Such differences in particle size and angularity would be expected from different sediment sources and further illustrate the need for comparative studies of the effects of different sediment types on fishes.

While direct mortality effects are obvious, sub-lethal effects on fish health, including physiological stress, are also important. Sub-lethal effects on health can ultimately result in population-level responses, such as through impacts to reproduction (Adams 1990). Sub-lethal effects on fish health represent the mechanisms explaining subsequent individual mortality and population-level declines. These mechanisms explaining observed patterns are often lacking and highlight the need for new studies examining sub-lethal effects on aquatic organism health from differing sediment concentrations and particle sizes.

High levels of suspended sediment reduce aquatic health in numerous ways. Excess sediment reduces light transmission, which diminishes the feeding success of trout and other fish species that rely on visual acuity (Sweka and Hartman, 2001). In addition, the decrease in transmission of photosynthetically active radiation (PAR) reduces the viability of submerged aquatic vegetation and periphyton and the heterotrophic communities they support (Yamada and Nakamura 2002; Wharton et al. 2006). Inorganic particles can also interfere with the operation of fish gills and macroinvertebrate feeding, reducing productivity (Ryder 1989; Newcombe and Jensen 1996). Silt and fine sand, which are often transported by saltation or near-bed suspension, may also affect benthic habitat through direct abrasion (Ryan, 1991). Moreover, gravel beds serve as spawning grounds for many species of fish, and the creation and maintenance of these coarse grained zones is of prime importance for stream restoration projects. Fine suspended sediments deposited on the stream bed can reduce hyporheic exchange and smother eggs, preventing recruitment (Carling and McCahon, 1987). Finally, the deposition of fines in gravel beds reduces the complexity of stream substrate by clogging the interstices between larger sediment particles (i.e., increasing embeddedness), which reduces the available habitat for benthic organisms (Wood and Armitage, 1997; Wood et al., 2005; Suttle et al., 2004).

There are many ways of measuring suspended sediment levels, including both direct measurements of concentration and proxy measures such as turbidity. Suspended matter causes light to be scattered and absorbed rather than transmitted; clay, silt, fine-grained particulate organic matter, bacteria, plankton, other microscopic organisms, and chromophoric dissolved organic matter (CDOM) can all contribute to turbidity and light attenuation (Davies-Colley et al., 1986; Belmont et al., 2007; MPCA, 2007b). Measurements of total suspended solids concentration are typically obtained from water samples that arefiltered and processed in a laboratory. Turbidity is an optical property of water in which the amount of light scattered by a given water sample is compared with that scattered by a standard sample. Attenuation is another optical property that accounts for absorption and scattering of light, typically measured for multiple wavebands (Bohren and Huffman 1983). Further methodological differences are related to the timing and location of measurements. Laboratory measurements are typically robust but cannot account for larger sediment particles, especially fine sands, that may be suspended in the water column in the field but will rapidly settle out in a cuvette.

It is possible to develop robust relationships between turbidity and suspended sediment concentrations in a river reach or system where the grain size distribution and optical properties of the suspended sediment are spatially and temporally constant. For example, Stubblefield et al. (2007) report a high correspondence ($r^2 = 0.91$ and $r^2 = 0.95$) between turbidity in the range 0-50 NTU and suspended sediment concentration in the range 1-100 mg/L in two creeks in the Lake Tahoe basin in California. At larger spatial scales landscape heterogeneity causes a more variable relationship. A large data set of paired suspended sediment and turbidity values from tributaries of the Minnesota, Lower Mississippi, Cedar, Des Moines, and Missouri rivers indicates a variable relationship between suspended sediment and turbidity: for the 25 NTU standard established by the US Environmental Protection Agency, a suspended sediment concentration of 60 mg/L in the Western Corn Belt Plains ecoregion or 100 mg/L in the Northern Glaciated Plains ecoregion constitutes a violation (Fandrei et al., 1988; MPCA, 2007a).

However, when the optical properties of the water and sediment mixture differ, which occurs as a result of differences in watershed slope, soil type, geology, precipitation, and other factors, turbidity will also differ, even if the overall suspended sediment concentration remains the same (Campbell et al., 2005; Belmont et al., 2007). As a result, it is often recommended that turbidity meters be calibrated for suspended sediment concentration within each basin. Even then, turbidity cannot always be used to predict suspended sediment concentrations accurately (Riley, 1998). In fact, particle size characteristics, which are closely tied to optical characteristics, can vary over a few hours through the course of a storm event, especially if flocculation is present (Williams et al., 2007). Moreover, methodological factors also affect the turbidity reading; these factors include different sensor technology and different sampling techniques (Effler et al., 2007;

MPCA, 2007b). In fact, identically calibrated instruments with different optical designs can measure turbidity levels that differ by a factor of two or more for the same sample (MPCA, 2007b). In practice, the brief delay that inevitably occurs between the time a sample is poured into a cuvette and the time that the turbidity measurement is made introduces an inherent limitation for measuring turbidity in water that contains sediment coarser than silt.

It is likely that different size distributions of particles, which often have different shapes and origins, have different biological impacts. Previous work has shown that decreases in water clarity can account for the biological impairment caused by fine particles (Davies-Colley et al., 1992). On the other hand, larger particles (silts and sands) would also be expected to directly abrade the bed surface and settle out, smothering the bed (Ryan, 1991; Wood et al., 2005). Turbidity is a metric that is well suited to account for effects caused by fine-grained particles that remain in suspension, but is less well suited to address sediment-related effects coarse-grained suspended sediment that travels near the bed. To our knowledge, however, no previous work has directly compared the biological effects of different particle size classes. As noted above, sediment impacts on stream systems result from both its effect on water clarity and its physical characteristics. Many states, however, including Minnesota, have a water quality criterion based on turbidity alone (MPCA, 2007a; MPCA, 2007b). In systems with spatial and temporal variability and thus weak correlation between turbidity and total suspended sediment, turbidity measurements may not provide adequate estimates of suspended sediment concentrations, indicating that alternative measures are needed (e.g., DFO 2000).

Despite widespread recognition of the negative impact of sediment loading in streams, explanations of the most important mechanisms of impacts are still speculative. In addition, few studies have isolated sediment loading from other co-varying physical factors such as flow velocity, turbulence, channel depth, and planform morphology. Finally, most studies to date of the effects of suspended sediment and turbidity on fishes have been completed on salmonid species with fewer studies on warmwater fishes (Waters 1995; Vondracek et al. 2003).

Here, we discuss several critical aspects of sediment pollution, with the goal of developing a better understanding of the information provided by each of the sediment-related metrics and informing future water quality management decisions. We test the null hypothesis that turbidity is the most important factor by experimentally manipulating turbidity levels within an outdoor stream ecosystem and observing impacts of turbidity on physical metrics (embeddedness, permeability, light attenuation) and macroinvertebrates. Trials were performed under high-flow conditions, which often accompany high turbidity levels and typically exert substantial stress on aquatic ecosystems.

METHODS

Experiments were performed during July and August 2008 in the Riparian Basin of the Outdoor StreamLab (OSL) at St. Anthony Falls Laboratory (SAFL), University of Minnesota, Minneapolis, Minnesota, USA. During 2008, the 40-m by 20-m basin was configured into a small sand-bed meandering stream within a floodplain (). Stream flow was initiated on June 19, 2009. Screened water from an impoundment on the Upper Mississippi River flows through the OSL, which makes the water quality of the experimental stream (0.5-2.0 mg/L NO_3-N, 20-90 µg/L PO_4-P in summer months) comparable to local small urban and agricultural warmwater streams (Metropolitan Council 2005). The mobile bed sediment is coarse sand with a median grain diameter of 0.7 mm.

The macroinvertebrate portions of the present project occurred in two 8-m-long constructed riffle regions with 2 1/2” non-mobile cobble located in the two meander bend crossings. Macroinvertebrates from Mississippi River drift colonized these riffle regions prior to the project start; additional taxa likely aerially recruited. Fish studies occurred in four 122-cm long, 67-cm wide, and 113-cm high completely enclosed 1.2-cm wire mesh pens installed side by side at each of the upstream and downstream ends of the stream, completely spanning the channel. To provide fish with natural substrate, pen bottoms were excavated below the local bed elevation, then bed material (limestone rock riprap) was placed within them. Pens protruded above stream flow at all times. A portion of each pen was shaded. Two upstream pens were stocked with smallmouth bass and two were stocked with white suckers; downstream pens were stocked in the same way.

Experimental manipulations consisted of simulated storm events, which were initiated by increasing the flow rate from the normal low flow to more stressful high flow bank-full conditions. During the first six hours of the high flow, suspended sediment was introduced halfway down the experimental stream, so that the upstream constructed riffle and upstream fish pens served as a control for the downstream reach. Six hours is the order of magnitude of the length of the peak of a storm hydrograph within local first-order streams (Metropolitan Council 2005). After six hours, the release of excess suspended sediment ceased, but high flows were sustained for an additional three hours, to approximate a typical storm hydrograph with a first flush of sediment. Storm events were separated by a 5- to 9-day-long period of low baseflow, which enabled stream organisms and to acclimate and recover from the stressful flood periods.

Four different combinations of dissolved and suspended particles were introduced during simulated storm events:

- Trials 1 and 5: clay with low organic content
- Trials 2 and 6: fine sand with low organic content
- Trials 3 and 7: fine loam from a nearby agricultural drainage ditch (County Ditch 78, Vernon Center, Minnesota)
- Trials 4 and 8: inorganic mixture with the same grain size distribution as the agricultural ditch sediment

During Trials 1, 4, 5, and 8, sediment was released just downstream of the control riffle (at the upstream end of a shallow pool) from six ports oriented longitudinally and distributed uniformly across the stream width. During Trials 3 and7, screened drainage ditch soil was released just downstream of the control riffle from two ports directed transverse to flow. During Trials 2 and 6, fine sand was released just upstream of the treatment riffle from eight vertical ports to prevent settling in the shallow pool between the riffles. Visual inspection and turbidity samples confirmed that the sediment was distributed across the width of the stream prior to entering the downstream riffle.

To provide replication of the measurements of physical habitat, all trials were repeated twice within the sampling season. Between Trials 4 and 5, a rake was used in both constructed riffles in an attempt to remove any deposited fine sediment prior to the second set of treatments.

Raking proceeded from upstream to downstream but did not result in any noticeable change in algal coverage or bed composition.

To verify that environmental conditions were adequate for our test organisms, consistent between trials, and similar between upstream-control and downstream-treatments during the acclimation and experimental periods, discharge, dissolved oxygen, water temperature and water velocity were measured throughout the study period. Stream discharge was calculated from stage data obtained at the upstream end of the stream at a 2-m-wide contracted weir at 15-minute intervals using a Massa M300 water level logger. In-stream dissolved oxygen and temperature measurements were logged at 15-minute intervals at upstream and downstream ends of the channel using a Hydrolab. The Hydrolab also contained a turbidity probe, but it rapidly fouled, and data failed quality checks except for immediately following daily cleaning events. Horizontal water velocity measurements were obtained using a two-dimensional sideways-looking acoustic Doppler velocimetry probe (Flowtracker, SonTek/YSI). The Flowtracker was used to acquire 1-min-long point measurements of water velocity at three different locations within each fish pen. Factory specifications state that the Flowtracker is capable of measuring between 0.1 cm/s and 500 cm/s with an accuracy of 1%.

Grab samples for turbidity analysis were obtained from both the upstream and downstream riffles, and turbidity was measured using USEPA Method 180.1 and an EPA-compliant Hach 2100N turbidimeter. Suspended sediment concentration measurements were obtained using an isokinetic point sampler and were then analyzed in the laboratory for dry weight and ash weight, using Standard Methods 2540B and 2540D (APHA 2005); the difference between these two measures is an index of organic content. Grain size distributions for all sediment types were obtained using a Horiba LA-920 laser-scattering particle size distribution analyzer. A LISST-StreamSide portable in-situ particle size analyzer was used to obtain in-stream samples of particle sizes and volumetric concentrations during Trials 7 and 8.

Bed composition and permeability were measured approximately 16 hr before and immediately (less than 1 hr after) each flood event at nine locations within each riffle. Bed composition in the two riffle regions was measured using a visual method, similar to U. S. Environmental Protection Agency Environmental Monitoring and Assessment Program methods, which rely on quantifying the average fraction of the perimeter of a larger grain that is surrounded by finer sediments (Kauffman et al., 1999, Peck et.al., 2000). At nine fixed locations within each riffle, a Nikon D70 digital SLR camera mounted 12-14" above the bed was used to obtain a photograph of the channel bed under base flow conditions before and immediately (less than 1 hour) after each flood event. A 20.3-cm square metal reference frame was placed on the channel bed centered beneath the camera, and an acrylic sheet was positioned to evenly break the water surface to eliminate surface distortion. Preliminary investigations showed that parallax contributed less than 5% error throughout the field of view, and no corrections were made for image distortion. To determine embeddedness from images, the region of the photograph inside the metal reference frame was broken up into 100 subregions, and each subregion was manually scored for dominant grain size (clay < 2 mm, silt 2 mm-2cm, and sand > 2 cm), using the reference frame as a known length scale.

Permeability was assessed using a 4.5"-diameter Horslov single-ring falling head permeater constructed of clear plexiglass (e.g., Landon et al., 2001). At nine positions within each riffle region, during base flow before and after each flood event the permeater was inserted vertically into the stream until the base of the permeater was 8 cm below the top of the sediment. Water was then placed within the permeater to a depth h1 (usually 25 cm) above the water surface, and the time t it took to drain to h0 = 15 cm above the water surface was recorded. This falling head velocity was converted to an index of benthic permeability based on a constant assumed vertical distance the water penetrated into the bed. The water also likely infiltrated laterally, which is not incorporated in this approach. The resulting permeability value is therefore taken as an index of relative bed permeability.

Spectral light absorption measurements were made using a Shimadzu 1601 UV-Visible dual beam spectrophotometer following the methods of Belmont et al. (2007). A total of 12 samples were collected for analysis during the experiments run on 7/28/08, 8/6/08, and 8/16/08 with samples being collected at approximately 10:00am and 2:00pm at each of the riffles. Samples were filtered with Whatman glass fiber filters (GF/F, with nominal retention size of ~ 0.7 µm). Filtrate was used to measure spectral absorption by dissolved organic matter using a 10 cm quartz Supersil™ cuvette referenced to air. The effect of light scattering by the cuvette was eliminated by subtracting the average optical density measured between 775 and 800 nm from the entire optical density (OD) spectrum (200 – 800 nm), because absorption by dissolved organic matter is negligible in these wavelengths and scattering is assumed to uniform across the spectrum. Dissolved absorption coefficients ($a_{d\lambda}$) were calculated using Equation 1 as 10 point averages centered on the wavelength of interest.

$$a_{\mathrm{d}}(\lambda) = (A_{\mathrm{d}}(\lambda) - A_{\mathrm{di}}(\lambda)) \times d_{\mathrm{p}} - A_{775-800}$$

Equation 1

where $d_{\mathrm{p}} = (LN(10) \times 100/L)$

A_d and A_{di} are the OD of the sample and deionized water, respectively and d_p accounts for the length of the cuvette where L is in centimeters.

Particulate absorption was measured using an adapted quantitative filterpad technique whereby particles are concentrated on a Whatman GF/F filter via filtration and absorption is measured in the dual beam spectrophotometer reference against a moist Whatman GF/A filter. For this method, approximately 50 mL of deionized is passed through the GF/F and GF/A filters and a baseline spectral OD measurement (280 – 800 nm) is made on the blank filters. Subsequently, the GF/F filter is removed and a measured volume of water is filtered and OD is again measured in reference to the clean GF/A filter. Geometric corrections are applied to account for pathlength and multiple scattering as the light passes through the filter and the particulate absorption coefficient ($a_{p\lambda}$) is calculated using Equation 2 (Roesler, 1998) with a pathlength amplification factor (β) of 2.0.

$$a_{\mathrm{p}}(\lambda) = (\frac{1}{\beta}) \times p \times \mathrm{OD}_{\mathrm{f}}(\lambda)$$

Equation 2

$$p = LN(10) \times (\frac{100}{d_g})$$

where

and d_g is the geometric pathlength, calculated as the volume filtered divided by the effective filter area (22 mm for the 25 mm GF/F filter).

The dissolved absorption coefficient, particulate absorption coefficient and absorption coefficient for pure water are summed to calculate the total absorption coefficient ($a_{t\lambda}$), which quantifies beam absorption. Following the rationale of Belmont et al. (2009), we apply a wavelength dependant correction factor μ, calibrated under blue sky conditions, to account for the diffuse nature of natural light to calculate attenuation coefficients ($K_{d\lambda}$):

$$K_d(\lambda) = \frac{a_t(\lambda)}{\mu(\lambda)}$$

Equation 3

Drift measurements were obtained at three cross-sections: (1) entering the constructed channel above the control riffle, (2) leaving the control riffle and entering the treatment riffle, and (3) at the exit of the constructed channel below the treatment riffle. Measurements were obtained using a commercially available drift net (45-cm wide by 30-cm high opening with 500 μm mesh) placed at the deepest portion of the channel cross-section, except for the flood on 7/17 when it was placed at midwidth. All macroinvertebrates from drift were collected and identified to genus, except for Chironomidae, which were identified to Family, and Diptera pupae, which were identified to Order. Sampling duration was long enough to collect approximately 70 individuals per sample. Most measurements were 8 min during flood conditions and 15 min during base flow. For all samples, the net was placed so that its base was level with the channel bottom. During flood events, the top of the net was at or just below the water surface. During baseflow, the top of the net extended above the water surface and only the wetted depth of net was used in calculating drift density. A Sontek Flowtracker was used to obtain 30s velocity records at middepth adjacent to each of the three net positions before and during 6 of the 8 flood events. Because the flow rate was very similar during all base flow and flood flow measurements, measurements of the horizontal flow speed (vector sum of mean horizontal flow components) at each location at each flow rate were pooled, and the uncertainty in flow rate was calculated as the standard error of these six different measurements.

Benthic density samples were taken before and during each flood event at two random locations within both the control and treatment riffles. To avoid resampling areas before recolonization, if the chosen location was within 0.5 m of a sample obtained within the past week the selection process was repeated until a novel site was identified. Measurements were obtained using a commercially available Surber sampler (base size 31 cm or 15 cm square with 500 μm mesh). Substrate within the sampling area was carefully cleaned of macroinvertebrates and fine sediment agitated in the flow. A subsample (approximately 50 individuals) was obtained using a commercially available subsampler and identified in the same manner as the drift samples.

Differences in invertebrate drift numbers and richness between upstream and treatment reaches, low flow and high flow without sediment (effect of flood only), and low flow and high flow within each sediment type were compared with one way ANOVAs. The difference in drift density across floods with different sediment types was evaluated by subtracting the mid reach samples from the downstream samples at sampling period S1 and S2. Density data were log (density+1) transformed for normality before analyses. Richness was assessed as presence or absence of individual taxa; richness data were not transformed.

During Trials 6 and 7, fishes were stocked in the net pens. White suckers were purchased from local bait shops, measured for total length, weighed, randomly placed in net pens that were separate from pens containing smallmouth bass, and given a unique fin clip. Twenty-four white suckers were placed in net pens for Trial 6 and 26 were used for Trial 7. Wild smallmouth bass were collected from Pool 4 of the Upper Mississippi River, Lake City, Minnesota, by electrofishing (fine-sand trial) and the Cannon River, Red Wing, Minnesota, by angling (agricultural-soil trial). For Trial 6, nineteen smallmouth bass were captured by electrofishing, transported in coolers to the OSL, measured for total length, weighed, given a unique fin clip, acclimatized for approximately 30 minutes by successive dilution in OSL water, and randomly placed in net pens. In an attempt to reduce handling stress, for Trial 7 we collected 24 smallmouth bass by angling at a location that was closer to the OSL facility.

Fishes were kept in pens for five days (white suckers) or six days (smallmouth bass) to acclimate to ambient conditions in the OSL before each simulated storm. Fishes that did not survive this period were removed from pens and not considered during further analyses. Mortality was moderate during the acclimation period. Eight to 13 individuals (average 12) were initially stocked into each of the upstream and downstream pens, but only five to seven individuals survived the acclimation period in each pen. Fishes had access to macroinvertebrates in stream drift and rock substrate and were also fed 4% of their combined body weight in bloodworms twice throughout each trial period. The majority of non-surviving fishes died within 1-2 days of introduction into the OSL.

Fishes surviving the acclimation period were a range of sizes. The 12 white suckers surviving for the fine-sand trial ranged from 164 to 208 mm with weights from 42 to 78 g. The 13 white suckers surviving for Trial 7 were smaller than those used in Trial 6, ranging in length from 122 to 155 mm and in weight from 18 to 32 g. Lengths of the 14 bass surviving for Trial 6 ranged from 188-329 mm, but most (79%) were less than 290 mm. Weights ranged from 85 to 409 g. The 13 bass surviving for Trial 7 ranged from 202 to 274 mm and weighed from 108 to 309 g.

Fishes were monitored during and immediately following addition of sediment to ascertain direct mortality effects. Fishes were considered dead if there was no movement of gill covers and if fishes were unresponsive to external nudging by biologists. Health assessment of fishes alive at the conclusion of each trial followed procedures in Goede (1991) and Adams et al. (1993), where visual examinations were made of fins, skin, eyes, parasites, gills, pseudobranchs, liver, hindgut, spleen, kidney, and thymus. Prior to health assessment, fishes were euthanized in a 300 mg/L solution of tricaine methanesulfonate (MS-222) in groups of three to five for processing. Each fish was individually identified by its unique fin clip, measured, and weighed. Gills were immediately examined to avoid any false observations from post-mortem changes. Fishes were then placed in a refrigerator while examinations were conducted on each individual fish.

Two workers conducted health assessment index (HAI) examinations using the rapid technique develop by Adams et al. (1993) to evaluate fish health under field conditions. Their fish HAI is intended to be a quantitative, yet rapid and simple, index to assess overall fish health based on visual observations of gross external conditions and internal autopsy of specimens (Adams et al. 1993). HAI observations began with evaluation of external components including fins, skin, eyes, and parasites. Internal examinations began near the head with evaluation of the pseudobranchs and thymus followed by the liver and spleen before concluding with the hind gut and kidney. Examinations were conducted with the naked eye and a dissecting microscope and usually required 2-4 minutes per fish to complete.

The determination of the condition of organs and structures was aided by descriptions in Goede and Barton (1990), Adams et al. (1993), and especially pictures in Goede (1991). Condition of fins, skin, eyes, gills, pseudobranchs, thymus, and parasites represented external health whereas liver, hindgut, spleen, and kidney indexed internal health. Each of the 11 internal and external variables was assigned a value between 0 and 30 depending on condition of the organ or structure (Adams et al. 1993). Summation of scores across variables provided a HAI score for either internal components, external components, or overall health. Normal conditions for all variables were assigned a 0, therefore lower HAI scores represented healthier fishes.

We examined short-term effects of sediment on mortality and health of white suckers and smallmouth bass with summary data and Kruskal-Wallis non-parametric tests. Comparisons were made among combinations of control vs. treatment cages and sediment types (i.e., Trial 6 control vs. Trial 6 treatment vs. Trial 7 control vs. Trial 7 treatment). Individual fishes were considered experimental replicates, and white suckers and smallmouth bass were analyzed independently for non-parametric tests. If mortality was observed, it was quantified as percent of individuals killed. HAI scores are often non-normally distributed and our data were similar, thus necessitating the non-parametric Kruskal-Wallis tests. Such tests have been used by others to compare HAI scores (e.g., Kovacs et al. 2002). Three separate Kruskal-Wallis tests ($P < 0.05$) were run for each fish species, one for each of the three health indices (i.e., overall HAI score, HAI for external components, HAI for internal components) following procedures in SAS (1991) and Neumann and Allen (2007). Selected individual health assessment index variables (e.g., scores for skin, gills, liver) were also summarized and interpreted (Adams et al. 1993).

RESULTS AND DISCUSSION

The precise physical control of the OSL enabled us to attain the same flood flow rate during each simulated storm event and similar conditions during each preceding acclimatization period (Figure 2, Figure 3, and Table 1). Benthic organisms were subjected to similar conditions in the two riffle regions, and fish experienced similar conditions in the two sets of net pens. Flow velocities during flood events were similar between the two riffles (Figure 4), with an average horizontal flow speed of 90 ± 30 cm/s and 15 ± 3 cm measured in the upstream riffle (mean ± standard deviation) and 80 ± 20 cm/s and13 ± 4 cm in the downstream riffle.

Depth and velocity conditions were also similar between trials and control and treatment fish pens and provided suitable habitat for the fishes used in this study. Average water depth in upstream pens was 29 ± 5 cm (mean ± standard deviation) under base flow conditions and 38 ± 8 cm during bankfull discharge; the average depth in downstream pens was 38 ± 2 cm under base flow conditions and 52 ± 6 cm during bankfull discharge. Depths between 25 and 125 cm are suitable for juvenile smallmouth bass, although adult smallmouth bass use deeper waters (50-250 cm; Aadland and Kuitunen 2006). Depths between 5 and 75 cm are suitable for the juvenile-sized white suckers used in this study (Aadland and Kuitunen 2006). Water velocity was not different between upstream and downstream pens ($P > 0.2$, n = 24). The mean velocity was 5 ± 3 cm/s under base flow conditions and 37 ± 13 cm/s during bankfull discharge, which provided suitable conditions for the smallmouth bass and white suckers used in this study (Aadland and Kuitunen 2006).

Environmental conditions were similar among trials and treatment and control locations, except for our primary variables of turbidity in the downstream end of the stream (Figure 3 and Table 1). Stream water temperature was between 22.2 and 28.5ºC, with approximately 1ºC diurnal variation and no consistent difference in temperatures between upstream and downstream ends of the 50-m-long stream. Dissolved oxygen levels were between 6.7 and12.6 mg/L and 82 and 158% of saturation, with approximately 2 mg/L diurnal variation and no significant difference between upstream and downstream ends. Turbidity in the upstream riffle during base and flood flow was between 5 and 56 NTU, with an average of 10.2 ± 0.4 NTU (mean ± standard error of the mean).-These temperature, dissolved oxygen, and turbidity levels are appropriate for a small warm-water stream.

Between 930 and 1450 kg of sediment was added to the stream during simulated storm events. The grain size distribution of the four different sediment types was quite different, ranging from a median grain size of 0.004 mm for the clay up to 0.1 m for the fine sand (Figure 5). The suspended sediment addition halfway down the OSL channel created elevated levels of suspended sediment within the downstream riffle; if all sediment released traveled as suspended load, the suspended sediment concentration would have been between 0.2 and 0.3 g/L (Table 1). This sediment addition markedly increased the observed turbidity within the downstream rffle during Trials 1, 3, 4, 5, 6, and 8 to between 89 and 160 NTU (Figure 6). The largest turbidity values were produced during Trials 1 and 5, when clay was added to the stream, even though these additions had the lowest amounts of mass added to the stream. The small clay grains were able to produce such high turbidity values not only because they had a slow settling velocity but also because the same mass of sediment will comprise many more particles and much more surface area that can scatter light. The smallest turbidity values in the downstream riffle measured using a laboratory meter were observed during Trials 2 and 6, when fine sand was added to the riffle. In fact, using a laboratory instrument, the measured turbidity for the downstream riffle during Trials 2 and 6 was the same as the levels measured in the upstream riffle. The low observed turbidity values likely resulted from rapid sand settling prior to measurement in a laboratory cuvette. Measurements of the in-stream grain size distribution confirmed that a significant fraction of the sand added to the system during the fine sand and agricultural soil trials was coarse, with typically 20% or more of sediment with a median grain size of 0.9 µm or smaller (Figure 7).

To further explore the effect of grain size distribution on measured turbidity values, we measured the turbidity associated with known concentrations of three of our four sediment types (clay, sand, and a mixture) using both standard laboratory procedures and also with an in-situ probe in a continuously stirred container, which simulates the high levels of turbulence observed in riffle regions where even coarser grain size fractions are more likely to become suspended in the water column. In-stream and laboratory measurements of the relationship between turbidity and concentration were indistinguishable for both clay and the inorganic mixture (Figure 8). The similar relationship for these two sediment types confirms that the finer size fraction (eg., the clay) is important in controlling the relationship between turbidity and suspended sediment concentration. The relationship for sand measured in situ was much different; for the same concentration of sand, a lower turbidity value was produced, likely because fewer grains are in suspension, and perhaps also because of the increased tendency to settle. Finally, laboratory measurements of sand turbidity were extremely low, much lower that comparable measurement in situ, confirming that laboratory techniques cannot provide an accurate assessment of the properties of coarser fractions.

Spatial and temporal patterns were observed in the benthic environment within riffle regions where macroinvertebrate populations were monitored. There was no difference in the average permeability of the two riffles (ANOVA, $p>0.05$) but, taking measurement date into account, the spatial position within each riffle was significant (two-way ANOVA, $p = 0.02$, $df = 17$, $F = 1.85$). There was a consistently lower fraction of sand in the upstream riffle (paired t-test, $p<0.001$, $n = 143$) and higher fraction of gravel in the upstream riffle ($p<0.001$, $n = 143$); the fraction of cobble was not significantly different between the two riffles at any point during the summer. Measurements of permeability and embeddedness were related; beginning with the fourth flood, log-transformed permeability was able to explain a significant amount of the variability in the observations of bed fraction covered by sand (ANOVA including date, $p = 0.02$; ANOVA with permeability alone, $p=0.005$). There was also consistent spatial patterning in bed characteristics within the riffle regions. In particular, the river right measurements within the upstream riffle and the river left measurements within the downstream riffle were more permeable than those on the upstream river left and center and downstream river right and center (pairwise t-test, $p=0.03$, $n =108$; Figure 9). This trend developed over time; it was not present within the first couple floods (paired t-test, $p>0.05$). A similar trend was evident in measurements of bed composition. For example, the overall fraction of cobble in the surface layer declined over the summer, but throughout the summer there was a higher fraction of cobble (lower embeddedness) on the outside of the bends (paired t-test, $p<0.001$; Figure 10), in regions where permeability was elevated.

Light absorption coefficients and attenuation coefficients are presented along with turbidity measurements for numerous samples in Table 2. We have chosen to present data from 320 nm and 440 nm as representative of the ultraviolet and visible wavebands. Dissolved absorption coefficients $a_{d\lambda}$ for 320 nm and 440 nm light are remarkably consistent (13.2 – 17.3 m^{-1} and 1.4 – 1.9 m^{-1}, respectively), indicating little variability in the optical properties of dissolved organic matter between 7/28/08 and 8/19/08. The downstream site exhibited consistently lower dissolved absorption by a small margin for each of the synchronously collected samples, though the short residence time in the system makes it highly unlikely that the difference between the two can be attributed to photodegradation of dissolved organic matter. Particulate absorption is moderately variable at the upstream site (1.1 – 5.2 m^{-1} and 0.7 – 2.9 m^{-1} for 320 and 440 nm light, respectively) and highly variable at the downstream site both within and among runs. For example, taking all experimental runs together, particulate absorption for 320 nm light at the downstream riffle ranges 3.8 to 32.7 m^{-1}. During the 8/19/08 experimental run particulate absorption ranged from 3.8 to 15.6 m^{-1}. Particulate absorption for 440 nm light was systematically lower, as should be expected the wavelength dependant nature of light absorption, and variability was less, ranging from 2.0 to 9.8 m^{-1} for all of the experiments taken together with the largest difference within any given experimental run occurring on 8/19/08 (2.0 to 7.8 m^{-1}).

The attenuation coefficient for any given wavelength was positively correlated to turbidity, however the slope of the relationship is wavelength dependant and the strength of the relationship is poor, with r^2 values ranging from 0.45 to 0.52 (Figure 11). The slopes from linear regression of turbidity and the attenuation coefficient for 320, 380, 440, and 675 nm light are 0.13, 0.08, 0.06, and 0.03, respectively, indicating that the wavelength dependence of the turbidity/attenuation relationship is nonlinear. The implication of this observation is that turbidity as a metric yields very limited information regarding the spectral optical environment in aquatic ecosystems. Aquatic organisms can exhibit waveband specific minimum light requirements or maximum light tolerances, though much work remains in developing an understanding of what those limits are for different taxa. Clearly the data here demonstrate that our ability to understand those limits and appropriately manage stream ecosystems is severely limited if only turbidity is considered. Although the short-term treatments performed in this study did not elicit an acute response by macroinvertebrates, we have demonstrated significant differences in the optical environment as a result of the introduction of different sediment compositions. Such differences in the optical environment are likely to be more important in streams that have chronically higher suspended sediment concentration.

Table 3 indicates the percentage of incident 320 and 440 nm light remaining at 10 cm water depth and the percent of light attenuation that can be attributed to suspended particles. Not having measured the amount of ambient radiation throughout the course of the experiments it is impossible to calculate the absolute light intensity on the benthos. However, for the purpose of this study the relative amounts of attenuation are more important. In the upstream, control riffle approximately 11-13% of incident 320 nm radiation and 50-70% of 440 nm radiation remains at 10 cm water depth. At the downstream, treatment riffle 320 nm light is reduced to <1% of incident radiation for most of the treatments. The percentage of attenuation that can be attributed to suspended particles varies substantially within and among experimental runs.

Overall there were 29 taxa sampled in each of the drift and the benthos. Invertebrates were observed associated with stream constructed riffle regions within three weeks of the start of flow on June 19 (Figure 12). Invertebrate drift leaving the flue showed significantly higher richness than the source water entering the flume when compared across all samples taken at every time period (t-test $p<0.06$, $n = 34$). This difference was mostly due to flood events, as there was no evidence of difference in richness during low flow (t-test $p>0.3$, $n = 9$). However on average only one more taxon was found in the downstream drift than the upstream drift making it unlikely that the flume was an ecologically relevant source of diversity to the river. While the additional taxon was not consistently the same, on 9 dates there were petrophila *ssp*. leaving the flume but not entering it, suggesting there could be localized recruitment in the flume of species not otherwise present.

Downstream drift had significantly higher invertebrate density than entering water (t-test $p<0.01$, $n=34$) and the flume was acting as a net source of invertebrates to the river. On average the outgoing water had 5.1 more individuals m^{-3} than incoming water for all samples. The low flow difference was significant (t-test $p<0.08$ $n = 9$ average 10.9 more individuals out m^{-3}) and high flow difference was significant (t-test $p = 0.02$ $n =25$ average 3 more individuals out m^{-3}). There was no evidence that richness varied between low flow to high flow sampled before sediment feed began ($p>0.5$). Similarly there was no evidence of a difference between low flow and high flow for density ($p>0.5$). When all sediment addition floods are taken together, there is no evidence of a difference between means of richness with the addition of sediment ($p > 0.9$). There was also no evidence for difference in density with the addition of sediment during high flow ($p >0.06$).

With the exception of Trial 2 (the first sand release) there was no difference between the control, pre-treatment and post treatment in the downstream reach. There may have been an initial colonization, or seasonal, impact on densities in the first flood, but following that, invertebrate density was fairly consistent across experimental floods. There was no evidence of reduction of densities as a result of the floods, and therefore no suggestion that densities failed to rebound before subsequent floods. This was different from another flume study of sediment impact on flume invertebrates (Connolly and Pearson, 2007), probably because the OSL is outdoors and is in close proximity to the Mississippi River.

Elevated levels of fine sand or agricultural soil had little effect on direct mortality or short-term health of smallmouth bass. No direct mortalities were observed during or immediately following either sediment treatment. Although at the sub-lethal level overall HAI scores were slightly lower for smallmouth bass in the agricultural soil trial (indicating better health), overall health effects were not significantly different among trials and control vs. treatment fish (Table 4). Neither external nor internal components of the HAI showed significant differences among trials and control vs. treatment fish (Table 4).

Elevated levels of fine sand or agricultural soil also had little effect on the mortality or short-term health of white suckers. As with smallmouth bass, no direct mortality of white suckers was observed during or immediately following either sediment treatment. Within the trial for each sediment type, white suckers in upstream control pens tended to have lower overall HAI, external HAI, and internal HAI scores, suggesting better health for those fish not exposed to elevated sediment levels (Table 4). Worse health scores in downstream treatment pens tended to be due to external parameters related to the fins, skin and gills, and possibly to internal parameters of the liver and spleen (Table 5). However, none of the differences between upstream-control and downstream-treatment pens were statistically significant for either sediment trial (Table 4).

In general, HAI scores tended to indicate that white suckers were in better health than smallmouth bass. This was primarily due to internal HAI scores (Tables 2 and 3), which were 17.8 points lower (better) for white sucker than for smallmouh bass. Liver condition accounted for most differences (Table 5). Most common liver anomalies observed in smallmouth bass were nodules. For external HAI scores, more smallmouth bass tended to have anomalies associated with fins, skin, and eyes whereas more white suckers had gill anomalies. Many white sucker gills were marginate or frayed. Finally, both species tended to have higher HAI scores indicating worse health in the fine sand trial than in the agricultural soil trial (Table 4). This was especially evident for smallmouth bass in the upstream control pen in the fine sand trial where these fish had the worst mean overall HAI score of any of the fishes used in the study. This suggests that there might have been a trial effect also.

CONCLUSION

This study found few effects on macroinvertebrate ricness or diversity or the mortality or health of white sucker or smallmouth bass that could be attributed to six-hour exposures of elevated levels of fine sand or agricultural soil sediments. This information lends some support to the Minnesota state turbidity standard of 25 NTU and contributes new information on the effects of these sediments on some common Midwestern communities that will be useful in future syntheses of biological responses to sediment pollution. Additional sampling effort and additional replicates would have allowed us to detect more subtle responses (e.g.., the impact on rare organisms) and also to further determine whether a small relationship exists. Even the sampling protocol outlined here, however, should have been able to determine whether a simulated storm event had a large effect on the downstream aquatic community.

The lack of aquatic community response to a short-term sediment release suggests that stream ecosystems may be able to response to acute sediment loading, especially in impacted urban and agricultural streams that have developed to face some amount of loading. These results should be considered when allocating resources for water quality improvement and stormwater best management practices. In addition, this observation suggests that downstream communities may be able to weather the short-term sediment pulse released in a small dam removal.

The observed lack of organism response may be due in part to the type of aquatic community tested here. The tolerant macroinvertebrate community that established naturally was supplied by drift and aerial recruitment from the highly impacted Mississippi River. The two species of fish examined, white suckers and smallmouth bass, are known to be tolerant of warm temperatures and relatively poor habitat conditions. In fact, both fishes are common throughout streams of the Midwest where non-point source sedimentation is prevalent. Different aquatic communities, especially those that are more sensitive to water quality, may respond differently to the experimental manipulations performed here.

Previous studies have observed sediment impacts on aquatic communities for turbidity levels of less than 100 NTU, which has led the MPCA to set 25 NTU as the state standard (MPCA, 2007a). By finding no community response as a result of short-term sediment loadings, the present study provides support for a more sophisticated water quality standard. One possible standard would consider exposure duration in addition to exposure severity; this separation of chronic from acute effects has been adopted in many federal regulations as the most defensible method to protect human health. Another approach is to adopt biologically based water quality criteria, which have been proposed in Minnesota and elsewhere. An improved understanding of the effects of suspended sediment on aquatic communities should help managers identify and mitigate probable ecosystem stressors. These results should assist federal and State agencies in modifying their TMDL program and better protect the water quality of America's rivers and streams.

Our study design used very specific constraints (i.e., six-hour duration, evaluation of immediate effects during and after sediment addition, and selected indices of macroinvertebrate community fish health), but within these constraints, our design strongly supported the notion of little acute effect of elevated levels of agricultural soil. This study did not address other effects of turbidity levels exceeding 25 NTU, such as on foraging success and reproduction, which may become important in a chronically impacted waterway. For example, the distance that smallmouth bass are able to detect prey items decreases significantly in waters with turbidity readings greater than 10 NTU (Sweka and Hartman 2003) and turbidity during floods can impact young smallmouth bass by inhibiting their ability to visually orient themselves (Larimore 1975). Observations of light attenuation confirm that the addition of suspended sediment decreased light availability at the bed, and it is likely that over time this change in the physical environment will lead to changes in the aquatic community.

Fine sand additions did not result in turbidity levels that exceeded the Minnesota state standard of 25 NTU. Because fine sand drops out of suspension within the measurement cuvette, turbidity measures may not adequately capture the effect of fine sand. If the fine sand traveled near the bottom of the water column, a benthic species such as white sucker may have had greater exposure than the smallmouth bass that dwell higher in the water column, which could explain the lower occurrence of external health problems within smallmouth bass. Others (e.g., UMRBA 2007) have suggested a need for standards to include bedload sediment in addition to suspended sediment, and our data provide some evidence to corroborate this assertion.

Results from fish health during Trial 7 support predictions of Newcombe and Jensen (1996) for the effect of suspended sediment on fish health. Our agricultural soil sediment was of a similar particle size (Fig. 2) to the model of Newcombe and Jensen (1996) for adult, freshwater, nonsalmonid fishes which considers sediment particles < 0.075 mm. For a 7-hr exposure to suspended sediment concentrations of 403 mg/L the model predicts sub-lethal effects only, ranging from moderate to major physiological stress and no mortality. Similarly, our 6-hr exposure of white sucker and smallmouth bass to up to 500 mg/L of agricultural soil resulted in no direct mortality and no significant effects to HAI for either species. Thus, our data corroborates the mortality predictions of Newcombe and Jensen (1996) for a previously unpublished intermediate level of suspended sediment concentration and exposure duration, supporting their model as well as results of other studies that use this model (e.g., Vondracek et al. 2003). Similarly, in his review of biological effects of stream sediment, Waters (1995) reported few published accounts of direct mortality of fishes due to elevated levels of suspended sediment, but also acknowledged the paucity of studies examining this aspect for warmwater fishes. Future studies could evaluate the physiological stress predictions of Newcombe and Jensen (1996) using behavioral indicators and blood chemistry.

Our study also presents some of the first information on the acute impact of coarse sediment loading on fishes. The Newcombe and Jensen (1996) model for adult freshwater nonsalmonid fishes was developed for sediment particles < 0.075 mm diameter, because empirical data for larger particles sizes were unavailable. Sediment particle sizes for our fine sand trial ranged from 0.1 mm to almost 1.0 mm in diameter. Although fine sand had a larger particle size, results from our 6-hr exposure to a concentration of about 200 mg/L were similar to the smaller agricultural soil particles (i.e., no immediate mortality) and suggests that results from the original Newcombe and Jensen (1996) model might be extendable to larger particles sizes. Previous studies have found effects of elevated sediment levels on fishes with similar body structures to those tested in this study, but those effects were only statistically significant at higher concentrations and longer durations (Bergstedt and Bergersen 1997, Lake and Hinch 1999, Sutherland and Meyer 2007). Future field assessments should consider using these body structures as supportive indicators of sediment pollution in rivers and streams.

Although no treatment effects were detected, fish HAI scores were higher (worse) following the fine sand trial as compared with the agricultural soil trial for both species (Table 4), possibly indicating a trial effect. There are a number of possible explanations for the difference in mean HAI scores between fish trials. The fine sand trial took place during a five-day cooling trend whereas the soil trial was during a five-day warming trend (though mean temperatures were not different). Elevated temperatures during the acclimation period for the fine sand trial may have caused unmeasured increases in stress to fishes. Alternatively, fishes may have been subjected to greater stress during collection for the fine sand trial, although efforts were made to minimize handling stress. This explanation is more plausible for smallmouth bass, which were collected by electrofishing from a location further from the OSL for the fine sand trial than for the agricultural soil trial; however white suckers were obtained from a local bait shop for both trials.

Differences in fish HAI between trials may also have been due to hysteresis; that is, the history of the batch of fish used for the fine sand trial (prior to collection) may have made them more susceptible to stress in experimental pens. Differences in internal components of the HAI were mainly due to nodules in the liver (Table 5), which supports hysteresis as a cause (since liver nodules were unlikely to have developed during the experimental period). Smallmouth bass collected from the Mississippi River for the fine sand trial may have had more liver nodules due to previous exposure to chemical pollutants, as suggested for other fish species (Peters et al. 1987). There were also differences in external components of the HAI between trials, generally due to abrasions to the gills, eyes, skin, and fins. External differences could not be attributed to sediment treatments, because of the lack of significant differences between fishes in upstream and downstream pens. However, overall conditions during the sand trial (such as an initially higher water temperature) may have caused fishes to be more agitated and rub against pens more frequently. Pre-sediment assessments of HAI scores may have improved our ability to isolate trial effects from sediment effects, but mortalities during the acclimation period reduced sample sizes to 5-7 individuals in each control and treatment pen and did not permit such pre-treatment HAI assessments.

In conclusion, the experimental setup at the OSL provided a novel opportunity to test the acute effects of sediment pollution. External confounding variables often impair our ability to distinguish the effects of suspended sediment from other factors (Waters 1995) but, by providing a nearly natural stream setting while maintaining similar environmental conditions between upstream-control and downstream-treatment environments, the OSL enabled us to isolate the effects of suspended sediment. Resulting observations support previous sediment-dose-fish-response models for small sediment grain sizes and contribute new information on the effects of larger-sized sediment particles on mortality and selected health indices for white sucker and smallmouth bass. Observations also suggest that a macroinvertebrate community suited to an urban or agricultural site is able to tolerate short-term sediment pulses. An improved understanding of suspended sediment effects on aquatic organisms will enhance our abilities to develop numeric water quality criteria, to determine factors contributing to biotic impairments, and thus to effectively manage water quality of streams and rivers.

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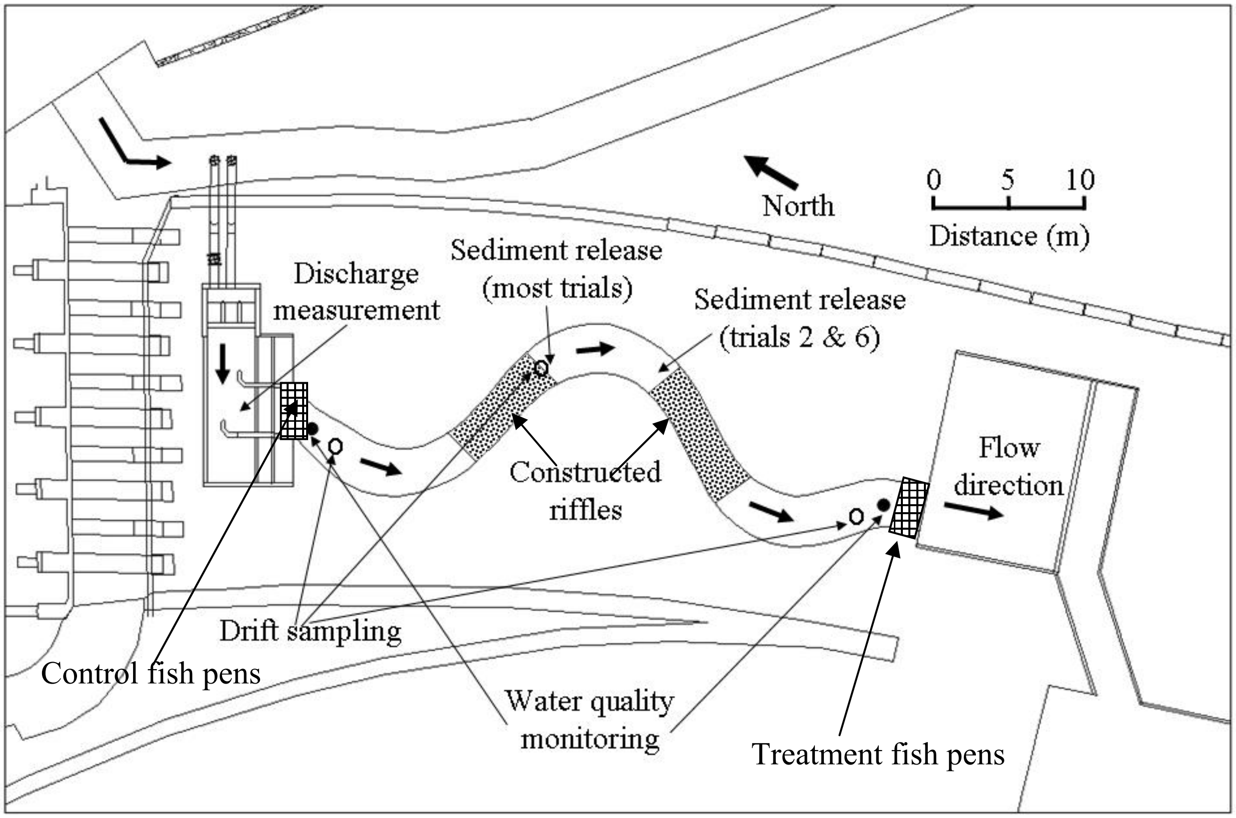

Figure 1: Plan view of the Outdoor StreamLab Riparian Basin at the St. Anthony Falls Laboratory, University of Minnesota. Suspended sediment release locations are marked by arrows. Response metrics were assessed within two constructed riffles, one upstream and one downstream of the releases. Fishes were held in the four fish pens at each of the upstream and downstream ends of the meandering stream.


Figure 2: Photograph of a suspended sediment release in the OSL on August 26, 2008. Flow is from right to left. Bridges provide access to riffle regions of the stream without disturbing the benthic environment.

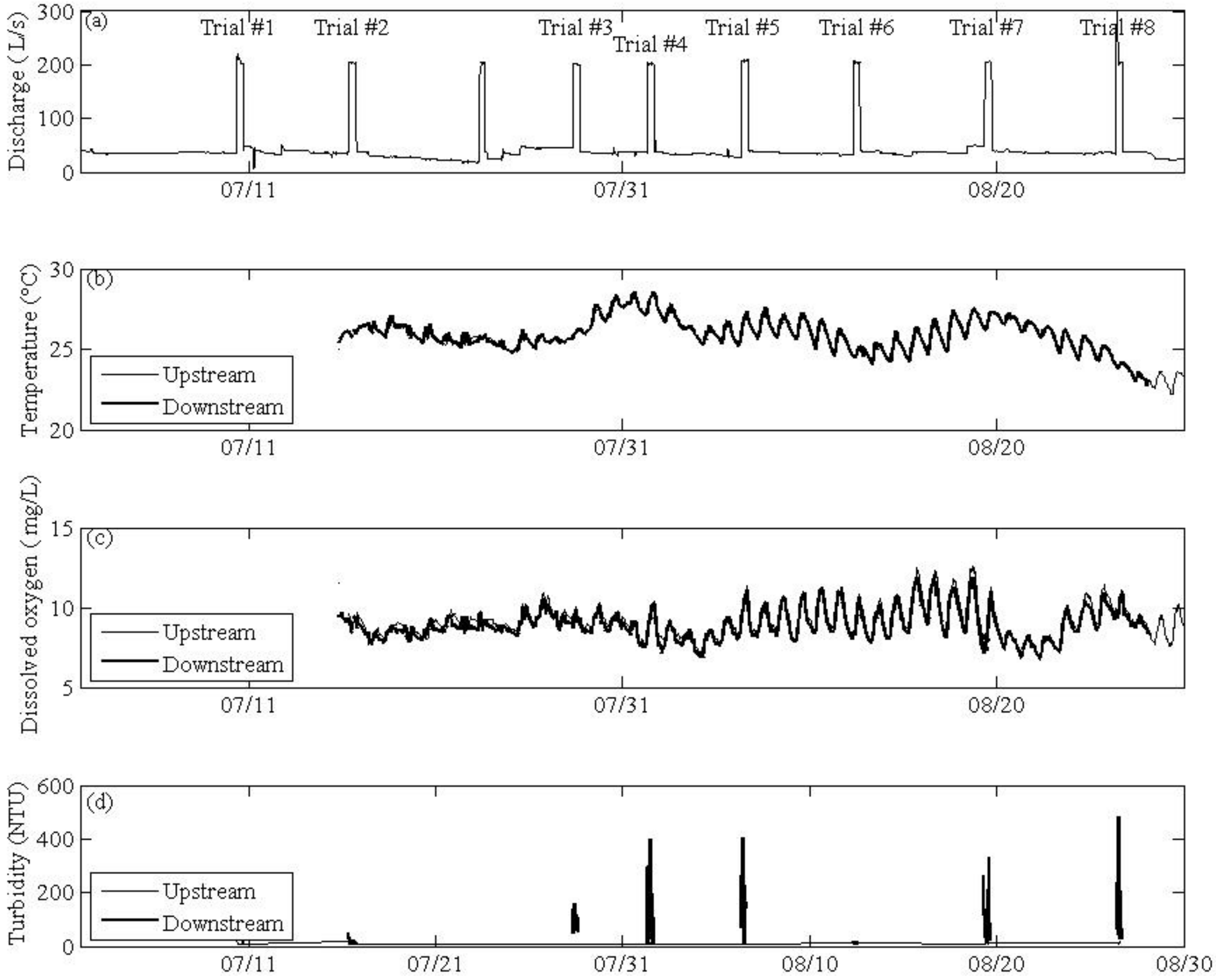

Figure 3: Environmental conditions within the Outdoor StreamLab during the study. (a) Discharge during the study period, including the eight experimental trials. (b) In-situ water temperature and (c) in-situ dissolved oxygen concentrations at the upstream and downstream ends of the OSL. (d) Laboratory measurements of turbidity from grab samples from the upstream and downstream riffles.

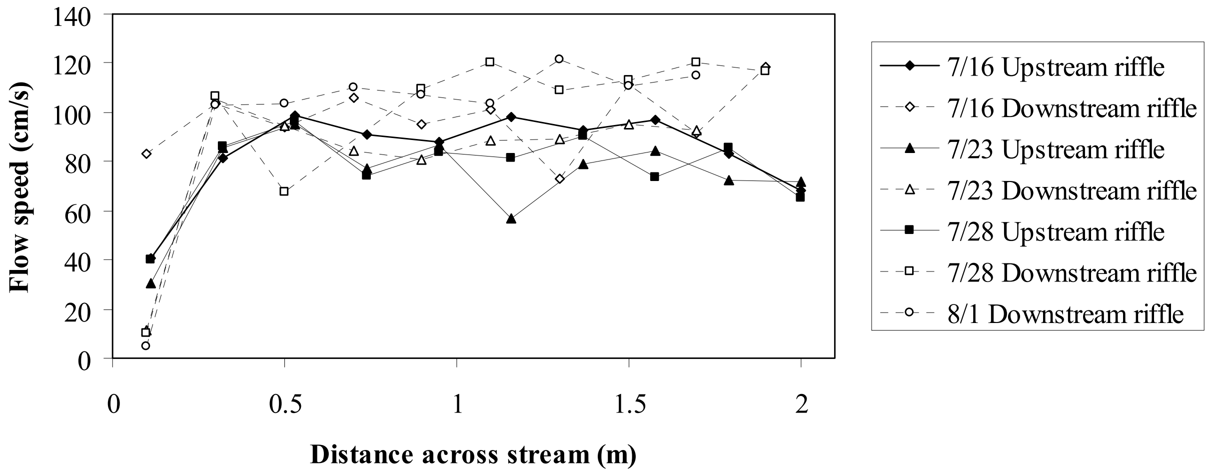

Figure 4: Horizontal flow speed near the middle of riffle regions during four different flood events. Distance across stream is measured facing downstream. Near-bank velocities were obtained near the left bank only.

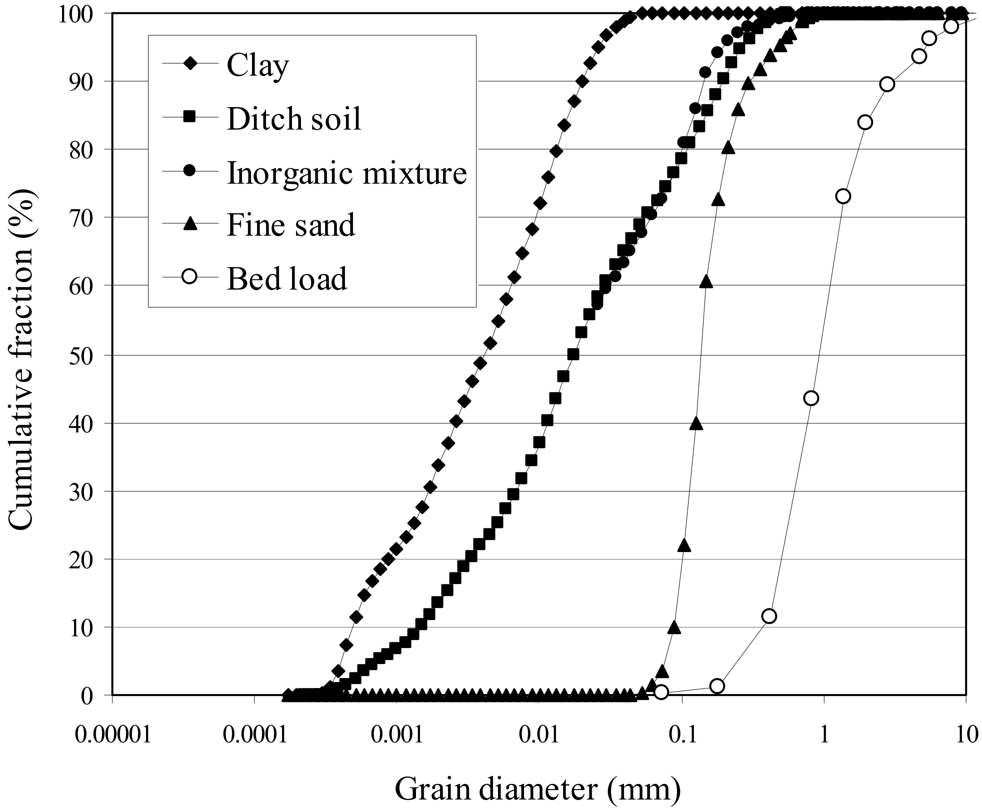

Figure 5: Grain size distributions for the suspended sediment releases and the bed load within the OSL.

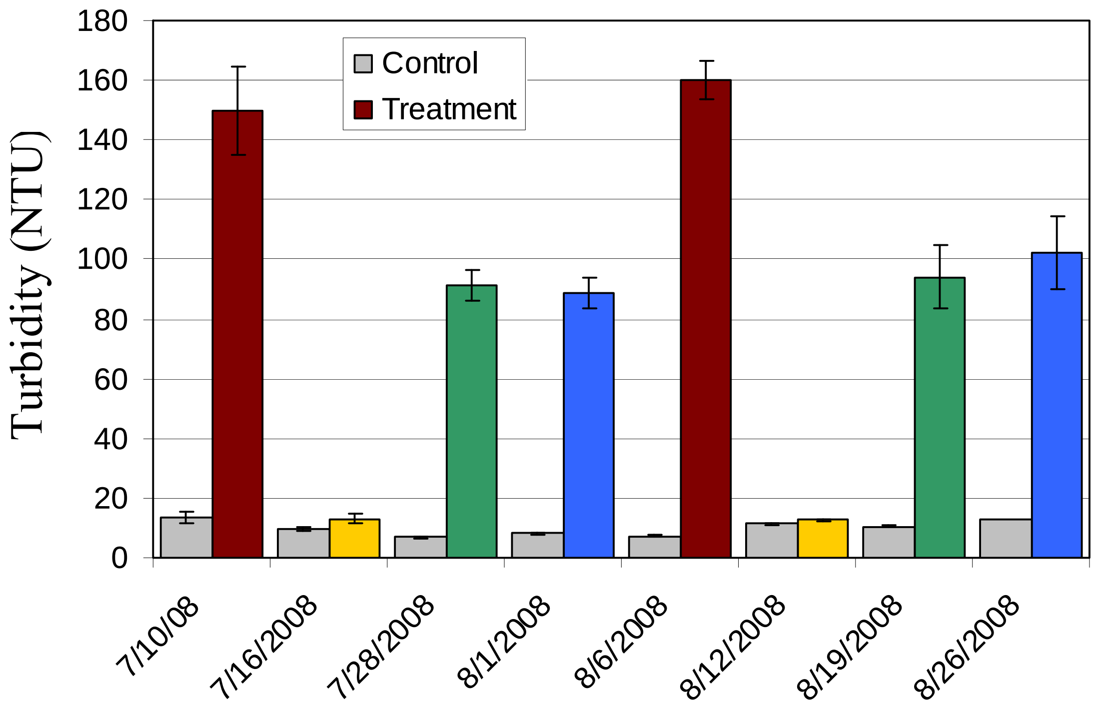

Figure 6: Turbidity as measured using a laboratory meter within OSL constructed riffles upstream (control riffle) and downstream (treatment riffle) of suspended sediment releases during high-flow events. Vertical bars indicate standard error of the mean.

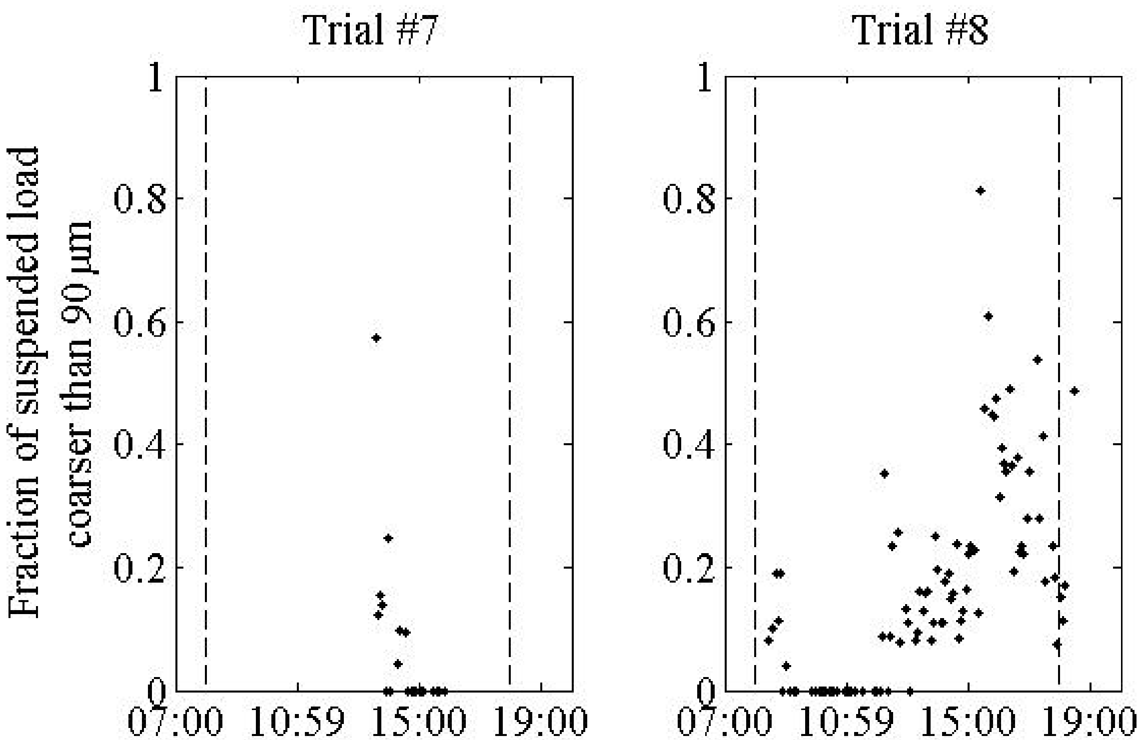

Figure 7: In-stream measurements (LISST StreamSide) of the volumetric fraction of suspended load larger than 90 µm during Trials #7 and 8. Vertical dashed lines indicate the beginning and end of the flood events on these dates.

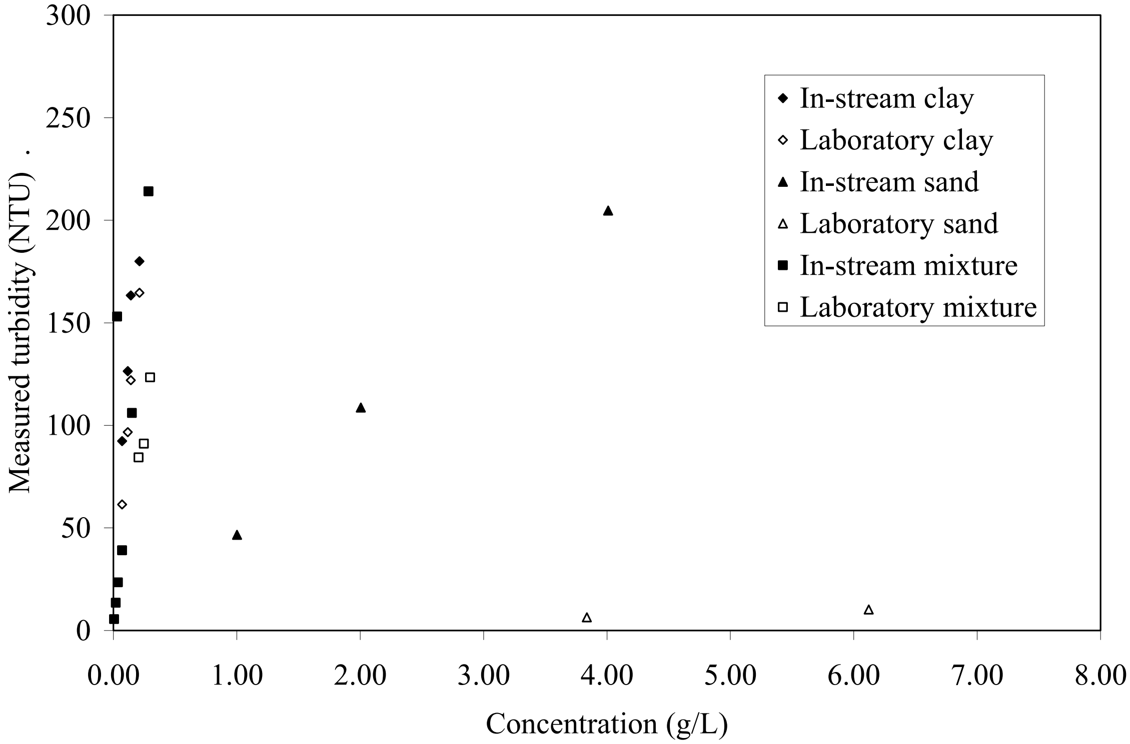

Figure 8: Comparison between turbidity values measured in situ and in the laboratory for sediment types with different grain size distributions.

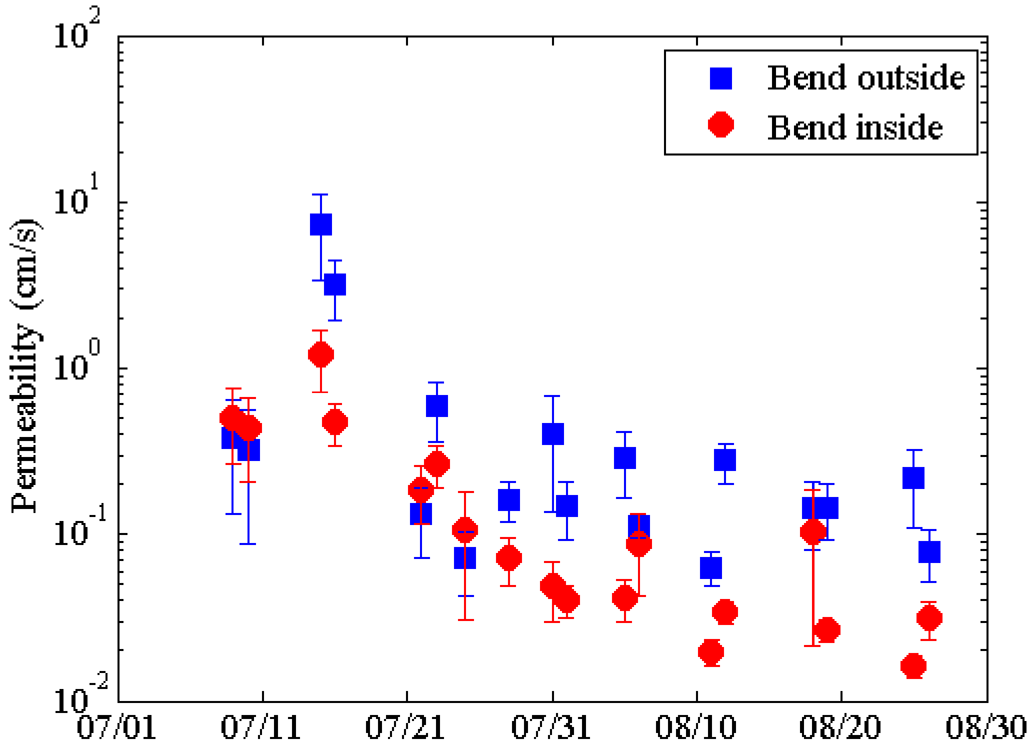

Figure 9: Spatial variability in bed permeability during summer 2008. Data from the outside of bends is the average of measurements from the river right of the upstream riffle and the river left of the downstream riffle; date from the inside of bends is the average of measurements from the river left and center of the upstream riffle and the river right and center of the downstream riffle. Vertical bars indicate the standard error of the spatial mean.

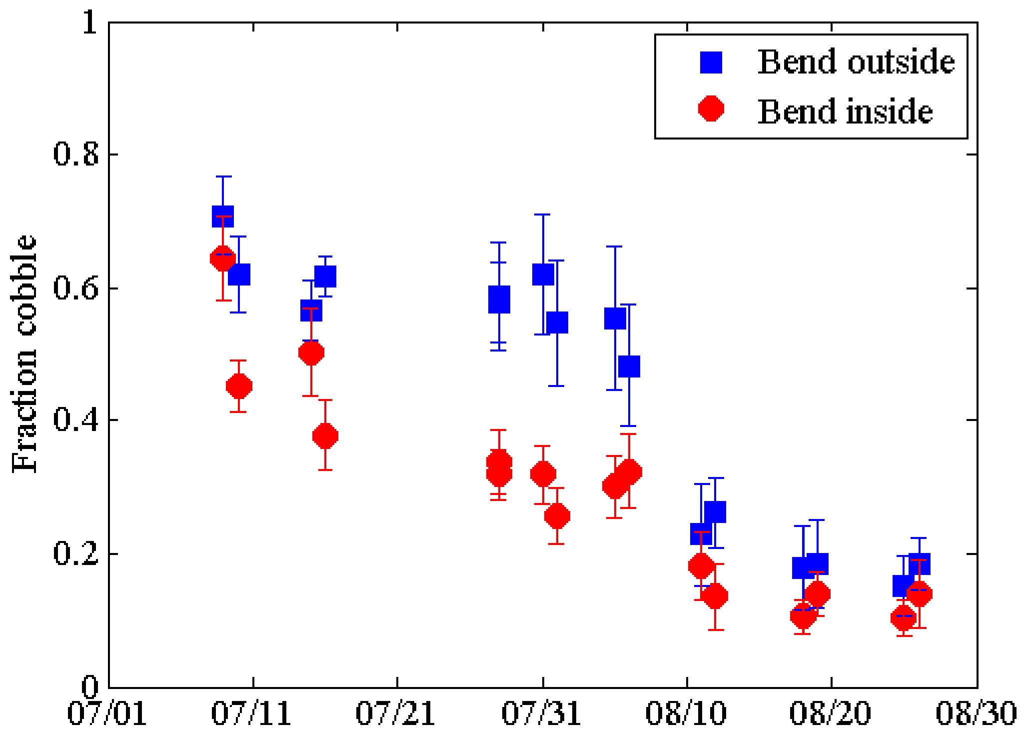

Figure 10: Spatial variability in riffle bed composition during summer 2008. Data from the outside of bends is the average of measurements from the river right of the upstream riffle and the river left of the downstream riffle; date from the inside of bends is the average of measurements from the river left and center of the upstream riffle and the river right and center of the downstream riffle. Vertical bars indicate the standard error of the spatial mean.

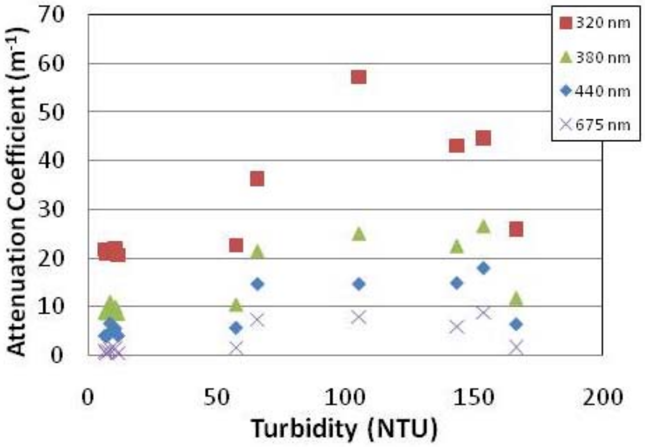

Figure 11: Light attenuation coefficients at four different wavelengths as a function of measured in-stream turbidity.

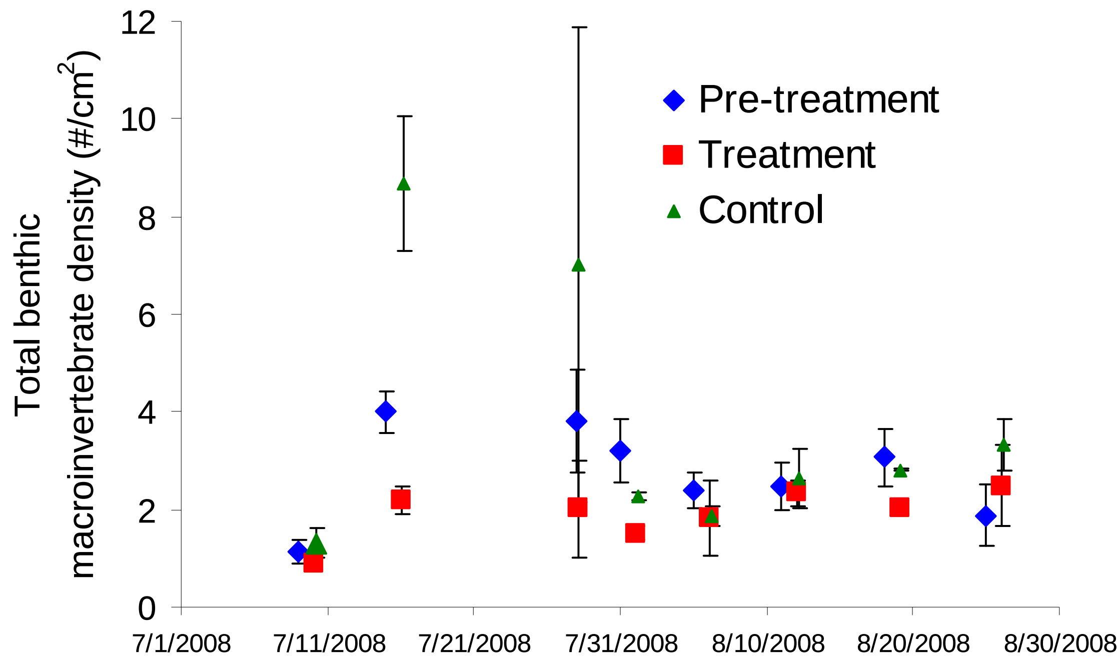

Figure 12: Density of macroinvertebrates per unit area of constructed riffle regions and drifting within stream water.

Table 1: Comparison of environmental conditions for experimental trials. Values are presented as mean ± standard error of the mean. Dashes indicate trials conducted before monitoring equipment was installed.

Parameter		Units	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7
Flood date		-	7/10/2008	7/16/2008	7/28/2008	8/1/2008	8/6/2008	8/12/2008	8/19/2008
Discharge	Baseflow prior to flood	L/s	1.17 ± 0.1	1.46 ± 0.1	1.60 ± 0.3	1.61 ± 0.1	1.47 ± 0.1	1.36 ± 0.1	1.34 ± 0.2
	Flood	L/s	7 ± 1	8 ± 1	8 ± 1	9 ± 1	9 ± 1	8 ± 1	7 ± 1
Temperature	Upstream	ºC	-	26.13 ± 0.04	25.51 ± 0.01	27.48 ± 0.03	26.21 ± 0.03	26.00 ± 0.03	25.79 ± 0.0
	Downstream	ºC	-	26.03 ± 0.02	25.50 ± 0.02	27.46 ± 0.03	26.17 ± 0.03	25.95 ± 0.03	25.78 ± 0.0
Dissolved oxygen concentration	Upstream	mg/L	-	9.30 ± 0.06	9.27 ± 0.02	8.94 ± 0.03	8.46 ± 0.04	9.44 ± 0.04	9.96 ± 0.0
	Downstream	mg/L	-	9.17 ± 0.03	9.08 ± 0.02	8.69 ± 0.03	8.30 ± 0.04	9.25 ± 0.04	9.56 ± 0.0
Dissolved oxygen as percentage of saturation concentration	Upstream	%	-	115.4 ± 0.8	113.1 ± 0.3	113.0 ± 0.4	104.5 ± 0.5	116.2 ± 0.5	122.2 ± 0.7
	Downstream	%	-	114.7 ± 0.4	110.7 ± 0.3	109.7 ± 0.5	102.5 ± 0.5	113.8 ± 0.5	117.3 ± 0.6
Turbidity	Upstream	NTU	14 ± 10	10 ± 3	7 ± 1	8 ± 2	7 ± 1	12 ± 1	11 ± 1
	Downstream	NTU	150 ± 81	13 ± 9	92 ± 29	89 ± 62	160 ± 60	13 ± 2	94 ± 76
Sediment released		kg	0.21 ± 0.01	0.22 ± 0.02	0.23 ± 0.10	0.27 ± 0.02	0.21 ± 0.01	0.23 ± 0.02	0.32 ± 0.1
In-stream concentration		g/L	930 ± 45	998 ± 91	1116 ± 446	1361 ± 91	975 ± 45	998 ± 90.7	1451 ± 446

Table 2: Light attenuation coefficients for OSL samples, including dissolved absorption coefficients (a_d) particulate absorption coefficients (a_p), and corrected attenuation coefficients ($K_{d\lambda}$) for each of two wavelengths.

			320 nm light			440 nm light			
date	time	location	a_{d320}	a_{p320}	K_{d320}	a_{d440}	a_{p440}	K_{d440}	Turbidity NTU
7/28/08	10:00	up	13.2	5.2	21.7	1.4	2.9	6.6	8
7/28/08	10:00	down	16.8	21.1	44.6	1.9	9.8	18.0	153
7/28/08	14:00	up	17.3	1.1	21.7	1.9	0.7	4.0	6
7/28/08	14:00	down	15.9	32.7	57.2	1.9	7.6	14.7	105
8/6/08	10:00	up	16.6	1.2	21.0	1.8	0.8	3.9	7
8/6/08	10:00	down	15.4	6.6	25.9	1.8	2.4	6.4	166
8/6/08	14:00	up	16.5	1.4	21.1	1.9	0.9	4.3	8
8/6/08	14:00	down	16.2	20.4	43.0	1.9	7.8	14.9	143
8/19/08	10:00	up	15.7	2.9	22.0	1.8	1.8	5.5	11
8/19/08	10:30	down	15.5	3.8	22.6	1.7	2.0	5.7	57
8/19/08	15:00	up	16.0	1.4	20.6	1.7	0.9	4.0	12
8/19/08	14:30	down	15.2	15.6	36.2	1.7	7.8	14.7	66

Table 3: Light attenuation in the OSL.

			Percent of incident light remaining at 10 cm depth		Percent of attenuation attributed to suspended particles	
date	time	location	320 nm	440 nm	320 nm	440 nm
7/28/08	10:00	up	11	52	6	28
7/28/08	10:00	down	1	17	67	80
7/28/08	14:00	up	11	67	56	84
7/28/08	14:00	down	0	23	28	67
8/6/08	10:00	up	12	68	56	80
8/6/08	10:00	down	8	53	8	33
8/6/08	14:00	up	12	65	7	30
8/6/08	14:00	down	1	22	30	57
8/19/08	10:00	up	11	58	16	50
8/19/08	10:30	down	10	57	20	54
8/19/08	15:00	up	13	67	8	35
8/19/08	14:30	down	3	23	51	82

Table 4: Health assessment indices (HAI scores) and statistical comparisons testing the short-term effect of two sediment types on health of fishes. A lower HAI score indicates better fish health. The χ2 statistic and P-value are from a Kruskal-Wallis comparison of HAI scores across the four trial and treatment categories.

	Smallmouth bass, Trial 6		Smallmouth bass, Trial 7		White sucker, Trial 6		White sucker, Trial 7	
Indices and Statistics	Upstream Control	Downstream Treatment	Upstream Control	Downstream Treatment	Upstream Control	Downstream Treatment	Upstream Control	Downstream Treatment
Overall HAI score (N)	54.3 (7)	38.6 (7)	26.7 (6)	32.9 (7)	18.3 (6)	38.3 (6)	2.0 (5)	14.3 (7)
SD	24.4	44.1	13.7	25.0	17.2	23.2	4.5	25.1
CV	44.9	114.2	51.3	76.0	93.9	60.4	223.6	175.5
Test statistic, (df), and *P*-value	$\chi^2 = 4.93$, (3), $P = 0.176$				$\chi^2 = 7.03$, (3), $P = 0.071$			
External HAI score	28.6	17.1	11.7	20.0	18.3	28.3	2.0	14.3
SD	16.8	37.3	14.7	19.1	17.2	19.4	4.5	25.1
CV	58.7	217.5	126.1	95.7	93.9	68.5	223.6	175.5
Test statistic, (df), and *P*-value	$\chi^2 = 5.62$, (3), $P = 0.132$				$\chi^2 = 5.56$, (3), $P = 0.135$			
Internal HAI score	25.7	21.4	15.0	12.9	0.0	10.0	0.0	0.0
SD	11.3	14.6	16.4	16.0	0.0	15.5	0.0	0.0
CV	44.1	68.3	109.5	124.7	.	154.9	.	.
Test statistic, (df), and *P*-value	$\chi^2 = 3.29$, (3), $P = 0.349$				$\chi^2 = 6.27$, (3), $P = 0.099$			

Table 5: Percentages of individual fish with internal and external anomalies by species, sediment trial, and fish pen location (US = upstream control pen, DS = downstream treatment pen) following sediment application midway between upstream and downstream pens.

Trial	Pen location	Fins	Skin	Eyes	Parasites	Gills	Pseudo-branchs	Thymus	Liver	Hindgut	Spleen	Kidney
Smallmouth bass												
Fine sand	US	71%	14%	57%	0%	0%	0%	0%	71%	0%	14%	0%
Fine sand	DS	14%	28%	14%	0%	14%	0%	0%	71%	0%	0%	0%
Agricultural soil	US	17%	33%	0%	17%	17%	0%	0%	33%	0%	0%	17%
Agricultural soil	DS	57%	14%	0%	43%	14%	0%	0%	43%	0%	0%	0%
White sucker												
Fine sand	US	0%	33%	0%	0%	50%	0%	0%	0%	0%	0%	0%
Fine sand	DS	33%	50%	0%	0%	67%	0%	0%	17%	0%	17%	0%
Agricultural soil	US	0%	20%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Agricultural soil	DS	14%	0%	14%	0%	28%	0%	0%	0%	0%	0%	0%

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PUBLICATIONS
No publications yet. We will shortly be submitting the following three papers for publication in peer-reviewed journals:

Merten, E.C., J. Loomis, A. Lightbody, and D. J. Dieterman. Effects of six-hour suspended sediment treatments on white sucker and smallmouth bass in an artificial stream. In preparation for submission to Environmental Biology of Fishes.

Lightbody, A., C. H. Orr, R. Bronk, and P. Belmont . Short-term effects of suspended sediment type on benthic macroinvertebrate community response. In preparation for submission to Hydrobiologia.

Lightbody, A., J. Marr, C. H. Orr, et al. The Outdoor StreamLab: a novel field-scale facility for the study of physical and ecological interactions. In preparation for submission to the Journal of the American Water Resources Association.

PRESENTATIONS

Orr, C. H., A. F. Lightbody, and R. Bronk. 2009. Determination of the short-term response of aquatic macroinvertebrate communities to suspended sediment loading. Oral Presentation. North American Benthological Society Annual Meeting, May 17-22, Grand Rapids, MI.

Lightbody, A., P. Belmont, J. Marr, C. Orr, and C. Paola. 2008. Determination of appropriate metric(s) for sediment-related total maximum daily loads. Oral Presentation. MinnesotaWater Resources Conference, October 27-28, 2008, St. Paul, MN.

Sayers, J. 2009. Outdoor StreamLab—From Construction Phase to Research Phase. Oral Presentation. St. Cloud State University Research Seminar, March 20, 2009, St. Cloud, MN.

Sayers, J. 2009. Outdoor StreamLab: From Construction Phase to Research Phase, including determination of groundwater flow with in-bank flood simulations. Poster Presentation. October 27, 2008, NorthStar STEM Alliance Student Research Symposium, University of Minnesota Bell Museum of Natural History, Minneapolis, MN.

Sayers, J. 2009. American Indian Science and Engineering Society (AISES) Region 5 meeting. Two 20 minute mini-presentations. Outdoor StreamLab and Outdoor StreamLab--from Construction Phase to Research Phase, including determination of groundwater flow with in-bank flood simulations. Tour SAFL with Diana Dalbotten and AISES Region 5 members. April 10, 2009, Minneapolis, MN.

STUDENT SUPPORT
This project was able to support salaries and/or supplies for the following students:

Undergraduate Students: June Sayers, Mary Presnail, Alex Nereson, Eric Johnson, Jordan Theissen, and John Wacloff

M.S. students: John Gaffney and Becca Bronk

Ph.D. students: Eric Merten and John Loomis

Post Doctoral Research Associates: Cailin Orr and Patrick Belmont

AWARDS
None

ADDITIONAL FUNDING
None directly continuing this line of research, but this project did enable us to obtain funding for several related projects. Most relevantly, we have obtained ~$60,000 from NSF (through the National Center for Earth-surface Dynamics Visitor Program) to consider the biogeochemical evolution of surface and subsurface environs, including the infiltration of fine sediment into the bed. We have also received ~$50,000 from Yonsei University in South Korea to perform behavioral studies on fish, in part leveraging our success with fish under the current project. Finally, we have been budgeted $106,000 for an EPA Section 319 grant (administered through the WRC) to examine surface-subsurface nutrient fluxes, though the money has not yet been released.

Integrating Biological Data into TMDL Assessments: Refining a Model to Determine Biologically Meaningful Target Levels for Dissolved Oxygen

Basic Information

Title:	Integrating Biological Data into TMDL Assessments: Refining a Model to Determine Biologically Meaningful Target Levels for Dissolved Oxygen
Project Number:	2008MN232B
Start Date:	3/1/2008
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Focus Category:	Water Quality, Non Point Pollution, None
Descriptors:	
Principal Investigators:	Leonard Charles Ferrington Jr.

Publication

Integrating Biological Data into TMDL Assessments: Refining a Model to Determine Biologically Meaningful Target Levels for Dissolved Oxygen

Principal Investigator
Leonard C. Ferrington Jr., Professor, Department of Entomology

Research Assistant
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ABSTRACT
Reduced levels of dissolved Oxygen (DO) can have severe and unacceptable consequences for productivity and biological diversity in surface waters. The Minnesota Pollution Control Agency has identified 64 stream/river segments with unacceptable DO conditions in their 2008 draft TMDL listing document (available on-line). All require development of a TMDL strategy to restore and protect productivity and diversity of aquatic organisms. Quantitative models are required in order to determine target levels of DO that will protect pre-determined percentages of aquatic organisms. This project was designed to continue developing models for integrating and interpreting data derived from a newly tested rapid bioassessment technique developed for assessing Chironomidae and using them as surrogates to measure biological conditions in streams that are listed for TMDL based on reduced DO conditions. Species thermal preferenda were developed and related to thermal patterns in stream segments that are controlled by groundwater/surface water interactions. In this project 20 stream segments from the MPCA 2008 draft list that are all warm-water segment, are listed for reduced DO, and have TMDL start dates of 2006, 2007 or 2008 were modeled. All streams occur in the Upper Mississippi, Minnesota River, Saint Croix River or Lake Superior drainage units. These models will serve to inform decisions about target levels for DO developed in the TMDL program and are constructed to allow predictions of the percentage of species that will be protected. The approach used to analyze data from this research project is patterned on concepts formulated by Posthuma et al. 2001 (and papers contained within) and is based on species sensitivities distributions derived from field data. This modeling approach relies on relationships of daily mean temperature to equilibrium saturation DO concentrations. The utility of the model derives from the ability to pre-determine a percentage of the chironomid community that is defined as the potentially affected fraction (PAF). In this modeling approach, the PAF is the percent of the total species that could potentially be affected (or extirpated) if water temperatures exceed their individual species preferenda during their emergence periods. Once the patterns of thermal preferenda are quantified, it will be possible to identify taxa that are extirpated, but should be present based on thermal regime, and develop predictive statements about species that should re-colonize a given stream if the TMDL results in improved DO conditions. This project will enable these species to be identified, and they can then be considered as species that will be indicators of improving DO conditions. This will enable and independent measure of improvement in biological conditions in addition to relying on physical and chemical indicators.

INTRODUCTION
Reduced levels of dissolved oxygen (DO) can have severe and unacceptable consequences for productivity and biological diversity in surface waters. The Minnesota Pollution Control Agency has identified 64 stream/river segments with unacceptable DO conditions in their 2008 draft TMDL listing document (available on-line). All require development of a TMDL strategy to restore and protect productivity and diversity of aquatic organisms. Chironomidae (Diptera) are predictably the most species-rich groups of aquatic insects in streams with reduced levels of DO and knowledge of their community composition, temporal patterns of emergence and species

thermal preferenda can be used to build quantitative models to determine target levels of DO that will protect pre-determined percentages of these aquatic organisms.

OBJECTIVES

Objectives are to determine the species composition, temporal emergence phenologies, develop and refine an Index of Biotic Integrity, and build thermal preferenda models for ten streams that have been identified by The Minnesota Pollution Control Agency as having unacceptable DO conditions. The sample sites are located on stream segments identified from the MPCA 2008 draft list, are all warm-water segments, are listed for reduced DO, and have TMDL start dates of 2006, 2007 or 2008. All occur in the Upper Mississippi, Minnesota River, or Saint Croix River drainage units. Results will be compared with data from five trout streams in order to develop models that can inform decisions about target levels for DO developed in the TMDL program, and are constructed to allow predictions of the percentage of species that will be protected.

METHODS, PROCEDURES AND MODEL DEVELOPMENT

Description of Methodology for Cost-effective Analysis of Chironomidae In this project collections of surface floating pupal exuviae (SFPE) were used to determine chironomid emergence periods at 10 stream segments per year. This monitoring technique is little known among and poorly understood by water quality managers in the United States. Detailed descriptions of the technique were included in earlier reports (Ferrington 1987, 1995, 2007) and are not repeated here. However, an introductory overview of the methodology follows.

Although not widely used in ecological investigations in the United States, collecting SFPE is not a new approach for gathering information about Chironomidae communities. It was first suggested by Thienemann (1910), but only occasionally used in taxonomic and biogeographic studies (Thienemann 1954, Brundin 1966) or ecological studies (Humphries 1938) until more recently. During the last 35 years, however, there has been increasing use of pupal exuviae collections in chironomid studies. Reiss (1968) and Lehmann (1971) used collections SFPE to supplement their larval collections when investigating Chironomidae community composition. In Western Europe and England collections of SFPE have been used extensively for surface water quality monitoring (McGill *et al.* 1979, Ruse 1995a, b; Ruse & Wilson 1984, Wilson 1977, 1980, 1987, 1989; Wilson & Bright 1973, Wilson & McGill 1977, Wilson & Wilson 1983). In North America the methodology has been successfully used in studies of phenology (Coffman 1973, Boerger 1981, Wartinbee & Coffman 1976), diel emergence patterns (Coffman 1974), ecology and community composition (Blackwood *et al*. 1995, Chou *et al*. 1999, Ferrington 1998, 2000, Ferrington *et al*. 1995, Kavanaugh 1988), microbial decomposition (Kavanaugh 1988), assessment of effects of point sources of enrichment (Coler 1984, Ferrington & Crisp 1989), non-point pesticide effects (Wright & Ferrington 1996), and effects of agricultural practices (Barton *et al*. 1995). In England and the United States SFPE collections have been used to study water and sediment quality (Ruse & Wilson 1984, Ruse *et al*. 2000, Ferrington 1993b), and used in Australia for measuring phenology (Hardwick *et al.* 1995) and effects of stream acidification on Chironomidae (Cranston *et al*. 1997).

Sampling Design: Ten stream segments were selected to compare sites with similar substrate and habitat, but potentially contrasting DO conditions. Samples were collected at three-week to one-month intervals during ice-free conditions. Lab work, consisting of sample sorting, identification and quantification, was completed in room 230 Alderman Hall. Voucher material has been curated and will be deposited in the insect museum of the Department of Entomology at the University of Minnesota, where it will be maintained for long-term study.

Species Composition and Temporal Emergence Phenologies: Composition and emergence phenologies are estimated from samples of SFPE collected using our standardized technique (Ferrington 1987, 1995, 2007) with the goal of collecting at least 100 specimens per sample. Relative abundance and phenologies were based on processing all the specimens in samples with 300 or less specimens. Samples exceeding 300 specimens were randomly subsampled to 300 specimens and then analyzed

Developing and refining an Index of Biotic Integrity: Fifteen metrics were calculated from samples of SFPE. The metrics are similar or identical to metrics calculated for aquatic macroinvertebrates collected with standard dip net sampling. Metrics were evaluated for independence and a subset of ten metrics was used to develop and refine an Index of Biotic Integrity. The ten metrics were scored using two different scoring techniques to determine which scoring strategy provided the best resolution of IBI for the ten stream segments.

Description of Species Sensitivity Models: The approach used to analyze data from this research project is patterned on concepts formulated by Posthuma *et al*. 2001 (and papers contained within) and is based on species sensitivities distributions derived from field data. Tolerances of individual species of Chironomidae to water temperatures and dissolved oxygen concentrations vary from species-to-species, and also within the life cycle of individual species. Two times that are critical in the life cycles of chironomids are (1) just before egg hatch, when embryogenesis is rapidly occurring and oxygen is rapidly being consumed, and (2) just before emergence of the adult, when adult tissues forming within the pupa require elevated amounts of oxygen. The adult portion of the life cycle of most species is very short (1-3 days) and pupation, emergence and embryogenesis of eggs by ovipositing females is therefore tightly linked over a short time span. By determining the emergence periods of individual species it is possible to assess the temperature and dissolved oxygen (DO) conditions that are preferred during both critical stages of the life cycle.

To develop species sensitivity distributions, we used historical data sets from 12 previous studies using the SFPE methodology in which 240 species have been linked to water temperatures. The temperatures at which 50% of specimens of each species in the data files emerged were calculated for each species, and this value has been defined as the respective **thermal preferenda.**

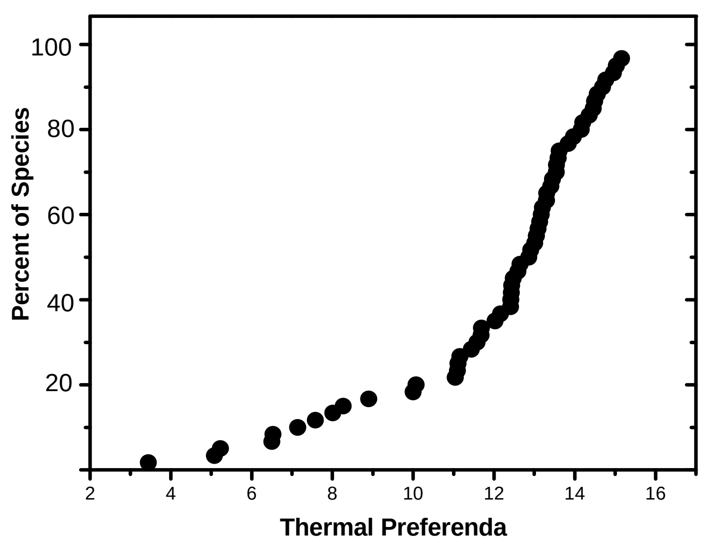

Figure 1: Conceptual model relating species emergence patterns to thermal preferenda, defined as the water temperature at which 50% of individuals of each species have completed their annual emergence.

In order to develop the models, species occurring in a stream segment will be arrayed by increasing temperature ranges during which they complete metamorphosis and molt from their immature to adult stage, and are plotted as a function of the species thermal preferenda (Fig. 1).

This modeling approach relies on relationships of daily mean temperature to equilibrium saturation DO concentrations. Because the molt from pupa to adult and embryogenesis are critical periods in the life cycle related to increased metabolic oxygen demands of their tissues, even transient exposure to reduced DO levels during the molt and/or egg embryogenesis can be lethal. Using Figure 2 the associated equilibrium saturation DO values can be calculated and linked to the thermal preferenda of each individual species. Furthermore, the model can be used to define different DO levels based on whether a species is cold-water adapted (and common in a trout stream) or warmer-water adapted.

The utility of the model derives from the ability to pre-determine a percentage of the chironomid community that is defined as the **potentially affected fraction** (PAF). In this modeling approach, the PAF is the percent of the total species that could potentially be affected (or extirpated) if water temperatures exceed their individual species preferenda during their emergence periods. For instance, in a model developed for a segment of trout stream (in Brown's Creek) a PAF of 50% (which would protect 50% of species) would correspond to a water temperature value of 12.9°C. By contrast, a PAF of 20% of species would correspond approximately to water temperature of 9.7°C. This lower water temperature value and associated equilibrium saturation concentration of DO would protect 80% of species.

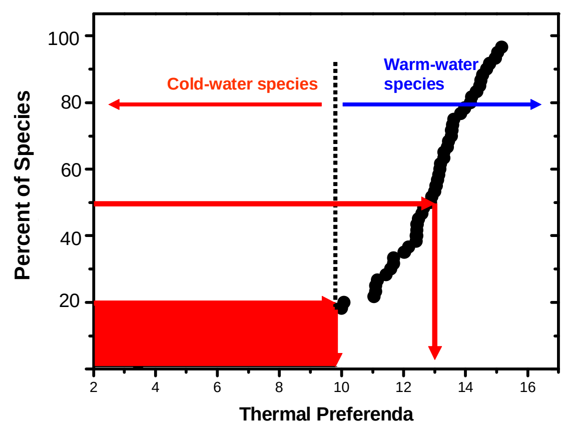

Figure 2: Conceptual model based on thermal preferenda for cold-water versus warm-water species in a single stream.

The actual mechanism that may produce deleterious effects on species in the PAF is not temperature *per se*, but is likely to be reduction in DO that occurs as oxygen concentrations equilibrate with water temperatures. Water temperatures that exceed a given species thermal preferenda are likely to result in less than adequate equilibrium DO levels. If these conditions coincide with embryogenesis and/or pupation and emergence periods of the species, mortality may result even if low DO conditions are transient. Consequently, once target levels for water temperature have been determined based on a pre-determined PAF, it is reasonably safe to assume that species with higher thermal preferenda will be protected. Thus a PAF of 50% (Figure 2) will protect all species in the upper right quadrat of the figure since it is known **from field data** that they can successfully pupate, emerge, oviposit, and undergo embryogenesis at higher temperatures and correspondingly lower equilibrium DO conditions than occur at 12.9° C during these critical portions of their life cycle. Alternatively, however, species below and to the left of the green arrows, which includes all of the cold-adapted species, would not be protected by the target level for temperature and corresponding equilibrium DO. The PAF of 20% would guarantee protection of at least two cold adapted species.

Model Development for Warm Water Streams: At the present time the models for this project have not been fully proofed and validated and, consequently, are not included in this report. However, a generalized conceptual model illustrating how species thermal preferenda are predicted to differ depending on whether a stream segment is groundwater dominated, such as in trout streams, or surface water dominated and has a warmer summer thermal regime has been provided in Figure 3. This figure shows a conceptual model for the patterns of thermal preferenda two types of stream segments, a cold water and warmer water segment. It is predicted that the thermal preferenda of species in the warmer water stream segment will be shifted along the X-axis to correspondingly higher temperatures. Although both types of streams will have both cold- and warm-water species, the percentages of total species that are cold adapted versus warm adapted will differ. Accordingly, a target PAF of 50% of species will result in the different stream temperature targets and corresponding equilibrium saturation DO levels as shown in Figure 4.

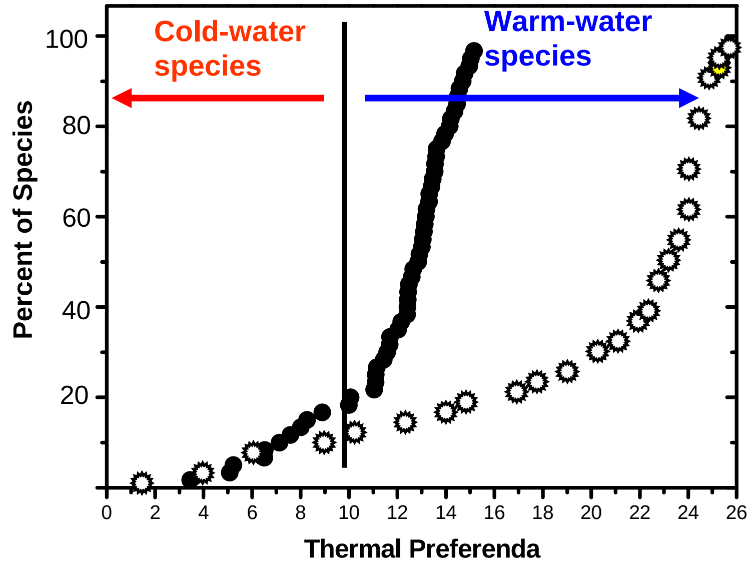

Figure 3: Graphical representation of species thermal preferenda for species occurring in a cold-water stream (solid dots) versus a warm-water stream (open dots).

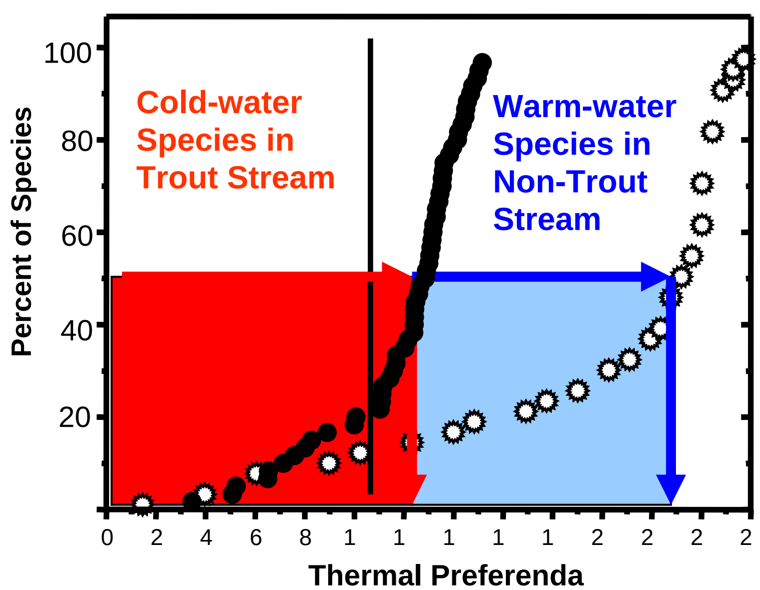

Figure 4: Graphical determination of water temperatures that do not exceed 50% of the individual species thermal preferenda of species occurring in a Trout Stream (solid dots) and Warm-water Stream (open dots).

Based on the hypothetical conceptual model in Figure 4, the 50% PAF of species occurring in a trout stream similar to Brown's Creek corresponds to a temperature of approximately 12.2° C. By contrast, the 50% PAF for the hypothetical warm-water stream is 22.8° C. Therefore, target levels for DO developed in a TMDL plan have the potential to be quite different, yet still able to protect a similar percentage of the species. Consequently, it is necessary to have models that are calibrated for both cold-water and warm-water segments of stream.

This modeling approach is strongly dependent on the equilibrium between stream water temperatures and attainment of equilibrium saturation concentrations in DO. Consequently, it is also necessary to determine the thermal preferenda of species in streams where DO is low and likely does not reach equilibrium saturation concentrations. Once the patterns of thermal preferenda are quantified, it will be possible to identify taxa that are extirpated, but should be present based on thermal regime, and develop predictive statements about species that should re-colonize a given stream if the TMDL results in improved DO conditions. This project will enable these species to be identified, and they can then be considered as species that will be “indicators” of improving DO conditions. This will enable and independent measure of improvement in biological conditions in addition to relying on physical and chemical indicators.

RESULTS

Table 1 summarizes the streams selected for study, coordinates of sampling locations on segments identified as having low DO and dates that samples were collected. Table 2 shows the dates samples were collected from each of the ten stream segments. The taxonomic compositions, cumulative species richnesses, and relative abundances of Chironomidae by subfamily or tribe are summarized by stream in Table 3. Table 4 and 5 summarize the ten metrics used to develop and refine the IBI for samples of SFPE based on two alternative metric scoring procedures.

Table 1. Locations of sample sites.

RIVER	COUNTY	LOCALITY	LATITUDE	LONGITUDE	ELEVATION
Clearwater River	Stearns	west of Clearwater	45° 24' 39.97" N	94° 03' 44.24" W	965 feet
North Fork Crow River	Wright	west of Rockford	45° 05' 45.69" N	93° 47' 21.62" W	904 feet
Silver Creek	Carver	41 HWY Bridge	44° 41' 29.21" N	93° 44' 09.37" W	861 feet
Ashley Creek	Todd	west of Sauk Centre	45° 46' 59.05" N	95° 01' 37.85" W	1253 feet
Moron Creek	Todd	south of Staples	46° 14' 20.78" N	94° 48' 45.27" W	1250 feet
Jewitt Creek	Meeker	west of Forest City	45° 11' 45.46" N	94° 31' 14.07" W	1074 feet
Grove Creek	Meeker	east of Manannah	45° 14' 47.18" N	94° 35' 53.84" W	1105 feet
Unnamed Creek	Chisago	south edge of Stacy	45° 23' 37.97" N	92° 59' 18.60" W	879 feet
Sunrise River	Chisago	north east of Stacy	45° 26' 57.43" N	92° 53' 12.45" W	852 feet
Mission Creek	Chisago	west of Pine City	45° 49' 54.72" N	93° 01' 14.56" W	938 feet

Table 2. Dates sample sites were collected in 2008.

RIVER	SAMPLE DATES					
Clearwater River	3 May	20 June	30 July	26 Aug	16 Sept	4 Oct
North Fork Crow River	4 May	21 June	31 July	26 Aug	17 Sept	5 Oct
Silver Creek	3 May	21 June	31 July	26 Aug	16 Sept	5 Oct
Ashley Creek	3 May	20 June	30 July	26 Aug	16 Sept	4 Oct
Moron Creek	3 May	20 June	30 July	26 Aug	16 Sept	4 Oct
Jewitt Creek	4 May	20 June	30 July	26 Aug	16 Sept	4 Oct
Grove Creek	4 May	20 June	30 July	26 Aug	16 Sept	4 Oct
Unnamed Creek	10 May	17 June	28 July	27 Aug	12 Sept	7 Oct
Sunrise River	10 May	17 June	28 July	27 Aug	18 Sept	7 Oct
Mission Creek	10 May	17 June	28 July	27 Aug	15 Sept	7 Oct

Table 3. taxonomic composition, cumulative species richnesses, and relative abundances of Chironomidae by subfamily or tribe.

SUBFAMILY/TRIBE	Moron Creek	Jewit t Creek	Grove Creek	Sunrise River	Unnamed Creek at Stacy	N Fork Crow R	Clearwate r River	Silver Creek	Mission Creek	Ashley Creek	PROJECT TOTALS
Total Specimens	1589	966	855	982	1252	1002	1486	1111	1130	1243	11616
Total Species	62	24	41	55	57	57	54	48	58	62	118
Chironomini Richness	19	4	14	16	18	26	10	10	13	16	39
Chironomini Abundance	316	40	88	232	219	458	79	87	148	118	1785
Percent Chironomini	19.9	4.1	10.3	23.6	17.5	45.7	5.3	7.8	13.1	9.5	15.4
Orthocladiinae Richness	23	12	14	21	22	17	29	30	29	29	48
Orthocladiinae Abundance	908	524	698	269	803	463	1235	897	697	994	7488
Percent Orthocladiinae	57.1	54.2	81.6	27.4	64.1	46.2	83.1	80.7	61.7	80.0	64.5
Tanytarsini Richness	11	6	8	10	8	7	7	6	11	10	14
Tanytarsini Abundance	334	399	54	383	134	35	112	117	200	106	1874
Percent Tanytarsini	21.0	41.3	6.3	39.0	10.7	3.5	7.5	10.5	17.7	8.5	16.1
Tanypodinae Richness	9	2	5	8	9	7	7	2	5	7	16
Tanypodinae Abundance	31	3	15	98	96	46	59	10	85	25	468
Percent Tanypodinae	2.0	0.3	1.8	10.0	7.7	4.6	4.0	0.9	7.5	2.0	4.0
Diamesinae Richness	0	0	0	0	0	0	1	0	0	0	1
Diamesinae Abundance	0	0	0	0	0	0	1	0	0	0	1
Percent Diamesinae	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.01
Prodiamesinae Richness	0	0	0	0	0	0	0	0	0	0	1
Prodiamesinae Abundance	0	0	0	0	0	0	0	0	0	0	1
Percent Prodiamesinae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Podonominae Richness	0	0	0	0	0	0	0	0	0	0	0
Podonominae Abundance	0	0	0	0	0	0	0	0	0	0	0
Percent Podonominae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4. Metrics and associated scores used for IBI based on rank assignment of scores (with ranges from 0 to 10).

METRICS AND ASSOCIATED SCORES	Moron Creek	Jewitt Creek	Grove Creek	Sunrise River	Unnamed Creek at Stacy	N Fork Crow R	Clearwate r River	Silver Creek	Mission Creek	Ashley Creek
Total Species	10	1	2	5	7	7	4	3	8	10
Chironomini Richness	2	10	6	5	3	1	8	8	7	5
Percent Chironomini	3	10	6	2	4	1	9	8	5	7
Diamesinae Richness	0	0	0	0	0	0	10	0	0	0
Orthocladiinae Richness	6	1	2	4	5	3	9	10	9	9
Percent Orthocladiinae	4	3	9	1	6	2	10	8	5	7
Tanytarsini Richness	10	2	6	8	6	4	4	2	10	8
Percent Tanytarsini	8	10	2	9	6	1	3	5	7	4
Percent of Three Most Abundant Taxa	6	1	2	8	4	9	3	5	10	7
Brillouin's Index Based on Site Total	5	1	3	8	6	9	2	4	10	7
Sum of Ten selected metrics	**54**	**39**	**38**	**50**	**47**	**37**	**62**	**53**	**71**	**64**

Table 5. Metrics and associated scores used for IBI based on proportional assignment of scores (with ranges from 0.9 to 10).

METRICS AND ASSOCIATED SCORES	Moron Creek	Jewitt Creek	Grove Creek	Sunrise River	Unnamed Creek at Stacy	N Fork Crow R	Clearwate r River	Silver Creek	Mission Creek	Ashley Creek
Total Species	9.8	3.9	6.6	8.9	9.2	9.2	8.7	7.7	9.4	10.0
Chironomini Richness	4.1	9.9	5.6	5.0	4.4	1.9	6.9	6.9	5.9	5.0
Percent Chironomini	6.9	9.2	7.9	5.3	6.5	0.9	8.9	8.4	7.4	8.1
Diamesinae Richness	0.9	0.9	0.9	0.9	0.9	0.9	10.0	0.9	0.9	0.9
Orthocladiinae Richness	7.7	4.0	4.7	7.0	7.3	5.7	9.7	10.0	9.7	9.7
Percent Orthocladiinae	6.9	6.5	9.8	3.3	7.7	5.6	10.0	9.7	7.4	9.6
Tanytarsini Richness	10.0	5.5	7.3	9.1	7.3	6.4	6.4	5.5	10.0	9.1
Percent Tanytarsini	8.4	10.0	1.4	9.6	3.4	0.9	2.8	2.9	5.0	2.7
Percent of Three Most Abundant Taxa	7.3	3.7	4.4	8.0	6.8	8.3	4.6	7.1	10.0	7.3
Brillouin's Index Based on Site Total	8.4	5.9	7.4	9.4	8.5	9.8	7.3	7.9	10.0	8.6
Sum of Ten selected metrics	**70.4**	**59.5**	**56.0**	**66.5**	**62.0**	**49.6**	**75.3**	**67.0**	**75.7**	**71.0**

DISCUSSION

A total of 118 species of Chironomidae were detected in the ten stream segments (Appendix table 1, available electronically on request), which provides significant basis for comparison of patterns in Chironomidae community structure with other streams in Minnesota. When considered across all ten stream segments, the taxonomic composition strongly contrasted with patterns typical of healthy trout streams with average DO values the are in dynamic equilibrium with water temperatures. The most pronounced difference compared to the fauna of trout streams consisted of reductions in species richness and percentage abundances of Diamesinae and Prodiamesinae. Only a single specimen of Diamesinae was collected from the stream segment, and Prodiamesinae were absent from all streams. By contrast, species of these two subfamilies are well represented in trout streams and account for substantial proportions of emergence in winter, early spring and late fall. They are among the most intolerant species of Chironomidae with regard to DO.

A second strong trend in taxonomic composition among the ten stream segments related to the higher species richness and proportional abundance of Chironomini relative to trout streams. Most species of Chironomini are moderately to strongly tolerant of reduced DO conditions, and Chironomini usually predominate in systems that are highly stressed by reductions of DO resulting from organic loading (Ferrington and Crisp, 1989). Although the Chironomini richnesses and proportional abundances are elevated relative to trout streams in Minnesota, the values are within limits observed for urban streams in the Minneapolis/Saint Paul metropolitan area such as Minnehaha Creek, Shingle Creek and lower portions of the Credit River, and there is little indication of very severe, chronic reductions of DO that can occur with excessive organic loadings in any of the stream segments.

This study represents the first attempt to develop and refine an IBI for evaluating responses to reduced DO based collections of SFPE in the United States. The ten metrics selected are easy to calculate from samples with more than 100 specimens, and appear to be robust at least up to subsample sizes of 300 specimens. The first approach used to score the metrics consisted of assigning a value from zero to ten based on the rank of each stream relative to the ten streams in this project. Scores were assigned as integer values and ties were treated by assigning the larger rank score. In this approach, the magnitude of difference among scores that are adjacent is always one full integer, except for ties, and the magnitude of difference does not influence the score. As and example, both Moron Creek and Ashley Creek had highest species richness values of 62 species and each received an integer score of 10. Mission Creek, with three fewer species (58), received an integer score of 8 due to the tie of Moron Creek and Ashley Creek. By contrast, the difference in species richness for the two lowest streams, Jewitt Creek at 24 species and Grove Creek at 41 species, resulted in integer scores of 1 and 2 respectively, although the absolute difference in species was 17 species.

The second method used to score the metrics was based on metric scores relative to our larger historical data files for streams that have been studies extensively in Minnesota. Scores were based on the proportion of the metric value for the stream segment relative to the best value in our data file. The proportional scores were scaled from 0.9 to 10 and were calculated to the second decimal.

The resulting IBI scores for both scoring strategies show very similar, but not identical, results. Although ranked scoring produced consistently lower final IBI scores relative to proportional scoring, only two stream segments, Ashley Creek and Clearwater River, switched in final rank. With ranked scoring the final score for Ashley Creek indicated it had the second best Chironomidae community with regard to DO, with the Clearwater River ranking third. The corresponding values based on proportional scoring placed Clearwater River second and Ashley Creek third.

In terms of the spread of final IBI scores, scoring based on rank provided slightly better differentiation of stream segment, ranging across 34 values from an IBI of 71 for Mission Creek to a low IBI score of 37 for the North Fork of the Crow River. By contrast, proportional scoring yield a spread of 26.1 values for IBI scores for these same two streams.

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PUBLICATIONS

None at this time. The following has been submitted to a Technical Advisory Committee related to TMDL development in Minnesota for review and comment before submitting to scientific journal, as required by granting agency.

Ferrington, L. C., Jr & R. W. Bouchard, Jr. (in technical review). Species Sensitivity Distribution Model for Emergence of Chironomidae in Trout Brook near Welch, Minnesota. 16 pp. (to be submitted to the Journal of the Kansas Entomological Society)

PRESENTATIONS

None

STUDENT SUPPORT

This project provided partial funding for one graduate student working toward an MS degree in aquatic ecology (Brian Schuetz). The student received training in aquatic Entomology and pollution responses of benthos in streams flowing through both urban and agricultural settings. The student developed technical skills identifying and quantifying Chironomidae larvae and pupal exuviae, determining phenological patterns, and building species sensitivity distribution models.

AWARDS

None

ADDITIONAL FUNDS

None

The Cultivation, Characterization, and Detection of Bacteria that Biodegrade Haloacetic Acids in Drinking Water Distribution Systems

Basic Information

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Principal Investigators:	Raymond M Hozalski, Timothy Michael LaPara

Publication

The Cultivation, Characterization, and Detection of Bacteria that Biodegrade Haloacetic Acids in Drinking Water Distribution Systems

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ABSTRACT
Drinking water distribution systems contain low levels of bacteria that can survive in an environment with very low nutrient concentrations and with a disinfectant residual. These bacteria can catalyze many reactions that impact drinking water quality. In the work discussed here, the diversity of haloacetic acid (HAA)-degrading bacteria and the corresponding metabolic genes were analyzed in drinking water distribution systems and other environmental systems, such as surface waters and soil. The research objectives related to this study were: (1) to isolate and characterize bacteria that can biodegrade haloacetic acids from numerous sources to expand our knowledge of the diversity of HAA-degrading bacteria and (2) to directly characterize the bacterial communities that biodegrade haloacetic acids in drinking water distributions by developing a novel use of terminal restriction fragment length polymorphism (tRFLP) targeting type I and type II dehalogenase genes (i.e. *deh*I and *deh*II).

We have satisfied about 50% of our first objective to date. We have used a filtration and direct plating isolation method and obtained several isolates from the Mississippi River and from agricultural soils. We will continue this work in the next year to obtain additional isolates from other sources. The second objective of this research is also about 50% complete, as we have developed a tRFLP method targeting short fragments (i.e., ~270 to 420 bp) of the *deh*I and *deh*II genes. The method was confirmed with previously obtained bacterial isolates and enrichment cultures and with a sample of granular activated carbon (GAC) from a prechlorinated GAC filter. In the next year, we intended to apply this method to several drinking water distribution system samples to better understand the prevalence and structure of HAA-degrading bacteria in these systems.

INTRODUCTION
The disinfection of drinking water and wastewater is needed for the protection of public health. Typically, chlorine is used to disinfect drinking water and wastewater because of its effectiveness and low cost, but chlorination also leads to the formation of disinfection byproducts (DBPs), the most dominant of which are trihalomethanes (THMs) and haloacetic acids (HAAs) (Krasner et al. 2006). The production of these DBPs is a critical water quality problem because many of these compounds are known or suspected carcinogens and some have suggested other deleterious effects such as increased risk of spontaneous abortion (Swan et al., 1998).

HAAs have been observed to decrease along the distribution systems in some cases (LeBel et al., 1997; Williams et al., 1995; Williams et al., 1997a). Williams et al. (1995) observed that dichloroacetic acid (DCAA) was significantly reduced in concentration in many samples collected from locations of relatively high residence times and this loss was later shown to be due to biodegradation (Williams et al., 1997b). We then demonstrated that bacterial cultures enriched on trichloroacetic acid (TCAA) and monochloroacetic acid (MCAA) could degrade HAAs at very low concentrations similar to those in the drinking water distribution systems (i.e., ~10-100 μg/L) (McRae et al., 2004).

Although there is growing information in the scientific literature about HAA-degrading bacteria, this information mainly comes from studies of soil and wastewater systems at relatively high concentrations of HAAs, such as those found at spill sites (*e.g.*, Olaniran, 2001; Lignell et al., 1984). Moreover, the very few HAA degraders isolated from the drinking water distribution system show that these bacterial isolates are different than those from soil and wastewater (Zhang et al., 2009). Also, the dehalogenase genes responsible for the biodegradation of HAAs are different than those that have been characterized in isolates from soil and activated sludge (Zhang et al., in review). Exploring the diversity of bacterial strains and HAA-dehalogenating genes from drinking water system is therefore important not only for expanding our scientific knowledge on the biodegradation of HAAs but also for being able to predict the fate of HAAs in drinking water distribution systems.

METHODS

Sample collection and preparation

Water and soil samples were aseptically collected from five different drinking water distribution systems (Minneapolis, MN (4 locations); St. Paul, MN; Blaine, MN; Bloomington, MN (2 locations); Bucharest, Romania), one source of surface water (Mississippi River) and from agricultural soil. Because tap water typically has relatively few bacteria in it (i.e. < 100 colony forming units per 100 mL), we filtered 10-20 liters of tap water on 0.2 μm sterile membrane filters to obtain useful quantities of bacteria for genomic DNA extractions. A similar filtration was used to concentrate the Mississippi River water, although only 50-100 mL were needed for bacterial strain isolation. In order to isolate HAA degraders from soil, 1 g of soil was placed in 9 mL of autoclaved phosphate-buffered saline (10 mM, pH 7.2) and vortexed for 5 minutes in order to dislodge bacteria. Serial dilutions were then prepared and 100 μL of each dilution were placed on agar plates supplemented with HAAs. Soil samples were obtained from an agricultural field in Blairsburg, Iowa where metolachlor was used in the past as herbicide. Metolachlor is known to photodegrade into MCAA (Wilson and Marbury, 2000).

Isolation and characterization of bacterial strains

Bacterial strains were isolated by directly placing filters or soil solutions on agar plates amended with 100 mg/L of MCAA, DCAA, or TCAA. The plates were incubated at room temperature for 7 to 14 days. A pH indicator (phenol red) was added at 0.03 % (w/v), which yields a yellow color at pH $\leq$ 6.6 and red at pH $\geq$ 8 (Kerr and Marchesi, 2006). Strains turning the red color to yellow, due to release of hydrochloric acid during HAA degradation, are presumed to be HAA-degraders. Single colonies were selected and streaked onto fresh plates. The isolates were re-streaked up to 3 times to ensure that the strains were pure.

The phylogeny of unique bacterial isolates was determined by nucleotide sequence analysis of nearly complete 16S rRNA genes. Nearly complete 16S rRNA genes specific to the domain *Bacteria* were amplified by polymerase chain reaction (PCR) using the 338F and 907R primers (Ghosh and LaPara, 2007). PCR products were purified (GeneClean; Qbiogene) and sequenced (BioMedical Genomics Center). Phylogenetic identification of nucleotide sequences was determined by comparison with sequences available in the GenBank database. Individual isolates were also assayed for the presence of group I and group II *deh* gene fragments via PCR using the primers and conditions described by Hill et al. (1999) (see below for more details).

Direct characterization of dehI and dehII genes via tRFLP

We attempted to characterize the HAA-degrading bacteria directly from our samples using a novel method of terminal restriction fragment length polymorphism (tRFLP). This approach was needed because there are numerous examples that have shown that the bacteria that are easy to culture from the environment are not necessarily the environmentally relevant organisms (e.g., Watanabe et al., 1998; McRae et al., 2004). More specifically, Marchesi and Weightman (2003) demonstrated a disconnect between *deh* genes found in pure or enriched cultures with those directly detected from the environment, suggesting that culturing introduces a large bias, not just in the bacteria isolated but also in the degradative genes that they contain (Marchesi and Weightman, 2003). Thus, we compared the dehalogenase gene sequences extracted from the original water samples with those of bacterial isolates previously obtained from the drinking water distribution system.

tRFLP patterns were generated by using PCR to amplify group I and group II *deh* gene fragments using the primers and specific methods described by Hill et al. (1999) (Table 1). The 5'-end of the forward primer was labeled with 6-carboxy-1,4-dichloro-2',4',5',7'-tetrachlorofluorescein (HEX). PCR products were initially resolved by agarose gel electrophoresis to ensure that a PCR product of the correct size was obtained. PCR products were purified using a Geneclean II kit (MPBiomedicals) to remove remaining enzyme, deoxynucleoside triphosphates, etc. The PCR products were then digested with the *Msp*I and *BfuC*I restriction enzymes to cut the PCR products at specific sequence locations. The two restriction enzymes were chosen based on a preliminary *in silico* study done with known *deh* sequences that were cut by all the restriction enzymes. The digested PCR products were then resolved on a 3130 XL capillary electrophoresis analyzer at the Biomedical Genomics Center at the University of Minnesota. This provided a sensitive, high-resolution fingerprint of the *deh* genes in our samples.

Table 1. Primers used for PCR amplification of Group I and Group II dehalogenase genes

Group I deh genes	
Primer	Sequence
$dehI_{for1}$	5'-ACGYTNSGSGTGCCNTGGGT-3'
$dehI_{rev1}$	5'-AWCARRTAYTTYGGATTRCCRTA-3'
Group II deh genes	
Primer	Sequence
$dehII_{for1}$	5'-TGGCGVCARMRDCARCTBGARTA-3'
$dehII_{rev1}$	5'-TCSMADSBRTTBGASGANACRAA-3'

IUPAC ambiguity code used: B = C, G, or T; D = A, G, or T; K = G or T; M = A or C; N = A, C, G, or T; R = A or G; S = C or G; W = A or T; Y = C or T. *Source*: Hill et al. (1999)

RESULTS

We previously obtained several HAA-degrading isolates from HAA enrichment cultures (Zhang et al., 2009). It is known, however, that the diversity of isolates is low when using an enrichment culturing technique. On the other hand, Kerr and Marchesi (2006) demonstrated that by improving the enrichment culture technique and by using direct plating as well, they were able to isolate novel bacteria able to degrade α-halocarboxylic acids. Therefore, our first goal was to use the direct plating isolation method in order to obtain more diverse HAA degraders.

We obtained 11 isolates on MCAA-amended plates and 15 isolates on DCAA-amended plates from Mississippi River water samples. Two of the MCAA-degrading isolates had both *deh*I and *deh*II genes; 3 isolates had only the *deh*II gene; and 6 isolates did not have either of these two genes. One of the DCAA-degrading isolates had both *deh*I and *deh*II genes; 5 isolates had only a *deh*II gene; and 9 isolates did not have either of these two genes. Two of the DCAA-degrading isolates were identified as being *Xanthobacter* spp. We further obtained 8 isolates on MCAA-amended plates and 8 isolates on DCAA-amended plates from agricultural soil samples. Of these, one MCAA-degrading isolate had both *deh*I and *deh*II genes; 4 isolates had only the *deh*II gene; and 3 isolates did not have either of these two genes. Also, 2 DCAA-degrading isolates had both *deh*I and *deh*II genes; 4 isolates had only the *deh*II gene; and 2 isolates did not have either of these two genes. These results show that the *deh*II gene is more prevalent than the *deh*I gene and suggest that novel dehalogenase genes could exist (carried by the isolates that grow on MCAA- or DCAA-amended plates but do not posses either the *deh*I or *deh*II genes). No isolates were obtained on TCAA plates, neither from the Mississippi river water nor from the soil samples. TCAA is a less favorable substrate that the mono- and di-halogenated HAAs because it is so highly oxidized that little energy is gained from aerobic degradation of this compound.

The second goal of our research was to directly characterize the bacterial communities that biodegrade haloacetic acids in drinking water distributions by using the tRFLP method targeting the *deh*I and *deh*II genes. We first did a preliminary theoretical analysis that showed that the tRFLP method can be used with small PCR fragments (~270 bp for *deh*I and ~420 bp for *deh*II) (Table 2). Moreover, the same *in silico* study suggested that the proposed tRFLP method can distinguish between the different phylogenetic *deh* groups and/or bacterial strains carrying *deh* genes. The tRFLP method was then confirmed using *Msp*I and *Bfu*CI restriction enzymes for the isolates listed in Table 2 as well as for a small number of other samples (activated sludge and granular activated carbon). The *deh*I gene was found in tap water samples collected from Minneapolis and the *deh*II gene was found in tap water samples collected from Minneapolis, St. Paul and Bucharest. The tRFLP profiles for the drinking water samples from Minneapolis are similar to those of *Afipia* spp. (having a group B *deh*I gene and a group C *deh*II gene), suggesting that this bacterial genus is the predominant HAA degrader in the water distribution system of Minneapolis.

Table 2. Prediction of the size of restriction fragments for several *deh*I (A) and *deh*II (B) sequences cut with *Bfu*CI and *Msp*I. GTS, GD1, EMD1, EMD2, GM1, GM2, GM3 and P1MI are isolates obtained in a previous study (Zhang et al., in review) from drinking water distribution systems.

A.

isolate or gene	species	*deh*I PCR fragment	phylogenetic *dehI* group	*Bfu*CI	*Msp*I
GTS	*Afipia broomeae*	272	B	123	151
GD1	*Afipia felis*	272	B	123	152
EMD1	*Afipia felis*	272	B	123	152
EMD2	*Afipia felis*	272	B	123	152
dehE	*Rhizobium* sp.	272	A	272	194
dehIAS1	*Xanthobacter* sp. *AS1*	272	B	118	64
dehIDA3	*Bradyrhizobium* sp. *DA3*	274	B	123	64
hadD	*Pseudomonas putida*	280	C	133	51
dehI17a	*Pseudomonas* sp.	281	C	119	36

B.

isolate or gene	species	*deh*II PCR fragment	phylogenetic *dehII* group	*Bfu*CI	*Msp*I
GM1	*Burkholderia glathei*	422	A	241	74
GM2	*Herminiimonas fonticola*	422	A	45	422
GM3	*Burkholderia glathei*	422	A	241	74
GD1	*Afipia felis*	416	D	104	175
GTS	*Afipia broomeae*	416	C	165	272
P1MI	*Afipia massiliensis*	416	C	135	175
EMD1	*Afipia felis*	415	C	163	173
EMD2	*Afipia felis*	415	C	164	174
dehH2	*Moraxella sp. B plasmid dehH2*	422	A	227	175
dehIIPP3	*Pseudomonas putida strain PP3*	422	A	255	422
dehH109	*Pseudomonas putida No.109 plasmid pUOH109*	422	A	305	175
L-dex	*Pseudomonas, YL*	422	A	114	99
dh1B	*Xanthobacter autotrophicus*	416	D	416	118
dehCI	*Pseudomonas* sp.	422	B	126	212
hdlIVa	*P.cepacia*	422	B	117	138

SUMMARY OF FINDINGS

The first outcome of our research was to establish an efficient bacterial isolation method in order to obtain HAA-degrading bacteria from drinking water distribution systems and environmental samples. We used a filtration and direct plating isolation method and obtained several isolates from the Mississippi River and agricultural soil. The second outcome of this research was to develop and optimize a tRFLP method for the study of the *deh*I and *deh*II gene diversity. We developed a tRFLP method targeting short fragments (i.e., ~270 to 420 bp) of the *deh*I and *deh*II genes. The method was confirmed with previously obtained isolates from drinking water systems as well as samples from an activated sludge bioreactor as well as a granular activated carbon filter treating chlorinated drinking water.

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Zhang P, RM Hozalski, LH Leach, AK Camper, EH Goslan, SA Parsons, Y Xie, and TM LaPara. Isolation and Characterization of Haloacetic Acid-Degrading *Afipia* spp. from Drinking Water, in review.

PUBLICATIONS
None

PRESENTATIONS
None

STUDENT SUPPORT
Name: Alina S. Grigorescu
Program: Civil Engineering, University of Minnesota
Degree being sought: Ph.D. (anticipated May 2010)

AWARDS
None

ADDITIONAL FUNDS
Hozalski R.M., A. Camper, S. Parsons, and T.M. LaPara. Biodegradation of haloacetic acids in distribution systems. American Water Works Association Research Foundation, January 1, 2006 – June 30, 2008. ($400,000)

Enhanced Degradation of Stormwater Petrochemicals within the Rhizosphere of Raingarden Bioretention Cells

Basic Information

Title:	Enhanced Degradation of Stormwater Petrochemicals within the Rhizosphere of Raingarden Bioretention Cells
Project Number:	2008MN235B
Start Date:	3/1/2008
End Date:	2/28/2009
Funding Source:	104B
Congressional District:	Fifth
Research Category:	Water Quality
Focus Category:	Water Quality, Non Point Pollution, None
Descriptors:	None
Principal Investigators:	Paige J Novak

Publication

1. Hozalski, R., G. LeFevre,, and J. Gulliver. 2009. Assessment of the Stormwater Infiltration and Pollutant Removal Capacities of Rain Gardens. Proceedings of the EWRI of ASCE Thailand 2009: An International Perspective on Environmental and Water Resources. Bangkok, Thailand, January 5-7, 2009.
2. Weiss, P., G. LeFevre,, and J. Gulliver. 2008. Contamination of Soil and Groundwater Due to Stormwater Infiltration Practices: A Literature Review. University of Minnesota, St. Anthony Falls Laboratory Project Report No.515. Available June 23, 2008.

Enhanced Degradation of Stormwater Petrochemicals within the Rhizosphere of Raingarden Bioretention Cells

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ABSTRACT
Traditional approaches to stormwater management, such as curb and gutter, fail to provide infiltration or water quality improvements and can act as conduits for pollutants. More municipalities and developers are turning to Low Impact Development (LID), which promotes on site infiltration as alternative stormwater management approaches. Raingardens (small, on-site, vegetated depressions to which runoff is directed) are a popular and promoted Best Management Practice (BMP) for urban stormwater quality. However, there is concern that contaminants present in runoff may accumulate and cause pollution of soil or groundwater. Little research has been done to examine the fate of hydrocarbons in alternative stormwater systems or to understand raingardens as a pollution control device. In order to truly be effective as a pollution control BMP, a raingarden must not only trap and detain, but degrade petrochemicals routed to them. Because most raingardens are vegetated, it is also vital to understand the role of plants in pollution control applications of raingardens.

We proposed to create simulated raingarden systems in columns and analyze the fate of benzene and toluene (gasoline components), and to determine what effects varying vegetation have upon the degradation capacity of these hydrocarbons. It was our hypothesis that legumes, which possess an enhanced microbial community in the rhizosphere of their roots, will facilitate an environment leading to greater biodegradation of these compounds.

PROGRESS REPORT
Petroleum hydrocarbons are a known constituent of urban stormwater, but little research has been conducted regarding their fate in bioretention areas. Hydrocarbon pollutant sources include leaking automobiles and leachate from asphalt sealants (van Metre et al. 2009); many of these compounds are carcinogenic, harmful to aquatic life, and carefully regulated. Therefore, determining the efficacy of bioretention treatment systems (raingardens) for the removal of petroleum hydrocarbons in stormwater is vital. Little is known about the ultimate fate of these contaminants in raingardens; therefore, initial work on this project has focused on determining whether biodegradation appears to be active in field-scale systems.

Soil samples were collected from raingardens at 75 sites in the Twin Cities metro area (Minneapolis/St. Paul, MN). Hexane extractions (Mohn and Stewart 2000) were performed on each sample, and the extracts were analyzed for petroleum hydrocarbons using gas chromatography. Results indicated that the total petroleum hydrocarbon (TPH) concentration in soil was very low (approximately 0.001 mg TPH / kg dry soil), and that there was no clear correlation between soil TPH and land use, loading factor (ratio of catchment area to infiltration area), or moisture content. Because the residual concentrations are lower than would be predicted based upon typical stormwater values, and concentrations observed do not correlate to loading, it is suspected that biodegradation may an important removal mechanism in these raingarden systems.

Currently, molecular biology methods are being adapted and optimized to allow for the quantification of the functional genes required for breakdown of petroleum hydrocarbons. This will help establish if raingardens with higher loading factors respond with higher populations of petroleum-degrading bacteria. Bacterial DNA was extracted from the raingarden soil samples analyzed above. Primer sets were chosen from the literature that had been developed and/or used to enumerate BTEX (benzene, toluene, ethylbenzene, and xylene)-degrading genes (Baldwin et al. 2003). Positive control organisms (*Pseudomonas putida* strains) were obtained and conventional polymerase chain reaction (PCR) performed on each of the target genes to test the primers. Although amplification of the functional genes was successful using conventional PCR, optimization with quantitative PCR yielded poor results. An alternative primer set is now being investigated and will be tested within the month.

In addition to the analysis of the field samples, laboratory column and batch reactors have been set up to test biodegradation of hydrocarbons in model raingardens. First, a naphthalene sorption equilibrium experiment was conducted to establish the sorption isotherm of naphthalene to a typical raingarden soil media mix. Biometer tests have been initiated and preliminary degradation experiments have also been conducted using radiolabelled naphthalene as a tracer. Finally, enclosed soil columns ("raingarden reactors") containing typical raingarden plants have been set up and will begin receiving naphthalene-contaminated feed this summer. These experiments will provide information on the fate of naphthalene in a model raingarden.

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van Metre, P. C..., Mahler, B. J., and Wilson, J. T. (2009). "PAHs Underfoot: Contaminated Dust from Coal-Tar Sealcoated Pavement is Widespread in the United States." *Environ.Sci.Technol.*, 43(1), 20-25.

PUBLICATIONS

Two publications (not peer reviewed) from the last year related to the project are listed below:

Weiss, P., G. LeFevre**,**, and J. Gulliver. 2008. *Contamination of Soil and Groundwater Due to Stormwater Infiltration Practices: A Literature Review*. University of Minnesota, St. Anthony Falls Laboratory Project Report No.515. Available June 23, 2008.

Hozalski, R., G. LeFevre, and J. Gulliver. 2009. *Assessment of the Stormwater Infiltration and Pollutant Removal Capacities of Rain Gardens.* Proceedings to EWRI of ASCE Thailand 2009: An International Perspective on Environmental and Water Resources. Bangkok, Thailand, January 5-7, 2009.

PRESENTATIONS

Two presentations were made during the past year related to this research:

2009 Minnesota Ground Water Association Conference: Impacts of Stormwater Infiltration on the Groundwater System (*Almer and LeFevre*).

2008 23nd Annual Conference on the Environment: Water Environment Association, Air & Waste Management Association (Minneapolis, MN). *Petrochemical Runoff into Raingarden Soils—Remediation or Residuals? (LeFevre, Novak, and Hozalski)*

STUDENT SUPPORT

No students are supported on this grant. The student working on this project is supported by a National Science Foundation fellowship.

AWARDS

None.

ADDITIONAL FUNDING

None.

Information Transfer Program Introduction

The WRC does not have any funded Information Transfer projects.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	9	0	0	0	9
Masters	6	3	4	0	13
Ph.D.	7	0	0	0	7
Post-Doc.	3	0	0	0	3
Total	25	3	4	0	32

Notable Awards and Achievements

Kevin Blanchet, Water Resources Center staff received the College of Food, Agriculture and Natural Resources Sciences Distinguished Professional and Academic Award.

Robert Dietz received a University of Minnesota Graduate School Fellowship for 2008 – 2011. Mr. Dietz is a PhD student in the Graduate Program in Water Resources Science at the University of Minnesota. He is supported by Dr. Emi Ito and Dr. Dan Engstrom (Department of Geology and Geophysics and Graduate Program in Water Resources Science).

Holly Dolliver received an Honorable Mention for the Universities Council on Water Resources (UCOWR) PhD Dissertation Award in Natural Sciences and Engineering. She was supported by Dr. Satish Gupta (Department of Soil, Water and Climate, and Graduate Program in Water Resources Science).

Allison Gamble received a University of Minnesota Graduate School Doctoral Dissertation Fellowship for 2008 – 2009. Ms. Gamble is an MS student in the Graduate Program in Water Resources Science at the University of Minnesota, Duluth. She is supported by Dr. Thomas Hrabik (Department of Biology, Duluth and Graduate Program in Water Resources Science).

Megan Kelly received a University of Minnesota Graduate School Fellowship for 2008 – 2010. Ms. Kelly is an MS student in the Graduate Program in Water Resources Science at the University of Minnesota. She is supported by Dr. Bill Arnold and Dr. Paige Novak (Department of Civil Engineering and Graduate Program in Water Resources Science).

Marian Kramer received a National Science Foundation GK-12 Fellowship for 2008 – 2009. Ms. Kramer is an MS student in the Graduate Program in Water Resources Science at the University of Minnesota, Duluth. She is supported by Dr. Erik Brown (Department of Geological Sciences, Duluth and Graduate Program in Water Resources Science).

Katsumi Matsumoto, Geology and Geophysics and Water Resources Science faculty, was named a McKnight Land-Grant Professor at the University of Minnesota.

Sarah Panzer received a University of Minnesota Graduate School Fellowship for 2008 – 2009. Ms. Panzer is an MS student in the Graduate Program in Water Resources Science at the University of Minnesota. She is supported by Dr. Kristen Nelson (Department of Forest Resources and Graduate Program in Water Resources Science).

Chris Paola, Geology and Geophysics and Water Resources Science faculty, received the Institute of Technology Distinguished Professor Award.

Sangwon Suh, Biosystems and Bioproducts Engineering and Water Resources Science faculty, was named a McKnight Land-Grant Professor at the University of Minnesota.

Jeffrey Buth, PhD student, Department of Chemistry, advised by Professors Kristopher McNeal and William Arnold received the EPA STAR Fellowship for 2006 – 2009.

Jeffrey Buth, PhD student, Department of Chemistry, advised by Professors Kristopher McNeal and William Arnold, received the 2008 ACS Graduate Student Award in Environmental Chemistry.

Kaitlin Steiger-Meister, Natural Resource Science and Management (NRSM) received funding by the International Association for Society and Resource Management for Steiger-Meister to present a paper on the project at the 14th International Symposium on Society and Resource Management.

Publications from Prior Years

1. 2000MNBN-04 ("Assessing the effects of endocrine disrupters from St. Paul Sewage treatment plant on sperm viability and testicular development in fish") - Other Publications - Schoenfuss, H.L. 2003. The need for novel approaches in assessing the biological impact of biologically active compounds. In: Proceedings of the 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals In Water, R. Masters (ed.), Minneapolis, MN, March 19-21, 2003, pp. 103-121.
2. 2000MNBN-04 ("Assessing the effects of endocrine disrupters from St. Paul Sewage treatment plant on sperm viability and testicular development in fish") - Articles in Refereed Scientific Journals - Schoenfuss, H. L., J. T. Levitt, G. Van Der Kraak, and P. W. Sorensen. 2002. Ten week exposure to treated sewage effluent discharge has small, variable effects on reproductive behavior and sperm production in goldfish. Environmental Toxicology & Chemistry 21 (10): 2185-2190.
3. 2000MNBN-04 ("Assessing the effects of endocrine disrupters from St. Paul Sewage treatment plant on sperm viability and testicular development in fish") - Water Resources Research Institute Reports - Schoenfuss, H.L., D. Martinovic, and P.W. Sorensen. 2001. Effects of exposure to low levels of water-borne 17b-estradiol on nest holding ability and sperm quality in fathead minnows. Water Resources Update 120: 49-55.
4. 2000MNBN-04 ("Assessing the effects of endocrine disrupters from St. Paul Sewage treatment plant on sperm viability and testicular development in fish") - Conference Proceedings - Martinovic, D., P.W. Sorensen and H.L. Schoenfuss. 2003. Low levels of water-borne estrogen suppress androgen levels and the ability to male fathead minnow to reproduce in the competitive spawning scenario. In: Proceedings of the 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals In Water, R. Masters (ed.), Minneapolis, MN, March 19-21, 2003, pp. 125-133.
5. 2000MNBN-04 ("Assessing the effects of endocrine disrupters from St. Paul Sewage treatment plant on sperm viability and testicular development in fish") - Conference Proceedings - Levitt, J. T., H. L. Schoenfuss, I. R. Adelman. 2001. Possible Effects of Endocrine Disrupting Compounds on Walleye, Stizostedion vitreum, near the Metro Sewage Treatment Plant, Saint Paul, MN. Proceedings of the Second International Conference on Pharmaceutics and Endocrine Disrupting Chemicals in Water, D. Guth (ed.), Minneapolis, MN, October 9-11, 2001, pp. 191-202.
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7. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Werner, J.J., K.H. Wammer, M. Chintapalli, W.A. Arnold, and K. McNeill. 2007. Environmental photochemistry of tylosin: efficient, reversible photoisomerization to a less–active isomer, followed by photolysis. J. Ag. Food Chem. 55(17): 7062–7068.
8. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Werner, J.J.; W.A. Arnold.; K. McNeill. 2006. Water Hardness as a Photochemical Parameter: Tetracycline Photolysis as a Function of Calcium Concentration, Magnesium Concentration, and pH. Environ. Sci. Technol. 40: 7236–7241.
9. 2003MN29B ("Arsenic in Minnesota Groundwater and its Impact on the Drinking Water Supply") - Articles in Refereed Scientific Journals - Edhlund, B. L., W. A. Arnold, K. McNeill. 2006. Aquatic Photochemistry of Nitrofuran Antibiotics, Environ. Sci. Technol. 40: 5422–5427.
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12. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Werner, J.J., K. McNeill, W.A. Arnold. 2005. Environmental photodegradation of mefenamic acid. Chemosphere 58: 1339−1346.
13. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Latch, D.E., J.L. Packer, B.L. Stender, J. VanOverbeke, W.A. Arnold and K. McNeill. 2005. Aqueous photochemistry of triclosan: Formation of 2,4−dichlorophenol, 2,8−dichlorodibenzo−p−dioxin and oligomerization products, Environ. Toxicol. Chem. 24(3): 517−525.
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