

**Water Resources Center  
Annual Technical Report  
FY 2012**

# Introduction

The Minnesota WRI 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 Resources Sciences (CFANS), University of Minnesota Extension, and the Minnesota Agricultural Experiment Station (MAES). 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 Science graduate major which offers both MS and PhD degrees and includes faculty and students from both the Twin Cities and the University of Minnesota – Duluth. 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 2-3 research projects each year, and the summaries of the current projects are found in the rest of this report.

# The Role of Sulfate Reduction in Sediment of the St. Louis River Estuary: Phase II

## Basic Information

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## NIWR Annual Technical Report Details

### The Role of Sulfate Reduction in the St. Louis River Estuary: Phase II

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Water Resources Science Graduate Program

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Sulfur and carbon controls on methyl mercury

in St. Louis River Estuary sediment

Phase II

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Research Report

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## **Abstract**

The presence of methyl mercury (MeHg) in freshwater aquatic systems is a concern to public health due to its bioaccumulative properties in aquatic food webs and neurotoxicity in humans. Transformation of inorganic mercury to MeHg is primarily driven by sulfate and iron reducing bacteria (SRB and FeRB) in anoxic sediment and water columns. The first objective of this research is to examine the production of MeHg in a sulfate-impacted freshwater estuary in the context of mercury-related geochemical parameters including organic carbon content and lability, sulfate concentrations, and bioavailability of inorganic mercury to methylating microbes. The second objective is to determine the significance of MeHg transport from sediment to the St. Louis River Estuary relative to upstream sources.

A laboratory sulfate addition experiment was performed using 20 cm intact sediment cores obtained from three characteristic sites in the St. Louis River Estuary. The intact cores from each site were exposed to a high (50 mg/L), medium (15 mg/L), and low (5 mg/L) overlying water sulfate treatment for an incubation period of six months. Over the six month laboratory study, MeHg/THg ratios in surface sediment (0-4cm) appeared to be insensitive to overlying water sulfate concentrations in all sites. However, at one site, MeHg/THg ratios in deeper sediment (4-10 cm) did appear to be related to overlying water sulfate concentration. Methyl mercury was strongly correlated with total mercury in sediment from all sites and all sulfate amendment conditions. Analysis of the bulk geochemical differences among sites suggests that MeHg concentrations are related to the total mercury in sediment and the quantity and type of organic carbon.

Laboratory flux measurements provided a means to compare MeHg loading from sediment relative to MeHg loading from the upstream St. Louis River. The estimated mercury loading from habitat zones represented by the three sites included in this study (45% of total estuary area) was small, but not insignificant relative to upstream sources during low flow conditions (8 – 13 %). During high flows, calculated sediment mercury loads are dwarfed by upstream loads (<0.4 %). These MeHg loading estimates suggest that MeHg transport from sediment may influence overlying water MeHg concentrations in the St. Louis River Estuary, particularly during low flows or in shallow, slow moving backwater bays or those areas with elevated sediment mercury contamination..

## 1. Introduction

Sulfate is elevated in many areas of the St. Louis River Watershed due to historic and ongoing mining operations. (Berndt and Bavin, 2009). Sulfate is of concern in the St. Louis River Watershed not because of its direct toxicological effects but due to its influence on the bioaccumulative form of mercury, methyl mercury (MeHg). Although dominant sources of inorganic mercury vary depending on the aquatic system of interest, it has been widely accepted that atmospheric deposition is a large source of mercury to most aquatic systems (Fitzgerald et al. 1998). In addition to atmospheric sources, the St. Louis River Estuary has been heavily impacted by industrial processes, resulting in localized, high mercury levels (Crane, 2006). The mercury form of greatest interest is MeHg, due to its ability to bioaccumulate in fish tissue and cause neurological damage to humans (Ratcliffe et al. 1996). In the St. Louis River Estuary, there is currently consumption advisory due to elevated fish tissue mercury concentrations (WI DNR. 2011)

The transformation of inorganic mercury to methyl mercury is biologically mediated by sulfate reducing bacteria (SRB) (Compau and Bartha, 1985), and requires organic matter, sulfate, inorganic mercury, and anoxic conditions. In fluvial systems, sulfate reduction occurs almost exclusively in sediment, since the overlying water does not favor anoxia (Hammerschmidt et al. 2004<sup>b</sup>; Mitchell et al., 2008a). In low-sulfate freshwater aquatic systems, it has been demonstrated that increasing sulfate concentrations increases the production of MeHg (Gilmour et al. 1992; Jeremiason et al. 2006); however, the biogeochemical processes controlling MeHg production by SRB are complex (Benoit et al. 2003) and involve many factors in addition to sulfate.

The specific area investigated in this study is the lower St. Louis River, a freshwater estuary located at the western tip of Lake Superior which has sulfate concentrations higher than unimpacted waters in the immediate region. In the Estuary, the river width increases considerably, water flow slows, and some exchange of water with Lake Superior occurs in the lower reaches. Since sulfate water concentrations (Berndt and Bavin, 2009) and methyl mercury in fish tissue (WIDnr, 2011) are elevated in the St. Louis River estuary, this study seeks to understand (a) whether elevated sulfate levels are driving methyl mercury production and transport in sediments, and (b) whether transport from sediments represents a significant source of MeHg to the estuary water column. To help answer these questions, laboratory experiments with controlled concentrations of sulfate in the overlying water were conducted on sediments collected from several major habitat zones in the St. Louis River Estuary (St. Louis River Alliance, 2002). Unlike other sediment sulfate addition studies using sediment slurries (Harmon et al., 2007; Gilmour et al., 1992), or episodic additions to field sites (Jeremiason et al. 2006, Coleman Wasik et al. 2012), this study utilized intact sediment microcosms exposed to a constant sulfate boundary condition in the overlying water. The use of intact sediment cores maintains realistic in-situ diagenetic redox zones and relies on diffusional transport to move sulfate into sediments, thus providing conditions analogous to those encountered in a freshwater estuary sediment environment.

## **2. Background**

### **Sediment Diagenesis**

In aquatic sediments, the consumption of oxidized compounds acting as electron acceptors occurs in a sequential pattern at increasing depth in the sediment (Froelich et al., 1979). Due to differences in Gibbs free energy associated with each reaction, the characteristic order of electron acceptor consumption in sediment is oxygen ( $O_2$ ), nitrate ( $NO_3^-$ ), manganese ( $Mn^{4+}$ ), ferric iron ( $Fe^{3+}$ ), and sulfate ( $SO_4^{2-}$ ) (Stumm and Morgan, 1996). The reduction of electron acceptors in sediment is primarily driven by heterotrophic bacteria which utilize organic carbon as an electron donor during metabolism (Jorgensen, 1982).

The primary driver of sediment diagenesis in freshwater and marine sediments is organic carbon flux into sediment (Meyers and Ishiwatari, 1993). In sediment systems that are organic rich, accelerated rates of microbial activity tend to compress microbial communities upwards, near the sediment water interface. Conversely, in organic poor aquatic systems, redox zones are stretched out, allowing the penetration of oxidized electron acceptors deeper into sediments (Katsev et al. 2006). In addition to concentration of organic carbon, the recalcitrance of the available organic carbon pool can limit or accelerate microbial processes (Meyers and Ishiwatari, 1993). The type of organic carbon transported to the sediment can be placed in two general categories, autochthonous and allochthonous carbon (Wetzel, 2002). Autochthonous carbon, which is typically considered the more labile of the two types of carbon, is produced at or near the site of consumption. Organic carbon produced outside the system of interest is considered allochthonous organic carbon (Wetzel, 2002). Since organic carbon in fluvial systems can be degraded during transport by microbial and macrobiotic processes, allochthonous carbon (produced elsewhere) tends to be more refractory (Vannote et al. 1980). Both the magnitude and type of organic carbon play major roles in controlling the rates of sediment diagenesis.

In addition to microbially driven reactions in the sediment column, abiotic reactions influence porewater and solid phase geochemistry. Abiotic processes that have an important role in sediment geochemistry are secondary redox, complexation, and precipitation/dissolution reactions. Secondary redox reactions involve many byproducts of microbial reduction reactions and oxidized species (Fossing et al., 2004). Certain electron acceptors are immobile while in their oxidized form (ferric iron and manganese(IV)), but are mobilized (ferrous iron, manganese (II)) once reduced by microbial communities (Brown et al. 2000). These reduced chemicals can diffuse into more oxidized zones and react with electron acceptors (Fossing et al., 2004). In addition to secondary redox reactions, complexation reactions have the ability to limit or enhance the reactivity or mobility of dissolved species and some complexation reactions produce a solid phase precipitate (sulfide-iron(II) precipitation to solid phase FeS). This collective sequence of reactions, including biologically mediated electron acceptor reduction and reactions among byproducts of microbial metabolism, is termed diagenesis.

### **Methyl mercury Production and Demethylation**

The transformation of inorganic mercury to methyl mercury has been shown to be a biologically mediated process (Compaeu and Bartha, 1985; Gilmour et al., 1992; Kerin et al., 2006; Fleming et al.,

2006). Sulfate reducing bacteria are widely considered the primary methylators of mercury in aquatic sediment (Gilmour et al. 1992; Jeremiason et al. 2006), while iron reducing bacteria have demonstrated the ability to methylate mercury in pure cultures (Fleming et al., 2006; Kerin et al., 2006). For sulfate reduction to occur, anoxic sediment conditions must be present. Otherwise aerobic bacteria out compete SRB.

In environmental systems, MeHg is also demethylated through biotic and abiotic processes. The balance between coincident methylation and demethylation processes results in a steady state MeHg concentration in sediment (Drott et al. 2008). Microbial MeHg degradation is thought to be the most important demethylation process in sediment (Benoit et al. 2003), and abiotic demethylation pathways, including photodegradation and oxidative demethylation, contribute to a lesser extent in sediment (Sellers et al. 1996; DiPasquale et al. 2000). It has been observed that %MeHg in the solid phase is a good measure of net MeHg production in sediment systems while instantaneous measurements of MeHg production rates, ( $k_m$ ) measured through spikes of enriched stable isotopes, is much better short term indicator (Drott et al. 2008).

### **Sulfate limitation**

A number of studies have investigated sulfate limitations in fluvial sediment and wetland systems by testing whether sulfate addition alone can stimulate mercury methylation (Braunfireun et al. 1999; Gilmour et al. 1992; Jeremiason et al. 2006; Harmon, 2004). Since SRB are the primary methylators of mercury in many environmental systems, sulfate addition to an anoxic systems starved for sulfate often increases SRB activity, thereby enhancing methyl mercury production (Benoit et al., 2003). A number of proxies and measures for increased SRB activity can be used to assess the magnitude of SRB response to sulfate additions including sulfate reduction rate (SRR), microbial community characterization, sulfide concentrations, CO<sub>2</sub>/CH<sub>4</sub> production, and AVS (King et al. 1999; King et al. 2000; Benoit et al. 2003).

A number of sediment sulfate addition experiments have been conducted in homogenized sediment amended with sulfate (sediment slurries) or pure cultures of particular SRB (Harmon et al. 2004; Gilmour et al. 1992; King et al. 2000). These studies have been useful in determining some of the biological mechanisms behind MeHg production in sediment but do not replicate intact diagenetic redox zones encountered in many field conditions. There have also been studies examining field scale sulfate additions to a wetland system which demonstrated significant MeHg response to episodic sulfate additions (Jeremiason et al. 2006, Coleman-Wasik et al. 2012). While performed under field conditions, the sulfate loading in these studies is not representative of constant increased loading of sulfate to sediment from the water column.

Although production of MeHg is limited by sulfate in many freshwater systems, a complex set of factors influences the production of MeHg (Harmon et al. 2007). Factors such as labile organic carbon availability, porewater inorganic mercury bioavailability, and competition among microbial communities can alter biogeochemical dynamics, creating situations in which an alternate set of limiting factors may arise (Hammerschmidt and Fitzgerald 2004a; Drott et al. 2007; Todorova et al. 2009; ). A conceptual model more complex than simple sulfate driven MeHg production is required to adequately explain

mercury dynamics in sediment.

### **Mercury Porewater Bioavailability**

Of all mercury present in aquatic sediments, typically less than 0.1% is present in sediment pore fluids, while the remainder is bound to the solid phase (Fitzgerald et al. 2007). The small amount of mercury present in the pore fluid is important, however, since it is the mobile fraction and able to participate in chemical and biological reactions. Porewater speciation of inorganic mercury is thought to have a large influence on the ability of a given system to produce MeHg efficiently (Benoit et al. 2003). Observations of systems with elevated dissolved sulfide concentrations and muted MeHg production provided early evidence of sulfide's influence on MeHg production (Gilmour et al. 1998; Benoit et al. 2001). Geochemical equilibrium modeling suggests that high sulfide concentrations can lower the concentration of uncharged mercury-sulfide complexes (primarily  $\text{HgS}^0$ ), which may diffuse passively across the membranes of SRB (Benoit et al. 1999<sup>a</sup>). The mercury-sulfide neutral-complex model is well supported (Gilmour et al. 1998; Benoit et al. 1999<sup>a</sup>; Benoit et al. 1999<sup>b</sup>; Benoit et al. 2001; Benoit et al. 2003), but the values of equilibrium constants used for the model are not well known (Skylberg, 2008; Drott et al. 2007), especially in relation to equilibrium constants for organic matter thiol groups, another important ligand for inorganic mercury (Skylberg et al. 2006). Low molecular weight organic compounds are capable of complexing mercury and may be transported into methylating bacteria via active transport (Golding et al. 2002). SRB culture studies (Graham et al. 2012; Schaefer et al. 2011; Schaefer and Morel 2009) and DOM modeling (Skylberg, 2008) have demonstrated that DOM can significantly increase the bioavailability of inorganic mercury and production of MeHg in some systems. Iron sulfide complexes may also have a role in limiting the bioavailability of porewater inorganic mercury (Skylberg, 2008, Skylberg and Drott 2010). Experimental additions of pyrite have demonstrated iron sulfide solids have the ability to remove inorganic mercury from solution (Bower et al. 2008) and inhibit MeHg production (Liu et al. 2009).

### **Organic Carbon Limitation**

Organic carbon is the basic metabolite for all chemoheterotrophic bacteria and is used as an electron donor (Froelich et al. 1979). Low quantities of labile organic carbon can limit the production of MeHg in sediments if a sulfate limitation has not been imposed on a system (Mitchell et al. 2008<sup>a</sup>). The addition of labile organic carbon to a system that is known not to have a sulfate limitation can yield high mercury methylation (Mitchell et al. 2008<sup>a</sup>). Carbon to nitrogen ratios can be used to determine the overall origins (autochthonous or allochthonous) of the organic carbon in a system and infer the capacity to drive microbial metabolism (Kim et al. 2011).

Solid phase organic carbon also binds mercury strongly and can inhibit the production of MeHg in sediments by lowering inorganic mercury in pore waters. In many systems, organic carbon is inversely correlated with porewater mercury, the fraction of the inorganic pool which is mobile and potentially available to methylating microbial populations (Hammerschmidt and Fitzgerald 2004a). Understanding the recalcitrance of the total carbon pool can assist with interpreting carbon's role in driving microbial activity and carbon's role in binding inorganic mercury (Kim et al. 2011).

### **Transport from sediments**

Many studies have sought to quantify the transport of inorganic and methyl mercury from sediments to the overlying water. Some have made direct measurements (in situ: Gill et al. 1999<sup>a</sup>, Benoit et al. 2009; or lab incubations: Covelli et al. 2008, Hammerschmidt et al. 2008a) and some have used estimates of sediment diffusion based on observed porewater concentrations (Hammerschmidt et al. 2008b). In general, estimates of flux from diffusion calculations have been lower than total flux measured in-situ or in lab measurements; however in some cases experimental measurements of total fluxes are smaller than diffusion estimates from porewater concentrations (Gill et al. 1999<sup>a</sup>, Choe et al. 2005). Total MeHg fluxes vary widely (from -100ng/m<sup>2</sup>/day to +2000ng/m<sup>2</sup>/day) and have been shown to vary seasonally (Corvalli et al. 1999) and even diurnally (Gil et al. 1999a) owing to factors including bioturbation (Benoit et al. 2009), redox boundary layers (Covelli et al. 1999), and dominant speciation (Jonsson et al. 2010). Recently, it has been suggested that sulfide may outcompete organic ligands for methyl mercury at high sulfide concentration and low pH (Jonsson et al. 2010). Although the complexation constants that predicts the predominance of methyl mercury bisulfide (CH<sub>3</sub>HgHS<sup>0</sup>) at high sulfide concentrations were not measured directly (Dyressen and Wedborg 1991), the species would exist as an uncharged molecule and have a substantially higher potential for transport with faster aqueous diffusion and potential for partitioning into dissolved gasses (Berndt and Bavin, 2011; Gray and Hines, 2009).

## **3. Experimental Design and Methods**

### **Site Selection**

The St. Louis River Alliance has outlined habitat zones which delineate and categorize zones of similar aquatic habitat type (St. Louis River Alliance, 2002). Representative sites for collecting sediment for the laboratory study were selected by choosing locations that (a) are within habitat zones that cover a large portion of the estuary, (b) are not influenced by regular dredging operations in the working harbor, and (c) have a range of organic carbon quantity and quality. Sites were chosen from the Lower Estuary Flats (LEF, 21% of estuary area), Upper Estuary Flats (UEF, 16% of estuary area), and Sheltered Bays (SB, 8% of estuary area). The three sites selected were in habitat zones that covered a total of 45% of the St. Louis River Estuary. Other habitat zones did cover a larger portion of the estuary but they were either highly influenced by industry or were located in the portion of the estuary that is regularly dredged, both factor that present a challenge to representative sampling. The LEF and SB habitat zones had prior information about bulk geochemical conditions, which demonstrated that there was a range of solid phase organic carbon (<2% to ~6% TOC, respectively, Johnson and Beck (2011)).

### **Study Design and Field Methods**

Sediment cores were collected from a small boat during August, 2011 using a custom designed sediment corer consisting of a 20 cm inner diameter polycarbonate tube. The sediment corer (Figure 3) was driven into the sediment using a drive rod operated by staff on the sampling boat. Once the core tube had penetrated 20-30 cm into the sediment, two ball valves were shut (ball valves remained open while core tube is driven into sediment) to create a hermetic seal that would preserve the sediment

water interface within the core tube once the core was removed from sediment. As the core was removed from the sediment, the bottom was capped immediately by a diver with an o-ring fitted polyethylene disk.

All twelve sediment cores were transported back to the laboratory where dissolved oxygen and pH were measured in the water overlying the sediment. Subsequently, two holes were drilled into the polycarbonate tubing 10 cm above the sediment water interface directly across from one another and fitted with 1/4" hose barbs. Within 4 hours of collecting cores, oxygenated water from each respective sample site was flowing over the sediment cores at 100 mL min<sup>-1</sup> to ensure the water near the sediment water interface would not become anoxic. Microcosms were then allowed to equilibrate at the incubation temperature (20 °C) for one week before any lab analysis was performed.

After a one week period, initial conditions for the sulfate addition experiment were measured in each microcosm using replicate voltammetric electrodes for redox-active species (Mn<sup>2+</sup>, Fe<sup>2+</sup>, total dissolved S<sup>2-</sup>, and O<sub>2</sub>) sediment porewaters and triplicate sub-cores to obtain samples solid phase and other porewater chemicals. The same methods were used to analyze the final conditions of the microcosms after the 6 month microcosm incubation. In an effort to reduce variability among cores, biota (zebra mussels and worms) were actively removed from the laboratory microcosms.

Once initial conditions for each microcosm were characterized, carboys containing water from each field site were disconnected from the peristaltic pump and connected to a reservoir of natural water amended with sodium sulfate. Each habitat zone had three different overlying water sulfate treatments applied for a period of 6 months. All treatments used water from the Cloquet River, since it has similar dissolved organic carbon concentrations to the main stem of the St. Louis River (10-20mg/L DOC) and low sulfate concentrations ( 2.5-5 mg L<sup>-1</sup>) (Berndt and Bavin, 2009). The three overlying water treatments applied to each habitat zone contained high (50 mg L<sup>-1</sup>), medium (15 mg L<sup>-1</sup>), and low (5 mg L<sup>-1</sup>) sulfate concentrations which simulated environmentally relevant sulfate concentrations in the St. Louis River Estuary (Figure 2). The medium (control) concentration was picked by estimating the sulfate concentration at the median discharge near the beginning of the St. Louis River Estuary in Cloquet, MN where a USGS continuous stream gauging station is located (Scanlon, #0402400). The median discharge at the gauging station for August (1,290 ft<sup>3</sup> s<sup>-1</sup>) was then compared to discharges and sulfate concentrations measured by Berndt and Bavin (2009) to determine an average sulfate concentration that would be used for the medium (control) treatment (15 mg L<sup>-1</sup>). The low treatment (5 mg L<sup>-1</sup> sulfate) was chosen based on observations in local, unimpacted tributaries, such as the Cloquet River (Berndt and Bavin 2009). Chloride was also added to the overlying water as a tracer (at concentrations 10-20x higher than in-situ observations) to ensure that diffusional transport was effectively delivering chemicals from the overlying water to sediment over the timescale of the experiment.

The reservoir of high sulfate water was used to recirculate water over the LEF high sulfate treatment, the UEF high sulfate treatment, and the SB high sulfate treatment. Similarly, medium and low sulfate reservoirs were recirculated over the respective microcosms from each habitat zone. Weekly monitoring of sulfate concentration in reservoirs ensured sulfate remained at initial concentrations. Fresh Cloquet water was obtained every two months during the experiment.

### **Laboratory Experimental Methods**

In order to make destructive measures of solid phase and porewater chemicals, sub-cores were collected from experimental microcosms in triplicate using one inch inner diameter polycarbonate tube. Before the 1 inch polycarbonate tubing was inserted, a 1 3/16 inch butyl tube was inserted into the sediment to prevent surrounding sediment from falling into the void created after removing a sub-core. Following extraction, sub-cores were immediately extruded using a 7/8 inch polyethylene rod wrapped with electrical tape to ensure a secure fit inside the sub-coring tube. Sediment was sectioned into 0-4 cm, 4-10 cm, and 10-20 cm intervals and placed directly into VWR trace metal clean glass jars. Each sediment sample jar was immediately filled with nitrogen to ensure that sediment redox conditions were not altered significantly. Within 15 minutes of sub coring sediment, samples were placed in a Coy glove box with an oxygen free atmosphere (97.5% nitrogen and 2.5 % hydrogen) where samples were homogenized and subsampled for solid phase and porewater analysis. All solid-phase samples that were not analyzed within 12 h of sub-coring were placed in a -20° C freezer until analysis.

To preserve the integrity of the experimental microcosm during initial sub-coring, the voids left from the extracted sub-cores were filled with sediment from a sacrificial sub-core taken from a fourth, sacrificial microcosm from each site. To ensure that sediment added to the experimental microcosms from the sacrificial microcosm was not substantially different relative to the experimental objectives, the sacrificial microcosm was also analyzed to determine its solid phase and porewater chemistry. To determine the variability within each habitat zone, triplicate one inch sub-cores were analyzed separately for one microcosm from each habitat zone. In the other two microcosms that did not have triplicate sub-cores analyzed separately, triplicate sub-cores were composited at each respective depth (0-4, 4-10, and 10- 20 cm).

Sediment THg and MeHg partitioning coefficients ( $K_D$  [L/kg]) are defined as the ratio of total solid phase mercury [ng/kg] to porewater mercury [ng/L] (passing 0.45 $\mu$ m filter) and were determined for each habitat zone at the close of the experiments. A 2.5" polycarbonate sub-core was removed from each microcosm and the top 10cm extruded into a 1 gallon Ziploc sample bag, transferred into an anaerobic glove box, and homogenized. Each homogenized sediment sample was transferred into 50 mL polypropylene centrifuge tubes (multiple required per microcosm), centrifuged at 10,000 rcf for 30 min, and filtered through a 0.45 $\mu$ m polyethersulfone filter (VWR, Supor). Water samples were filtered into clean PETG bottles and acidified (0.5%) using ACS trace metal grade hydrochloric acid. A small aliquot of homogenized sediment was also removed prior to centrifugation and placed in a VWR trace metal clean scintillation vial for solid phase analysis.

Flux experiments were conducted at the end of incubations to determine the magnitude of mercury flux out of sediment from each habitat zone. To conduct these experiments, fresh water from the Cloquet River was obtained in 50 L carboys. To remove any previous overlying water, five volumes of new water were allowed to flow over each microcosm to ensure fresh Cloquet River water was present on top of each microcosm. After fresh water was supplied to the overlying water of each microcosm, pumping was stopped, and water remained on top of sediment for 36-48 hours under well-mixed conditions created by a slow bubble of air. Overlying water was collected at the beginning and end of flux experiments using acid cleaned polypropylene syringes and immediately filtered using

disposable 0.45  $\mu\text{m}$  polyethersulfone filters directly into PETG trace metal clean bottles. Flux experiment water samples were acidified with 0.5% trace metal hydrochloric acid.

## **Analytical Methods**

### *Porewater*

Porewater sulfide, the sum of  $\text{H}_2\text{S}$  and  $\text{HS}^-$  (denoted  $\Sigma\text{S}^{2-}$ ), ferrous iron ( $\text{Fe}^{3+}$ ), and manganese ( $\text{Mn}^{2+}$ ) were measured with mercury gold amalgam voltammetric electrodes using methods similar to Brendel and Luther (1995). A Cloquet River water matrix was used to calibrate electrodes for  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$  at pH 4.5 with 0.6 mM and 1.2 mM acetic acid buffer, respectively. Sulfide calibrations were conducted in a 1.2 mM HEPES buffer (pH 8.6) in a Cloquet River water matrix. A two point oxygen calibration was performed in Cloquet River water assuming zero oxygen after nitrogen purging and equilibrium oxygen concentrations with atmospheric conditions after air bubbling. All anoxic calibration ( $\text{Mn}^{2+}$ ,  $\text{O}_2$ ,  $\text{Fe}^{2+}$ , and  $\text{S}^{2-}$ ) solutions were degassed by purging the solution with nitrogen gas at  $80\text{ cm}^3\text{min}^{-1}$  for 20 minutes and maintaining nitrogen headspace during analysis. Square wave voltammetric scans were used for  $\Sigma\text{S}^{2-}$ ,  $\text{Mn}^{2+}$ , and  $\text{Fe}^{2+}$ , and consisted of a potential range of -0.1 to -2.1 volts (V) vs silver/silver chloride (Ag/AgCl) reference electrode, 15 mV step height, 200 mV s<sup>-1</sup>, and 2.25 mV step increment. In the presence of  $\Sigma\text{S}^{2-}$  a -0.8 V conditioning step was applied before the acquisition scan, while in the presence of  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$  a -0.2 V conditioning step was applied for 10-30 seconds to remove any iron, manganese, or sulfur deposited during scans. Oxygen was determined with linear sweep voltammetric scans with a range of -0.1 to -1.8 mV and 200 mV s<sup>-1</sup> scan rate.

Porewater samples for other analytes (sulfate and DOC) were obtained by placing an aliquot of sediment from a sub-core section into a 50 mL centrifuge tube under an  $\text{N}_2$  atmosphere and centrifuging at 10,000 rpm for 30 minutes. Samples were then filtered through 0.45  $\mu\text{m}$  polyethersulfone filters directly into acid cleaned 15 mL polypropylene centrifuge tubes and stored short-term in an  $\text{N}_2$  atmosphere to avoid oxidation. A 5-10 mL aliquot of filtered porewater was acidified to pH 4.5 using 0.1 N nitric acid to convert all dissolved sulfide species ( $\text{H}_2\text{S}$ ,  $\text{HS}^-$ , and  $\text{S}^{2-}$ ) to  $\text{H}_2\text{S}$ , and prevent sulfide oxidation to sulfate. Acidified samples were purged of dissolved gas by bubbling with oxygen free nitrogen gas. Sulfate and chloride samples were analyzed by ion chromatography on a Dionex ICS-1100 IC system (IonPac AS22 4x250mm column) connected to an AS-DV Autosampler. Samples for dissolved organic carbon (DOC) were acidified to pH 2 to remove any dissolved inorganic carbon (DIC) and analyzed in a Shimadzu TOC<sub>VSH</sub> high temperature carbon analyzer with a sparging time of 3.5 min.

### *Solid Phase Analysis*

All solid phase sediment that was analyzed on a wet basis (AVS and Ferrous Iron) was normalized by a dry/wet weight ratio. A quantitative mass of sediment was weighed in an aluminum weight dish and heated to 105 °C for 24 hours to dry completely. Once dried, it was weighed again to obtain the dry weight and percent solid content of each sample. Each sediment section was analyzed separately for percent solid content to ensure that all solid phase samples were normalized correctly.

Acid Volatile Solids (AVS) was measured in the sediment solid phase using the Brower diffusion method (Brower and Murphy, 1994) and quantified with an Ion Selective Electrode (Eaton et al. 2005).

Solid Phase ferrous iron was extracted using an oxalate extraction outlined in Phillips and Lovley (1987). Total sulfur (TS) and total carbon (TC) sediment samples were dried for 48 hours in a 60°C oven to remove any moisture and quantified with thermal conductivity in a CHN analyzer. SRB abundance was characterized by quantitative real time polymerase chain reaction (qPCR) using a Mo Bio Powersoil extraction kit. Methods for analysis and quantification of the sediment extraction results are outlined in Schippers et al. (2006) as modified by Hicks and Oster (2012).

Methyl mercury and total mercury sediment aliquots were removed from the original sample container before all other analytes and placed in VWR trace metal cleaned glass scintillation vials in an oxygen free atmosphere. An acid-cleaned Teflon spatula was used to transfer sediment for mercury analysis. Samples were placed in a freezer (-20° C) within 30 minutes of sub-sampling sediment core sections. Before analysis, sediment was freeze dried at the lab in which it was analyzed. Total- and methyl- mercury analysis was performed by the University of Toronto using isotope dilution ICP-MS and analytical methods are outlined in detail in Mitchell and Gilmour (2008<sup>b</sup>), Hintelmann and Evans (1997), Hintelmann and Ogrinc (2003), and Horvat et al. (1993).

## 4. Results and Discussion

### Geochemical setting

Differences in sediment carbon content and quality are illustrated in Figures 4a and 4b for each of the LEF, UEF, and SB habitat zones. Abundant carbon is capable of driving fast rates of diagenetic activity if the carbon is present in a labile form (Kim et al. 2011). The SB had more abundant solid phase TC concentrations (4-6% TC) relative to LEF and UEF (2-4% TC) (Figure 4a). In the SB and UEF sites, TC decreases with depth suggesting a continuous supply of depositional carbon that is consumed during burial. Dissolved organic carbon concentrations in sediment pore water did not differ among habitat zones and were relatively consistent with depth in the SB (24.5±0.5 mg/L DOC), LEF (22.8±4.5 mg/L DOC), and UEF (26.2±5.9 mg/L DOC) sediment.

C/N ratio can be used as a proxy for the recalcitrance and source of organic carbon in sediment. In the water column, freshly produced particulate organic carbon (POC) exhibits C/N values of 6-11 (Kim et al. 2011). In the SB habitat zone, C/N ratios (12.8-15.0) were slightly higher than values characteristic of freshly produced organic matter. The observation that C/N ratios (indicating carbon recalcitrance) increase with depth in SB and UEF sediment suggests that labile carbon is degraded in surficial sediment during burial. C/N ratios demonstrate that organic carbon in the LEF sediment is more recalcitrant than that in the SB and UEF sediment which may lead to lower microbial activity. Since the carbon that is delivered to the LEF sediment appears to be recalcitrant, the consequent slower microbial utilization of this carbon would explain the lack of a significant increase in C/N ratio with depth.

Despite areas of localized mercury contamination in the estuary, total mercury in the sites sampled is not highly contaminated (>500 ng/g THg) (Benoit et al. 2003) but is higher than many sites impacted only by atmospheric deposition (Fitzgerald et al. 1998). The study sites have significant differences in total mercury (THg) concentrations with the more organic rich SB sediment containing the

highest concentrations ( $260 \pm 102$  ng/g). The LEF and UEF have lower THg concentrations of  $112.3 \pm 49.9$  ng/g and  $70.7 \pm 26.5$  ng/g, respectively. Solid phase total mercury was the most variable in cores collected from the LEF habitat zone and exhibited a significant decreasing trend with depth and variability amongst cores. Sediment solid phase THg concentrations agree with the first year bulk geochemical analysis of THg in the LEF and SB sites (Johnson and Beck, 2011).

## **Experimental Results and Discussion**

### *Sulfate and Chloride*

At the beginning of incubations, sulfate penetrated to a sediment depth of 5-10 cm in all treatments, indicating that sulfate reduction is occurring in this zone in all habitat zones (Figure 5 a-c). After the 6 month incubation, sulfate penetration into the sediment was greater in the high sulfate treatments (20-30 mg/L at 2cm depth; 1-4 mg/L at 7 cm depth) relative to the medium treatment (5-10 mg/L at 2 cm depth; <0.1 mg/L at 7 cm depth) and low treatment (0.1-3.8 mg/L at 2 cm depth and <0.1 mg/L at 7 cm depth). To ensure that there was a diffusive flux into the sediment, chloride was added to the overlying water as a conservative tracer at a concentration of 200mg/L. The initial (uniformly  $9.6 \pm 1.1$  mg/L) and final (150-200mg/L) porewater chloride profiles indicate that diffusion effectively transported chloride from the overlying water to 20cm depth in experimental microcosms over the 6 month experiment. Chloride and sulfate observations (Figure 5) demonstrate that sulfate reducing bacteria in surface sediment were exposed to varying amount of sulfate which was a main objective of the experiment design. However, the resolution of sulfate measurements makes it difficult to ascertain the depth at which sulfate reduction began in sediment.

### *Porewater Redox Conditions*

Initial and final porewater profiles for redox-active dissolved chemicals are shown in Figures 6 and 7. For Figures 6-7, the rows of figure panels correspond to different habitat zones (a-c LEF; d-f UEF; g-i SB). Columns of figure panels correspond to different sulfate amendments (a,d,g low; b, e, h medium; c, f, i high). Initial porewater measurements of  $\Sigma S^{2-}$ ,  $Fe^{2+}$ , and  $Mn^{2+}$ , illustrate a clear geochemical difference between the sediment from flats habitat zones (LEF / UEF), and the SB habitat zone. Porewater in LEF and UEF habitat zones have high  $Mn^{2+}$  (200-350  $\mu M$ ) with no detectible  $Fe^{2+}$  or  $\Sigma S^{2-}$  (Figure 6a-f) and are similar for all sediment microcosms. A sediment profile consisting of  $Mn^{2+}$  with no detectible  $Fe^{2+}$  or  $S^{2-}$ , suggest the LEF and UEF habitat zones have less labile or lower concentration of organic carbon (effectively stretching redox boundaries to deeper depths) or a limited supply of  $Fe^{3+}$  and sulfate. In SB sediments (Figure 6 g-i), porewater  $Fe^{2+}$  concentrations were high in porewaters (200-400  $\mu M$ ) (Figure 6g-i), with small amounts of detectible  $\Sigma S^{2-}$  (1-5  $\mu M$ ). The SB habitat zones are backwater sites, which leads to hydrologic conditions similar to lakes and the potential for anoxia in the water column. An oxygen-depleted hypolimnion could lead to  $Mn^{2+}$  transport to the overlying water (in the absence of oxygen) allowing iron and sulfate reduction to move into the surficial sediment. A higher abundance of labile organic carbon in the SB habitat zone, in conjunction with periodic anoxic overlying water could be responsible for a shift upward in iron and sulfate reduction relative to UEF and LEF sites (Figure 6).

The final porewater concentrations for redox-active chemicals in the LEF and UEF sediment did not change relative to the initial conditions (Figure 7a-f). Porewater concentration for redox active chemicals changed markedly in the SB habitat zones from the initial to final sampling (Figure 7g-i). Porewater  $Mn^{2+}$  concentrations in SB sediment were below detection limit in the initial conditions while it increased to 100-200  $\mu M$  in the final conditions. This change in redox conditions was most likely driven by a change in overlying water oxygen concentrations. The field conditions of the overlying water may have been anoxic due to a thermal stratification and significant sediment oxygen demand. After pumping water saturated with oxygen over the sediment for 6 months and altering the redox boundary at the sediment water interface, the less thermodynamically favorable reduction reactions (iron and sulfate reduction) appear to have been lowered in the sediment column (Figure 7g-i). Changing redox conditions can change the locations and activity of microbial communities that mediate the production of MeHg (Johnson et al. 2010).

### *Solid Phase Redox Conditions*

Figures 8-11 contain initial and final solid-phase sediment concentrations of AVS, %TS,  $Fe^{2+}$ , and SRB abundance. In each of these figures, initial conditions are depicted with dotted lines and final concentrations with solid lines. Columns of panels contain concentrations from each habitat zone, while rows represent different sulfate amendment levels in the overlying water. The AVS extraction method quantifies the least recalcitrant pool of solid phase iron sulfide minerals ( $FeS$ ), while not measuring pyrite ( $Fe_2S_3$ ), and can be considered the more recently formed or loosely bound iron sulfide minerals (Brouwer and Murphy 1994). Initial conditions for AVS (Figure 8) show that sediment from the SB habitat zone has the greatest abundance of solid phase AVS (Figure 8 b, e, h) with a peak near the sediment water interface (SWI) ( $50 \pm 2.4 \mu mol/g dw$ ) and a decrease with depth (to  $8.0 \pm 2.4 \mu mol/g dw$ ). An opposite AVS profile is seen in the sediment from the LEF and UEF habitat zones, where higher concentrations occur deepest in the sediment profile (Figures 8 a, d, g; c, f, i). The increase of AVS with depth in sediment from UEF and LEF habitat zones, suggests slow accumulation of loosely bound, reduced iron sulfides during burial.

For SB microcosms, the concentrations 6 months after amendments display a marked change in AVS in surficial sediment which is consistent with porewater redox changes observed in voltammetric electrode measurements (Figure 7) and decreases in total sulfur in the 0-4 cm interval (Figure 9, 30  $\mu mol/g \sim 0.1\% S$ ). The surficial sediment (0-4 cm) appears to have been exposed to less reducing conditions leading to the oxidative dissolution of iron sulfide minerals, while deeper areas in the sediment (>10cm) appeared not to be affected by this change to the upper boundary condition. Sediment between 4-10cm had an increase in AVS relative to initial conditions in high and medium sulfate amended microcosms. The oxidation of iron sulfide minerals may have created a situation in which both internal loading of sulfate from the sediment and external loading of sulfate (diffusion from overlying water) were significant. For LEF microcosms, little change was observed in AVS following the 6 month incubation, but slight decreases were observed at depth for low and medium sulfate treatments below 4cm (Figure 8a, d, and g). AVS in sediment from the UEF habitat zone increased relative to the initial conditions for all sulfate treatments between 4-10cm and for low and medium sulfate treatments

between 10-20cm. Although one explanation for this increase is sulfate diffusion from the overlying water, reduction and immobilization, a parallel increase in TS was not observed in UEF sediment (Figure 9) and would have been quantifiable (20 $\mu$ mol/g  $\sim$ 0.06%). Additionally, estimates of sulfate diffusion based on observed gradients are far less than necessary to account for the increase of AVS over 4-10cm. It is possible, therefore that over the course of the experiment, recalcitrant iron sulfide minerals, such as pyrite, were transformed into more labile reduced species that AVS is capable of measuring.

Measurements of total sulfur (TS), which quantifies all sediment sulfur, is presented Figure 9. The SB sediment solid phase TS (Figure 9, panels b, e, h) follows a similar trend as AVS for the initial and final experiment conditions (Figure 8). For sediment underlying a seasonally anoxic water column, this similar behavior between TS and AVS might be expected in surficial sediment as recalcitrant solid phase iron sulfides (resistant to AVS extraction) require permanent anoxic conditions to form. There was a decrease relative to initial conditions in TS from 0-4 cm in the low and medium LEF sulfate treatments but no change in the high LEF treatment. TS was not measured initially in the UEF high sulfate treatment sediment. However, if assumed to be similar to initial TS content in medium and low sulfate treatment sediment, an increase in TS was observed between 0-4 cm and 4-10 cm relative to initial conditions (Figures 8c, f, and i).

Ferrous iron was measured in the solid phase and is depicted in Figure 10. Solid phase Fe<sup>2+</sup> increased between 4-10 cm in all SB treatments relative to initial conditions (Figure 10b, e, and h) likely due to a redox change in the upper oxygen boundary, not a result of sulfate treatments. As discussed previously, the release of iron sulfide minerals could produce ferrous iron that can be measured with the extraction method used in this study. Porewater Fe<sup>2+</sup> concentrations decreased from the initial conditions (Figure 6 g-i), implying that there was not an increase in iron reduction in the SB sediment. Little change was seen in solid phase Fe<sup>2+</sup> for the top 10cm of LEF microcosms over the course of the study, while increases were observed from 10-20 cm (Figure 10). The UEF high treatment sediment in the 0-4cm interval displayed a marked decrease in solid phase ferrous iron, although it began with a magnitude four times larger than any other sediment microcosm (Figure 10).

#### *Sulfate Reducing Bacteria Microbial Abundance*

The abundance of sulfate reducing bacteria (SRB) was measured in the solid phase and is displayed in Figure 11. In the high-sulfate treatments for the SB and UEF habitat zones, the top 4 cm displayed an increase in SRB abundance relative to initial conditions and data suggests that this increase was statistically significant (Figure 11 b and c). For the medium sulfate treatments, SRB abundance in the 0-4cm interval increased relative to initial conditions for all habitat zones, but was not significant for SB sediment. For low sulfate treatments, a decrease in SRB abundance relative to initial conditions was observed at all depths for SB and LEF sediment, but UEF showed little difference (or a moderate increase) from initial conditions.

#### *Mercury Experimental results*

Solid phase THg concentrations varied among habitat zones and even among cores collected from the same habitat zone, (Figure 12, 13 and 14). Differences were most notable for microcosms collected in the LEF habitat zone, but also were present in the initial UEF medium (higher THg between 0-4 cm). Variability in total mercury necessitates normalization of the MeHg data to total mercury concentration if treatments are to be compared to one another in terms of methyl mercury production efficiency. In sediments that are not highly contaminated with mercury (<500 ng/g), MeHg and THg are often strongly correlated (Figure 15). A strong correlation was observed in this study, suggesting that no sediment conditions (among all habitat zones and overlying water sulfate amendments) were producing methyl mercury much more efficiently than others. A unit commonly employed to determine a sediment's capacity to methylate mercury under its in-situ geochemical setting is %MeHg (Drott et al. 2008). Since %MeHg is a measure of the sediment's capacity to methylate mercury, it will be used exclusively to compare limiting factors in the production of MeHg. Sediment profiles of MeHg, %MeHg, and THg are displayed in Figure 12, 13 and 14 for the initial treatments and treatments after 6 months. Error bars represent one standard deviation from the mean as calculated by the analysis of triplicate sub-cores from each habitat zone at the beginning of experiments. Panels a-c give concentration at the end of the experiment and panels d-f show initial conditions.

In sediment from the LEF habitat zone, %MeHg in the 0-4cm interval appeared to be unrelated to overlying water sulfate after the 6 month lab experiment (Figure 12b); however, %MeHg in the 4-10cm interval was consistent with increased with increasing overlying water sulfate. Although SRB abundance was not directly related to overlying water sulfate concentrations in LEF sediment (Figure 11), the increased %MeHg in the 4-10cm interval is consistent with SRB-driven methylation below the oxidized surface layer of sediment.

In sediment from the SB habitat zone % MeHg was not significantly different than the initial conditions in the high, medium, or low sulfate treatments ( $p > 0.05$ ) and did not differ amongst sulfate treatments (Figure 13). The SB has the greatest abundance of AVS and SRB suggesting that there is SRB mediated sulfate reduction producing MeHg in the sediment. However, the oxidation of reduced sulfides in the top 10 cm as a result of experimental manipulation likely caused internal loading of sulfate which could have lessened the influence of overlying water sulfate. Although this was an artifact of the experimental design, it may reflect conditions in the field if periodic anoxia exists in the bottom waters of SB environments. There was a decrease in sulfate reducing bacteria in the surficial sediment of the SB low sulfate treatment, (particularly between 4-10cm, Figure 11) which suggests that sulfate in the overlying water did influence SRB. However, a coincident decrease in %MeHg was not observed, suggesting that other factors such as the availability of inorganic mercury to methylating microbes may be limiting methyl mercury production in sediment of the SB. The SB sediment

did have the lowest porewater total mercury concentrations of any habitat zone despite the highest solid-phase total mercury (Table 3).

In the initial and final experimental conditions, sediment from the UEF habitat zone had higher %MeHg concentrations (Figure 12, 13, and 14) than sediment from other habitat zones at all depth intervals. The low UEF sulfate treatment displayed a large increase in %MeHg in the 0-4cm interval (Figure 15) relative to initial conditions, though geochemical trend analyses indicate that this point may be an outlier when compared to the entire data set (Figures 17 and 18). A sediment system in which methyl mercury does not depend on sulfate reduction can indicate that there is limited inorganic mercury available for methylation or that iron reducing bacteria (FeRB) could be the primary methylators of mercury (Mitchell and Gilmour, 2008<sup>b</sup>).

#### *Microbial and Geochemical Controls on MeHg production*

In the St. Louis River estuary, evidence suggests that there are different factors limiting sediment MeHg production in each site. Organic carbon can influence the production in MeHg via two separate mechanisms. A lack of labile organic carbon can limit the production of methyl mercury by limiting the microbial activity of SRB in sediments (Lambertsson et al. 2006). Organic carbon can also limit the bioavailability of inorganic mercury to SRB, thereby decreasing mercury methylation (Hammerschmidt and Fitzgerald 2004<sup>a</sup>). Both carbon related MeHg limiting process appear to be occurring in the St. Louis River Estuary sediment, depending on the habitat zone. In the LEF habitat zone, organic carbon in the sediment is refractory (high C/N ratio, Figure 4). Figure 18 depicts a significant ( $p < 0.05$ ) negative correlation between total carbon and %MeHg in the LEF system, similar to the trend observed by Hammerschmidt and Fitzgerald (2004<sup>a</sup>). Since the organic carbon supplied to the LEF habitat zone is refractory, organic carbon at this site may bind inorganic mercury (thus limiting the supply of bioavailable inorganic mercury) while not providing the energy to drive methylating microbial processes. The net effect of carbon in LEF sediment, therefore would be a lower in production of MeHg.

The second organic carbon limiting mechanism is proposed for the SB and UEF habitat zones. A significant relationship between organic carbon and %MeHg ( $p < 0.05$ ) was observed in the SB habitat zone (Figure 18) but was not significant ( $p > 0.05$ ) for the UEF sediment. Since the organic carbon supplied in the UEF and SB habitat zones is not as recalcitrant, it may function as a ligand for inorganic mercury but also drive microbial activity. Both UEF and SB sediment had much lower porewater total mercury than LEF (2.3x and 6x smaller, respectively), but consistently higher %MeHg. Despite having porewater total mercury concentrations 2-3x lower than UEF, SB had similar %MeHg, possibly due to greater overall activity of sulfate reduction driven by a larger quantity of carbon.

Many studies have cited a limitation of inorganic mercury available for methylation due to high porewater sulfide concentrations (Benoit et al. 1999<sup>a</sup>; Benoit, 2003; Gilmour et al. 1998). Additionally, transport of MeHg from sediments is believed to be accelerated in the presence of dissolved sulfide (Berndt and Bavin, 2011; Jonsson et al. 2010). In the St. Louis River estuary, sulfide concentrations were uniformly below the detection limit (1-5 $\mu$ M), which is below the proposed threshold of sulfide limitations on MeHg production (<5-10  $\mu$ M sulfide, Benoit, 2003); however, it is possible that iron sulfides may be limiting the bioavailability of inorganic mercury to SRB by binding it to the solid phase (Bower et al. 2008; Liu et al 2009). The sulfide concentration at which inorganic- and methyl- mercury speciation shifts from organic ligands to sulfide ligands is dependent upon the sulfur content of organic carbon (Skylberg 2008) which is not known for the St. Louis River Estuary. However, the low sulfide conditions observed in the estuary would likely favor organic carbon as a ligand relative to sulfidic environments encountered elsewhere in the St. Louis watershed (Berndt and Bavin, 2011).

#### *Porewater Mercury Partitioning Coefficients and Flux Estimates*

Porewater total- and methyl- mercury was measured in bulk sediment from the top 10cm in each of the microcosms at the close of the experiments (Table 1). No discernible trend was observed for porewater THg or MeHg among high, medium, and low sulfate treatments, and values from each habitat zone are averaged for flux analysis and calculations. Porewater total mercury concentrations appeared to be strongly related to the total organic carbon at the three study sites, with SB having the lowest concentrations (4.5 $\pm$ 1.8 ng/L), UEF having higher porewater THg (12.3 $\pm$ 5.4 ng/L), and LEF having the highest porewater THg concentrations (26.3 $\pm$ 21.6 ng/L). Porewater methyl mercury was less variable, with all sites averaging between 0.32 and 0.46 ng/L. Sediment from the LEF habitat zone had the most variable solid-phase and dissolved-phase total- and methyl- mercury concentrations.

Partitioning coefficients for THg and MeHg are presented in Table 2 for each habitat zone. For THg, the UEF and LEF have the lowest log  $K_D$  values of 3.9 $\pm$ 0.1 and 3.9 $\pm$ 0.2, respectively, while the SB had the largest log  $K_D$  value of 4.70 $\pm$ 0.2. The lower total mercury log  $K_D$  values for LEF and UEF may be due to lower TC values relative to the SB sediment. TC and  $K_D$  tend to be positively correlated since THg sorbs strongly to organic matter (Hammerschmidt and Fitzgerald 2004<sup>a</sup>).

MeHg partitioning coefficients are all fairly similar among habitat zones with the UEF habitat zone having the lowest  $K_D$  value (3.2 $\pm$ 0.5) relative to the SB (3.6 $\pm$ 0.1) and LEF (3.4 $\pm$ 0.5) habitat zones. In addition to inorganic mercury, organic carbon also binds MeHg very strongly and may be controlling the partitioning of MeHg to the solid phase (SB had highest  $K_D$  for both THg and MeHg). Sulfide concentrations remained low (<5 $\mu$ M) in all estuary sediment,

potentially resulting in conditions that favor organic ligands over sulfide in MeHg speciation (Dryssen and Wedborg 1991).

THg flux measurements to the overlying water made at the close of lab experiments are summarized in Table 1 for each habitat zone. The change in mass of mercury in the overlying water over a 36-48 hour time period was normalized for surface area according to Equation 1.

$$Flux_{Hg} = \frac{\Delta C \cdot V_w}{\Delta t \cdot A} \quad \text{Equation 1}$$

Where

- $\Delta C$  is the change in overlying water mercury concentration
- $V_w$  is the volume of the overlying water
- $\Delta t$  is the time of the flux experiment, and
- $A$  is the surface area of the sediment microcosm.

Consistent with porewater measurements, no discernible trend was observed in flux measurements among high, medium, and low sulfate treatments, so results were averaged for each habitat zone. THg flux out of the St. Louis River Estuary habitat zones was between 15 and 38 ng/m<sup>2</sup>/d (Table 1), and is in agreement with estimated fluxes from other sediment systems (Gill et al. 1999<sup>a</sup>). THg flux out of the sediment, similar to porewater concentrations, seems to be influenced by organic carbon with SB and UEF having the lowest flux (15.5 and 18.5 ng/m<sup>2</sup>/day, respectively), and LEF having the highest flux (38 ng/m<sup>2</sup>/day). Since the LEF and UEF habitat zones have similar Log(KD) values, larger solid phase THg concentrations appear to be driving larger fluxes in the LEF habitat zone (Table 1).

MeHg flux was measured in the sediment microcosms, but differences between initial and final MeHg were not adequate to calculate fluxes. In order to estimate MeHg fluxes, the effective mass transfer coefficient (effective diffusion) observed during total mercury flux experiments was employed. Flux across the sediment water interface can be given by:

$$F \left[ \frac{ng}{cm^2 - day} \right] = k_{mt} \left[ \frac{cm}{day} \right] \left( C_{pw} - C_{overlying} \right) \left[ \frac{ng}{cm^3} \right] \quad \text{Equation 2}$$

Since THg flux, overlying water concentration, and porewater concentration are known for THg experiments, an effective mass transfer coefficient ( $k_{mt}$ ) can be calculated for each THg flux experiment. MeHg flux was estimated by assuming that mass transfer resistances for MeHg are similar to that of THg. Mass transfer coefficients for the three sulfate treatments in each habitat zone were then averaged and used in MeHg flux calculations according to Equation 2. Calculated MeHg flux values for the LEF, UEF, and SB habitat zones are displayed in Table 1 and are in agreement in magnitude with those estimated in the Long Island Sound (CT) system

(Hammershmidt et al 2004<sup>b</sup>). The SB, having the largest kmt, has the largest MeHg flux value (1.75 ng m<sup>-2</sup> d<sup>-1</sup>) while the UEF and LEF have lower values of 0.15 and 0.72 ng m<sup>-2</sup> d<sup>-1</sup>, respectively.

Experiments utilizing sediment from each habitat zone was scaled to the total area of the Habitat zone within the estuary. MeHg flux values and areas were used to calculate sediment MeHg loading (ng hr<sup>-1</sup>) to the St. Louis River Estuary (Table 3). MeHg loading from the upstream St. Louis River (Figure 3) was calculated using MeHg concentrations measured by Berndt and Bavin (2009 and 2012) and discharges from USGS gauge stations located at the Scanlon Dam at multiple time points (St. Louis River discharges were taken from the same day MeHg water samples were obtained). The estimated MeHg loads from the UEF, LEF, and SB habitat zones were 1.2, 7.6, and 6.3 mg d<sup>-1</sup>, respectively. The incoming MeHg load at the Scanlon Dam was calculated to be 173 mg d<sup>-1</sup> during low flow conditions and 7780 mg d<sup>-1</sup> during high flow conditions (Table 3). Median flow conditions (around 28000 L s<sup>-1</sup>) carried a MeHg load of 400 mg d<sup>-1</sup> from upstream sources. If summed, the total MeHg load from the three habitat zones examined in this study (representing 45% of the total area in the St. Louis River Estuary) represents about 13 % of that being delivered from upstream sources during low flow conditions, but is very small compared to upstream sources during high flow conditions.

Some habitat zones not included in this study contain sediments that are contaminated with respect to mercury (>1000 ng g<sup>-1</sup> THg), which could result in small portions of the estuary contributing disproportionately large amounts of mercury to the overlying water column. In other contaminated sediment systems with THg concentrations greater than 1000 ng g<sup>-1</sup>, sediment MeHg and THg fluxes have been reported up to three orders of magnitude greater than those measured in the LEF, UEF, or SB habitat zones. If habitat zones with high concentrations of solid phase mercury (THg) in the St. Louis River estuary have MeHg fluxes similar in magnitude to those in other contaminated systems, sediment MeHg flux from these small, heavily contaminated areas could contribute a substantial amount of MeHg to the overlying water.

Since mercury flux was not measured in half of the estuary and in no heavily contaminated locations, it is still uncertain how important sediment is as a source of MeHg to the St. Louis River estuary. If other habitat zones contribute MeHg fluxes similar or greater to those estimated in this study, the MeHg load from sediment in the estuary could exceed 25% of total loads to the estuary during low flow conditions but will likely be overshadowed by upstream sources during high flow conditions. Understanding the magnitude of MeHg flux from sediments in the estuary will help to inform watershed managers whether active management to control MeHg production and flux from estuary sediments is necessary.

## 5. Conclusions and Implications

Results from the sulfate addition experiments suggest that MeHg production in sediment of the St. Louis River Estuary was largely controlled by carbon except in one case when sulfate in the overlying water appeared to be important. Over the timescale of these experiments (6 months), no habitat zones exhibited a significant change in sediment %MeHg in the surficial sediment (0-4cm) as a result increased or decreased sulfate in the overlying water. However, measurements in the LEF habitat zone showed that %MeHg in deeper sediments (4-10cm) appeared to be related to sulfate in the overlying water (Figures 12-14).

Carbon controls on MeHg production in each habitat zone was related to the type and quantity of carbon present. In the SB habitat zone, large quantities of organic carbon and iron sulfides (represented by AVS) are likely limiting the amount of inorganic mercury available to microbial communities for methylation. The UEF and SB had similarly low C/N ratios which could drive higher microbial activity (Figure 4); however lower total carbon in the UEF may be the cause of higher porewater inorganic mercury (relative to the SB sediment) available for methylation and transport (Table 1). It should be noted that the %MeHg in the 0-4 cm depth of the UEF low sulfate treatment (Figure 14) appears to be an outlier (Figure 17 and Figure 18) and calls into question the significance of the higher %MeHg observed in response to a decrease in overlying water sulfate.

The Sheltered Bay (SB) and Upper Estuary Flats (UEF) habitat zones initially were the most efficient methylators of mercury, with %MeHg values (0.8-1.3 %MeHg) similar to those found in other aquatic systems (Kim et al. 2011). The UEF site had the highest %MeHg possibly due to two factors: low  $\log K_D$  ( $3.9 \pm 0.13$ ) indicating significant porewater concentrations; and low C/N ratio ( $15.9 \pm 2.1$  C/N) indicating availability of labile organic carbon. Both the UEF and SB habitat zones, according to the data presented, may be limited in the mercury methylation process by porewater bioavailable inorganic mercury, not sulfate. Although strong associations with organic carbon may limit MeHg production in the high organic SB habitat zone, significant flux was still observed as indicated by the high effective diffusion (mass transfer) coefficient (Tables 1 and 3).

Another conclusion from the data presented is that decreasing sediment THg concentrations could result in a decrease in MeHg production (Figure 16). It has been observed in both the dataset presented here for the St. Louis Estuary and in the literature (Benoit et al. 2003) that MeHg concentrations are strongly related to THg concentrations in sediment that is not highly contaminated by mercury (<500 ng/g). In all sites for this study, there was a significant positive relationship between THg and MeHg. Although scientifically answering the question of sulfate and carbon limitations to sediment MeHg production and transport could help to evaluate management alternatives, decreasing atmospheric and terrestrial mercury

loading to the St. Louis River Estuary could lead to an decrease in sediment MeHg concentrations.

MeHg and THg flux was estimated for each habitat zone to quantify the mass loadings of mercury to the St. Louis River Estuary System. It was calculated that the three habitat zones examined in this study (44.9% of the entire estuary area) could contribute a MeHg load up to 13% of that delivered from upstream sources during low flow conditions. Although the residence time of different parts of the estuary is not well known to support a complete mass balance, these calculations indicate that sediment contributions could influence MeHg concentrations in the overlying water, particularly during low flows or in shallow, slow moving backwater bays with elevated mercury concentrations in surficial sediment.

This study answered multiple scientific and management questions related to the production and transport of MeHg in the St. Louis River Estuary. The first question answered was whether changing sulfate concentrations in the overlying water would influence MeHg concentration in sediments in light of the various factors limiting MeHg production in each habitat zone. From a management standpoint, results suggest that sediment loading could be important for the overall mercury balance in the water column, relative to upstream loading in certain locations (isolated bays) or certain times (low flows). Although further investigation of sediment-related mercury dynamics and hydrodynamic mixing in the St. Louis River Estuary will be necessary to achieve a full mass balance for MeHg, the results presented here have illuminated the role of sulfate on the production and transport of MeHg in the St. Louis River Estuary sediment.

## 6. References

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## Figures & Tables

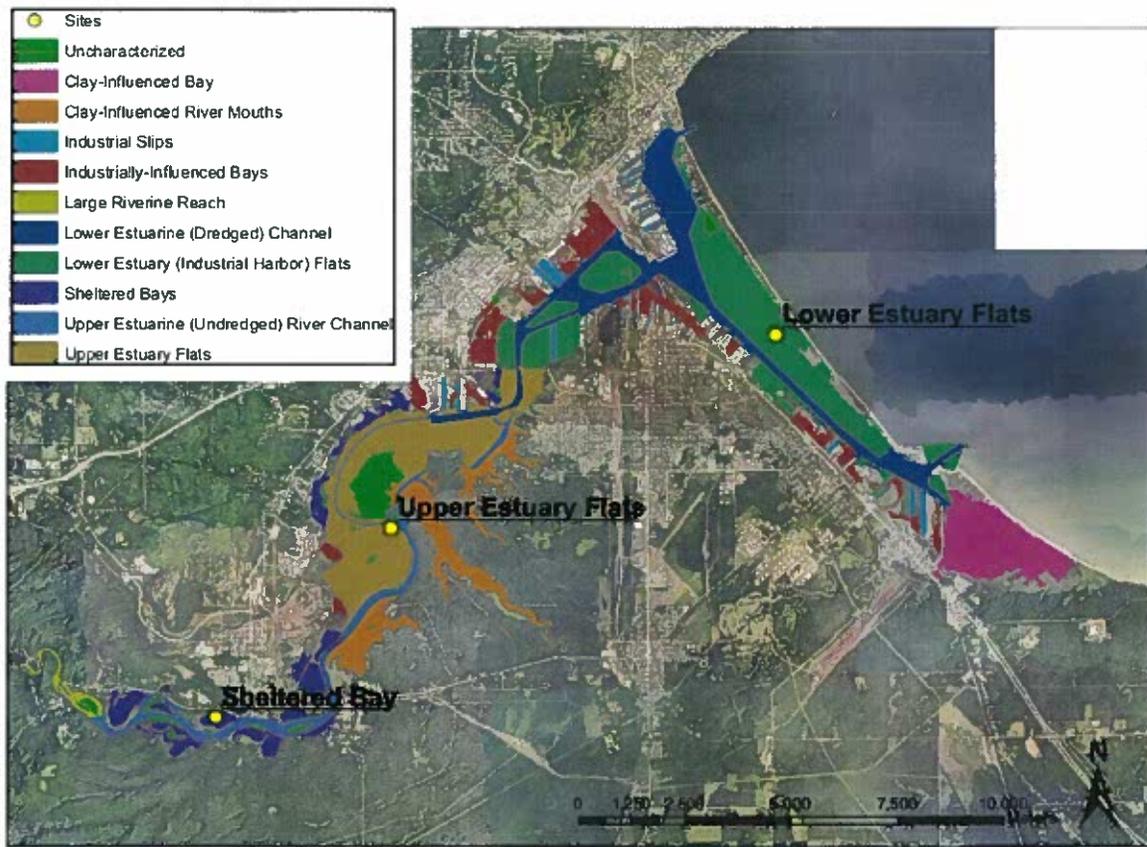


Figure 1. St. Louis River Estuary with Habitat Zones delineated by the SLRA. Sites sampled in August 2011 are highlighted in yellow.

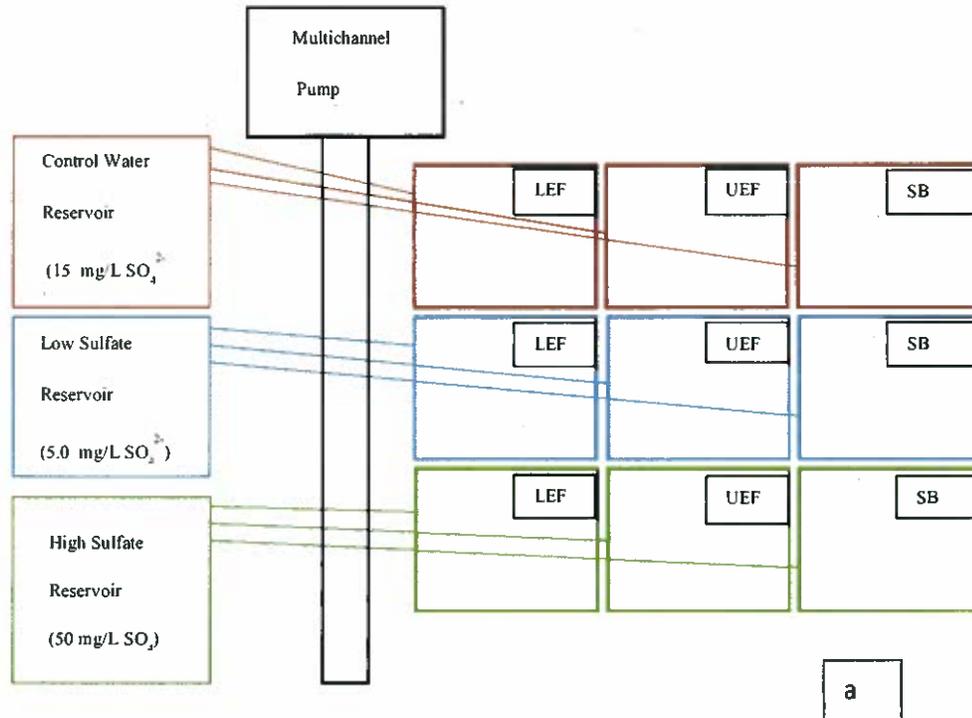


Figure 2. Schematic of experimental design including treatment concentrations (a) and a photo of the experimental setup within the temperature control chamber (b).



Figure 3. Sediment Core tube attached to sediment corer and drive rod. Ball valves displayed in an open position, which was used when driving core tube into sediment and closed 90° when core had reached its desired depth.

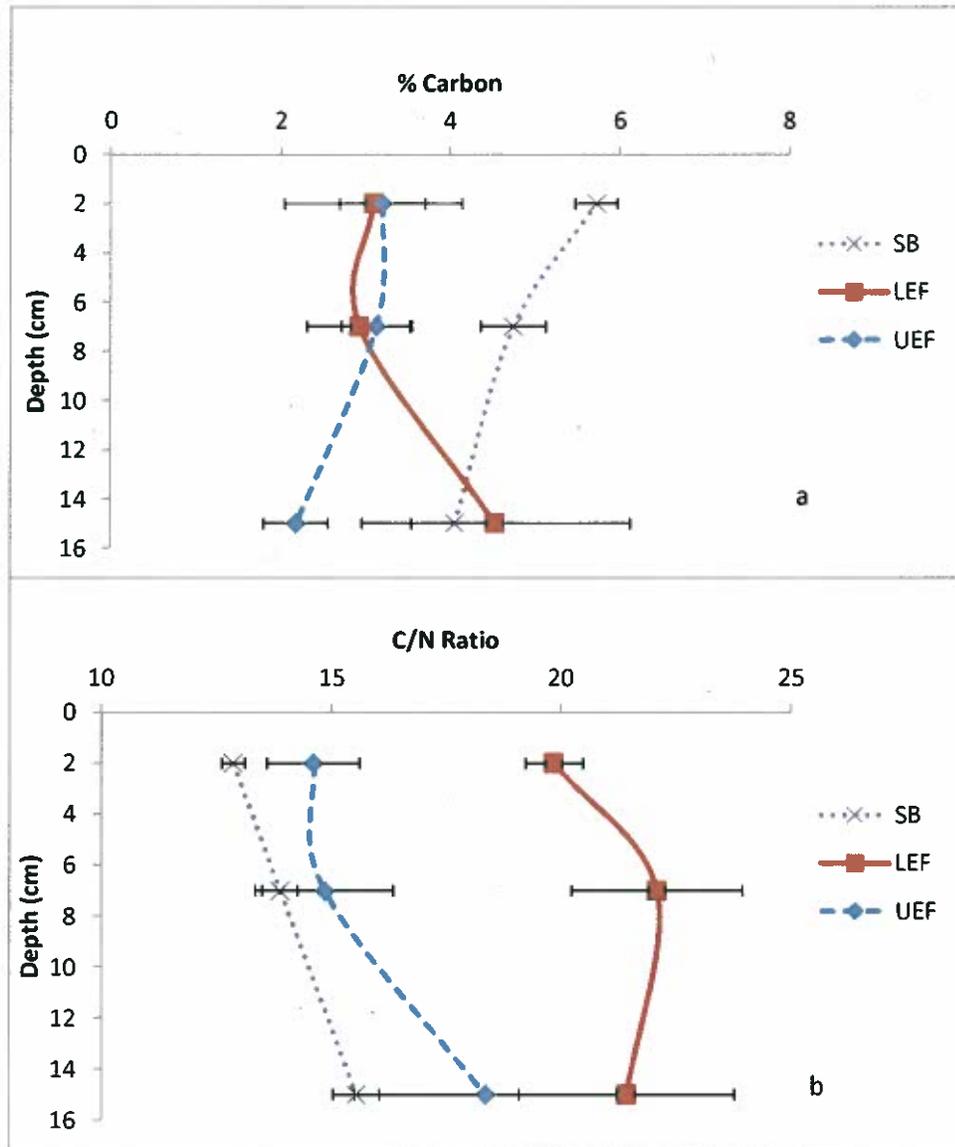


Figure 4. Total Carbon (TC) (a) and C/N ratio (b) sediment profiles. Sediment profiles were averaged for each site since TC and C/N ratios went unchanged during the experiment time period.

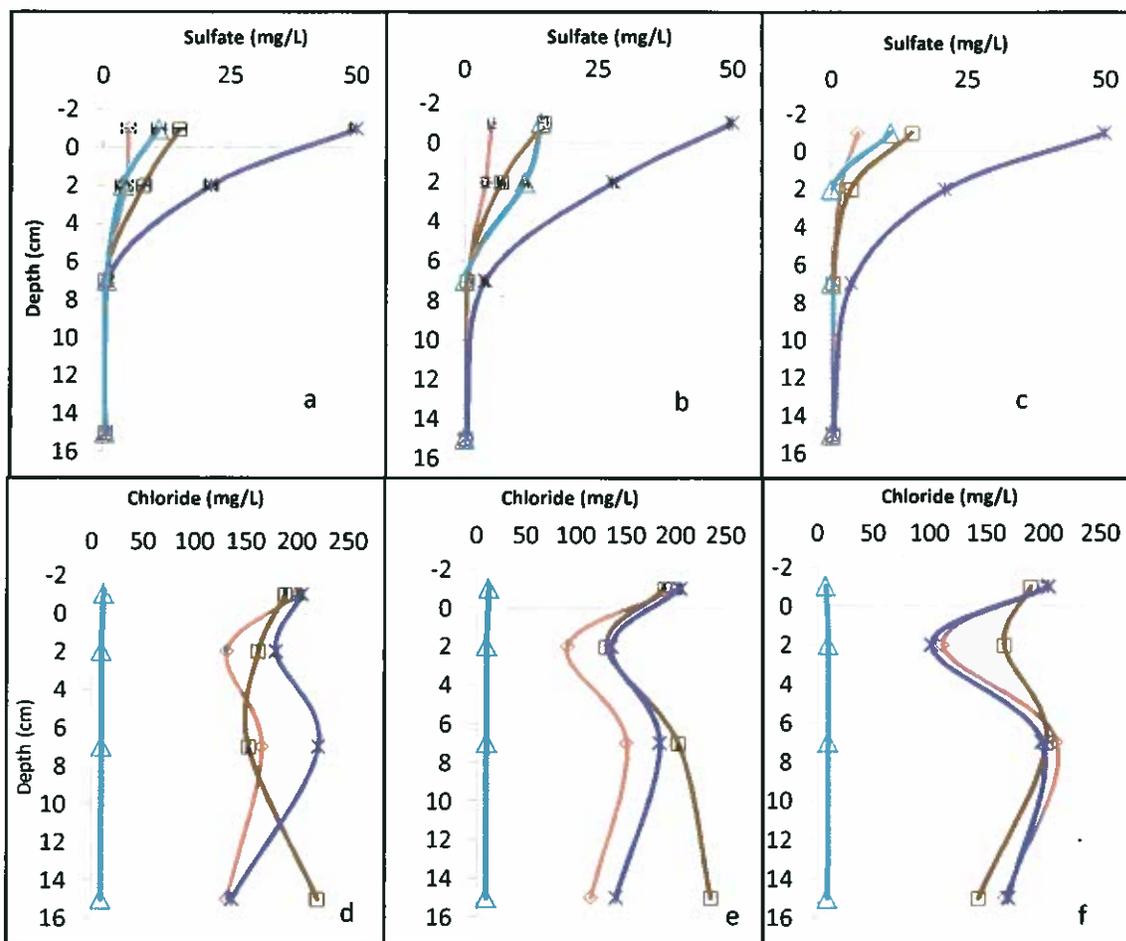


Figure 5. Sulfate and chloride sediment profiles for UEF (a and d), LEF, (b and e), and SB(c and f) sites including overlying water concentrations for high (purple crosses), low (pink diamonds), medium (brown squares), and initial profiles (blue triangles).

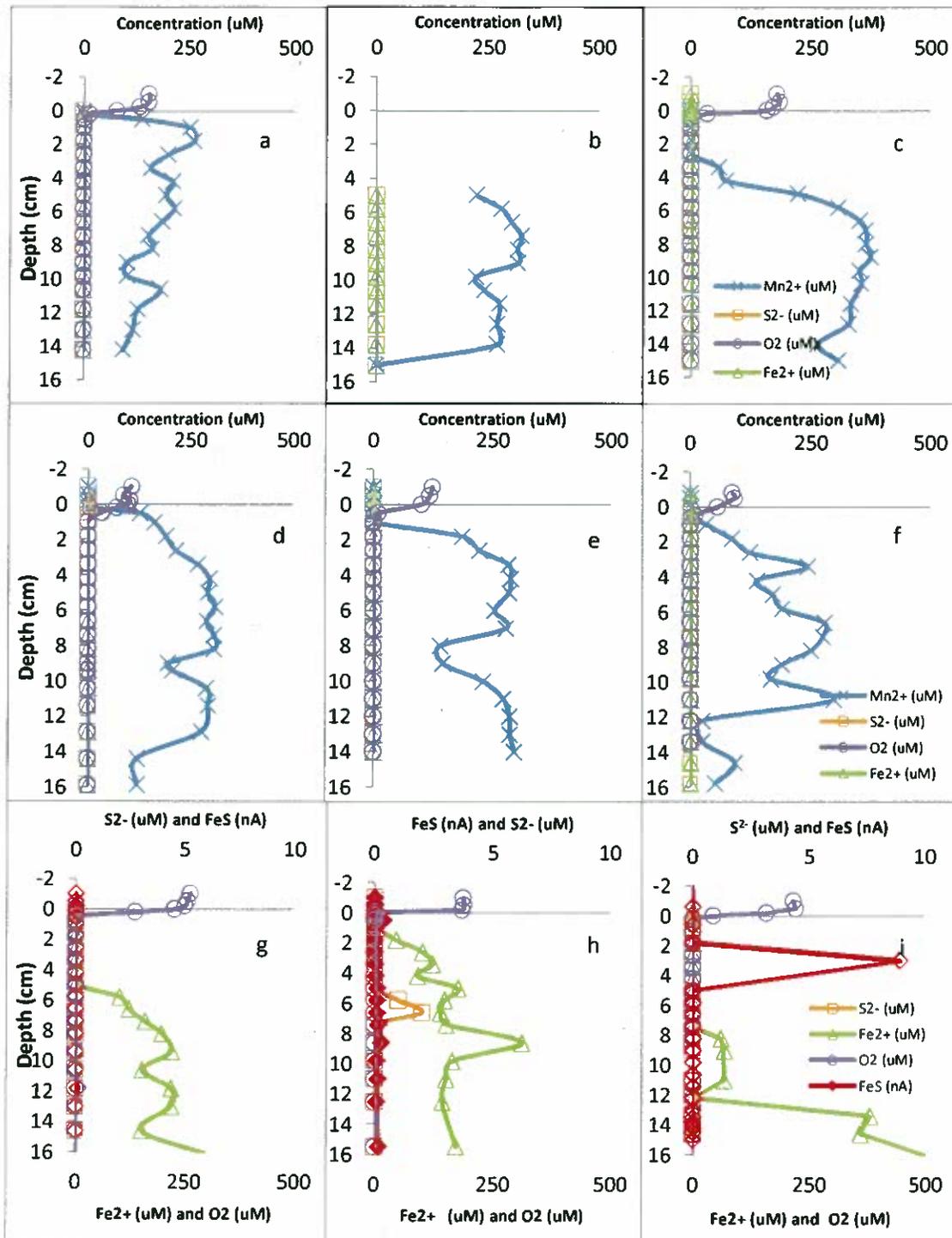


Figure 6. Initial condition porewater redox profiles for the LEF (low (a), medium (b), high(c)), UEF (low(d), medium(e), and high(f)), and SB (low (g), medium (h), and high(i)) experiments.

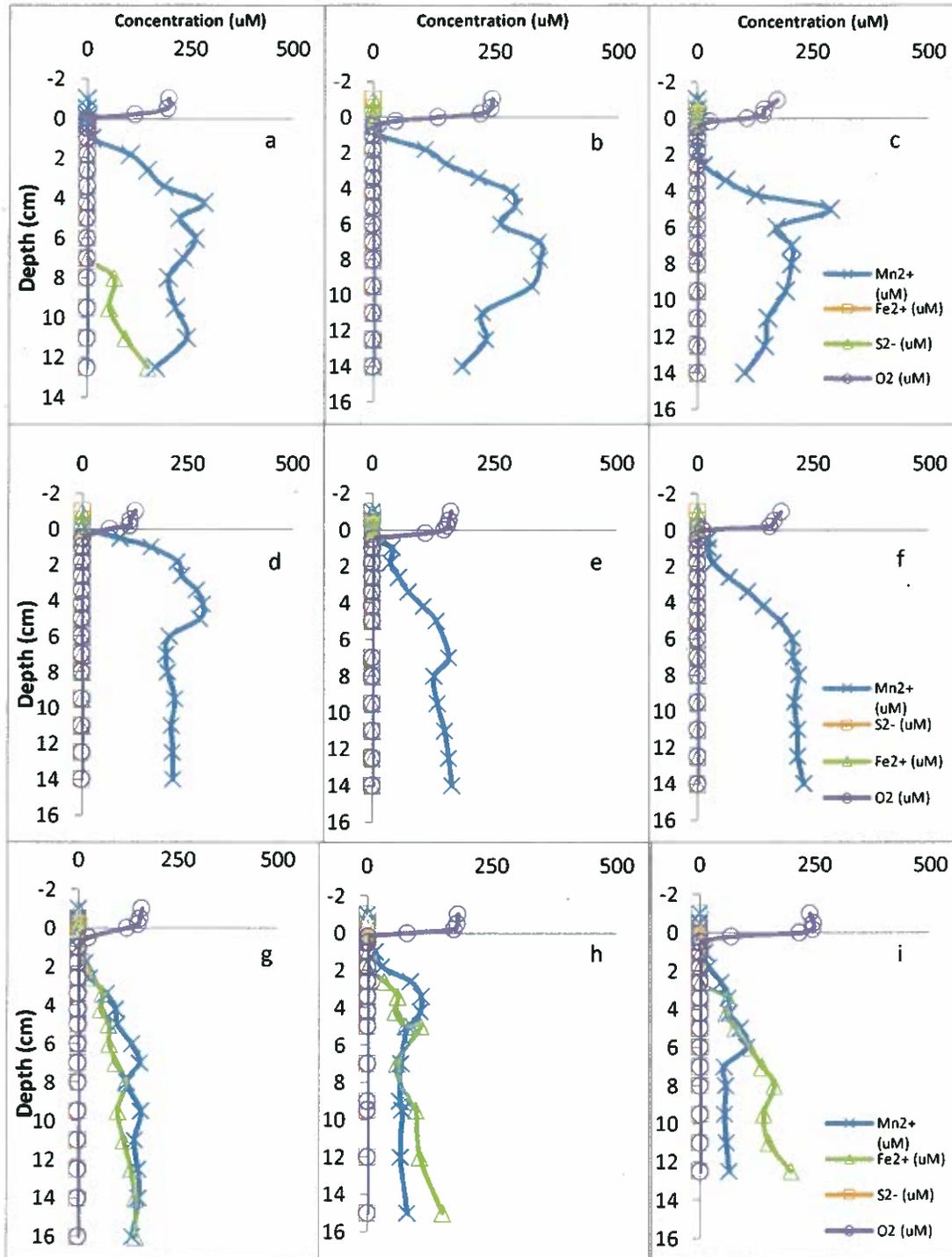


Figure 7. Final condition porewater redox profiles for the LEF (low (a), medium (b), high(c)), UEF (low(d), medium(e), and high(f)), and SB (low (g), medium (h), and high(i)). FeS was not added to this figure since there was no detectable FeS in any of the microcosms.

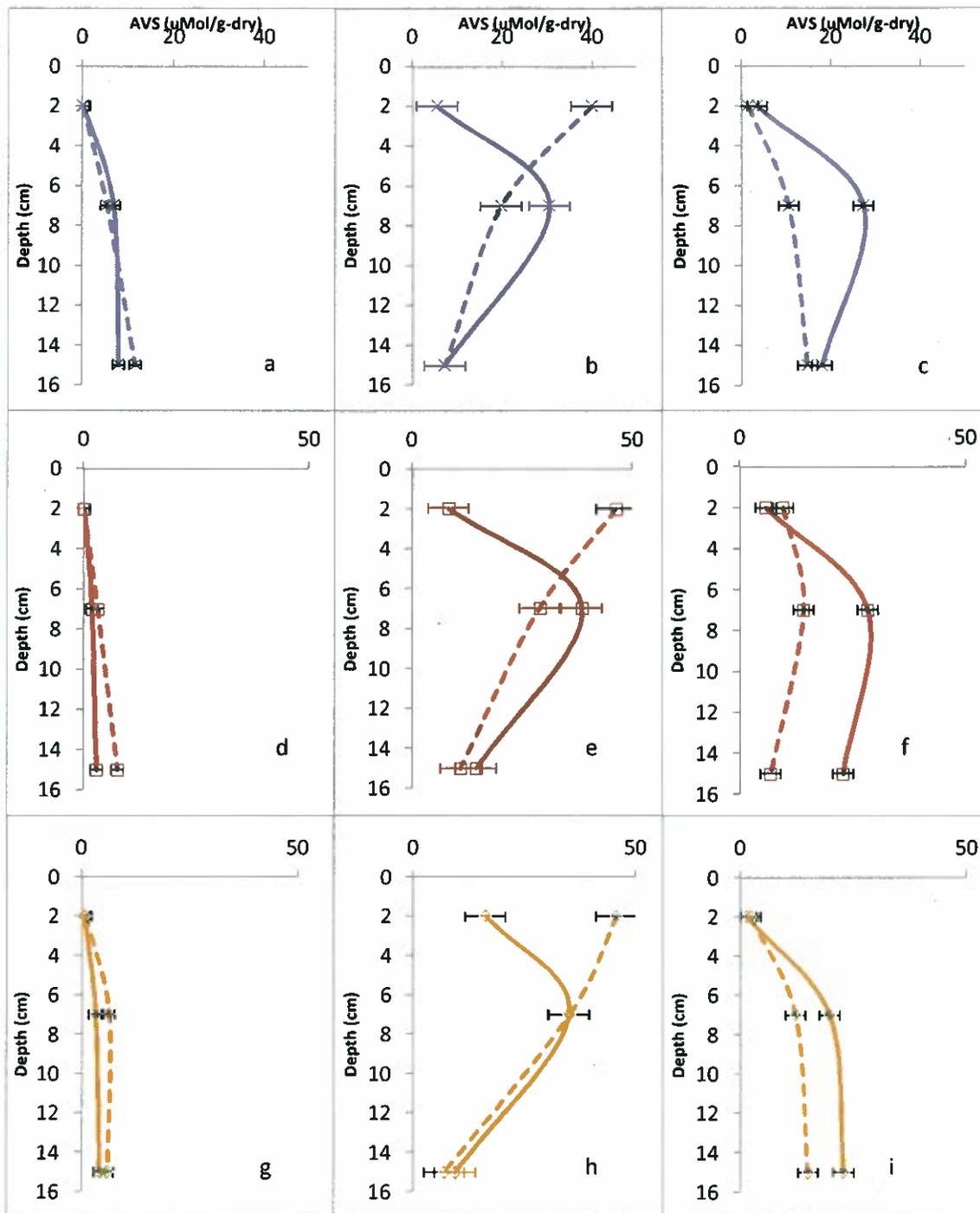


Figure 8. Sediment AVS profiles of Initial (dotted lines) and final (solid lines) for LEF (a, d, and g), SB (b, e, and h), and UEF (c, f, and i). High sulfate treatments (a-c) are displayed in purple crosses, medium treatments (d-f) are displayed in red squares and low treatments (g-i) are displayed in yellow diamonds.

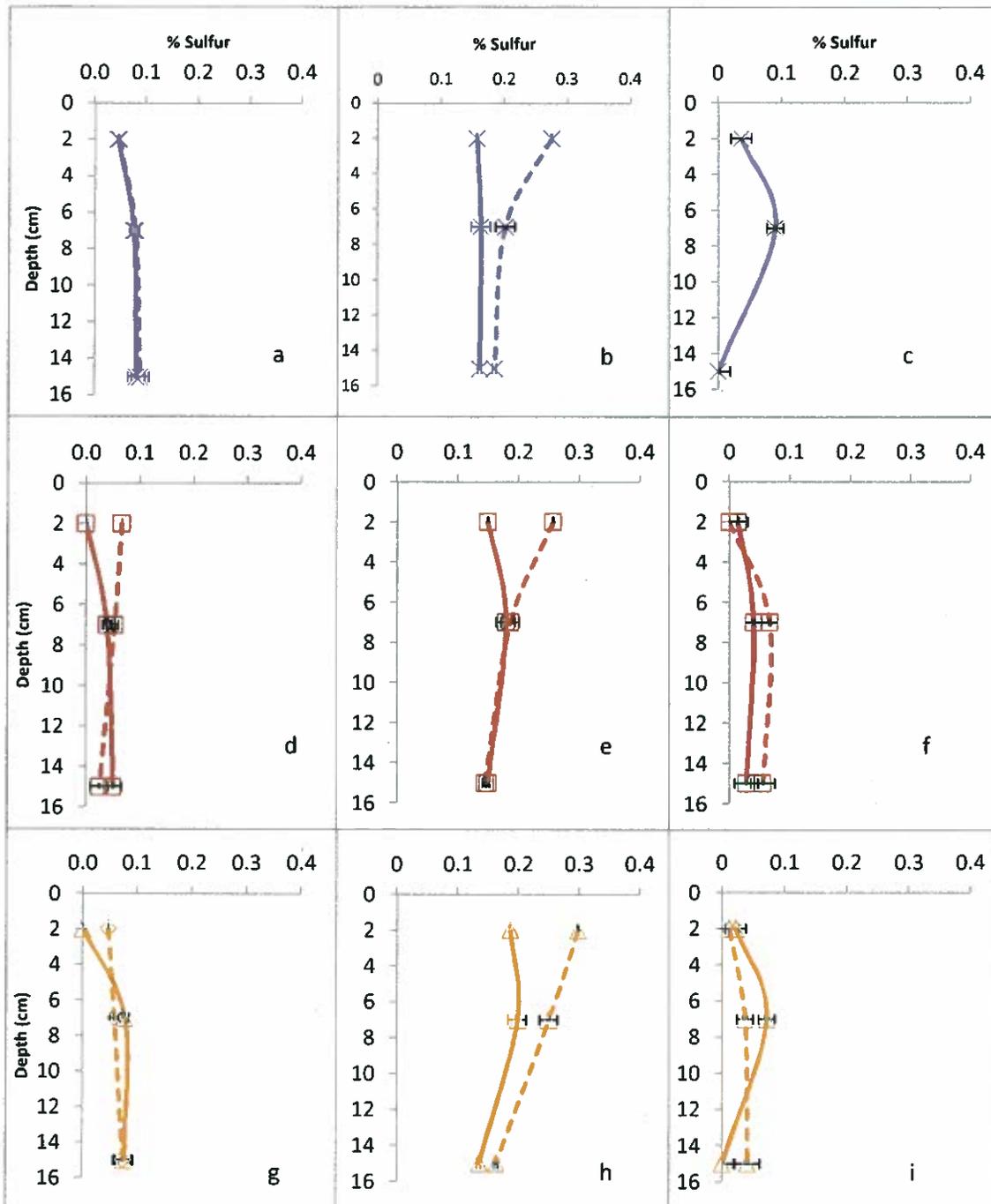


Figure 9. Sediment profiles of Initial (dotted lines) and final (solid lines) for LEF (a, d, and g), SB (b, e, and h), and UEF (c, f, and i) % sulfur concentrations. High sulfate treatments (a-c) are displayed in purple crosses, medium treatments (d-f) are displayed in red squares and low treatments (g-i) are displayed in yellow triangles.

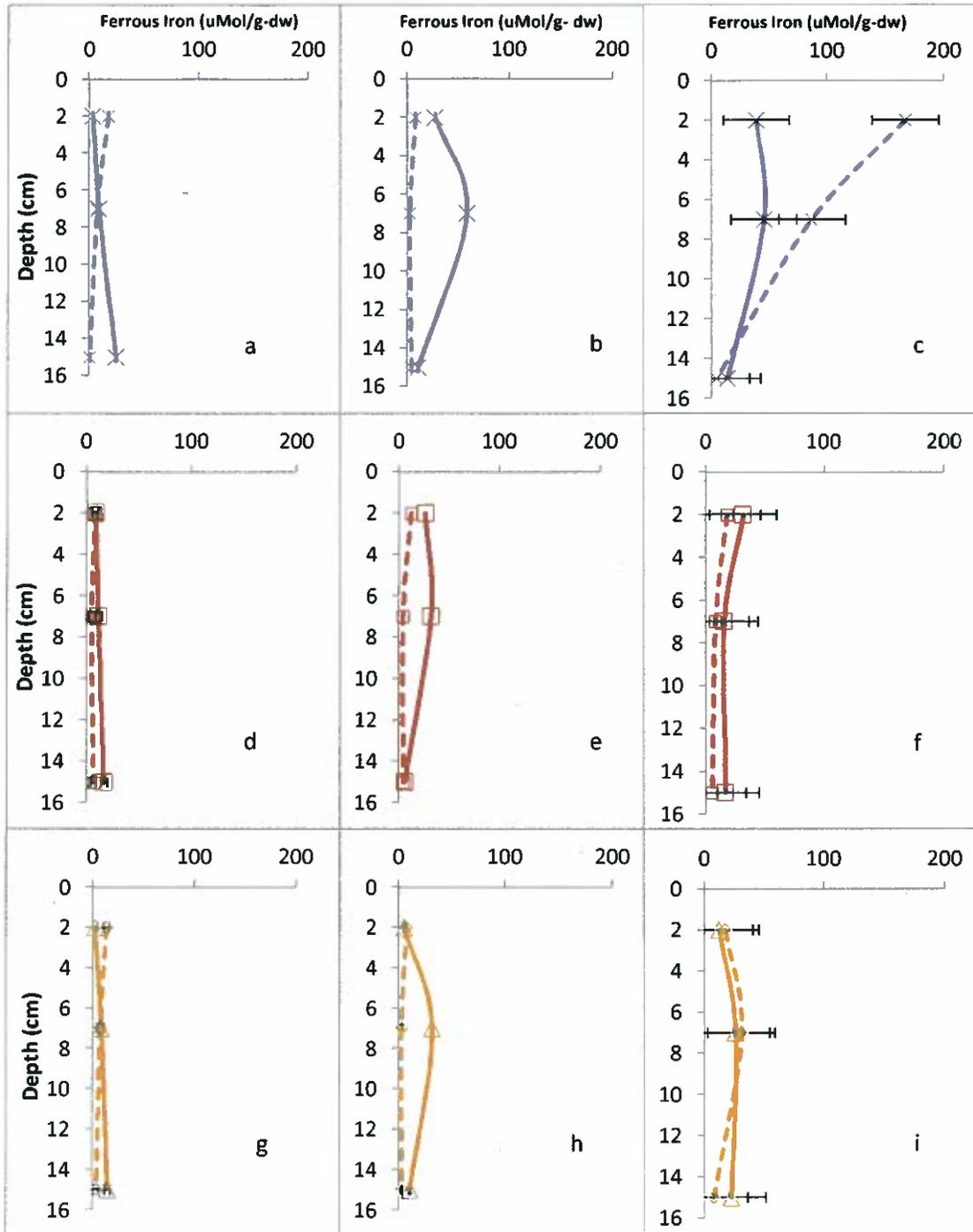
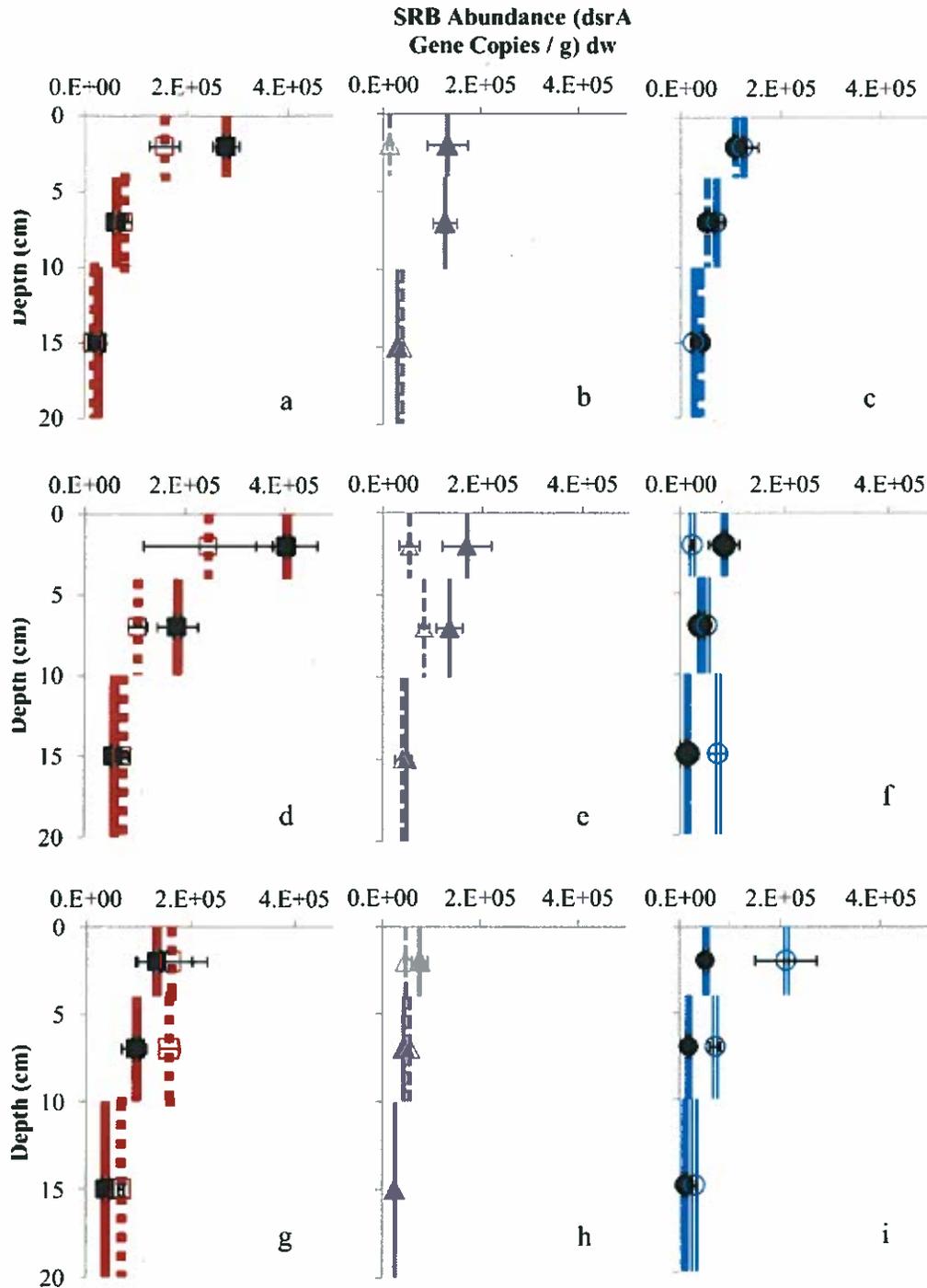


Figure 10. Sediment ferrous iron concentration profiles of Initial (dotted lines) and final (solid lines) for LEF (a, d, and g), SB (b, e, and h), and UEF (c, f, and i) experimental conditions. High sulfate treatments (a-c) are displayed in purple crosses, medium treatments (d-f) are displayed in red squares and low treatments (g-i) are displayed in yellow triangles.



**Figure 11.** SRB abundance at initial (empty markers; 0 months) and final (black markers; 6 months) laboratory conditions for sediment from (a-high sulfate, d-control sulfate, and g-low sulfate) SB microcosms, (b-high sulfate, e-control sulfate, and h-low sulfate) UEF microcosms, and (c-high sulfate, f-control sulfate, and i-low sulfate) LEF microcosms. Values depicted represent averages ( $n=3$ ) from triplicate analysis. Horizontal bars represent the standard deviation from triplicate analysis of the DNA extract. Vertical bars represent the composited interval represented by each data point.

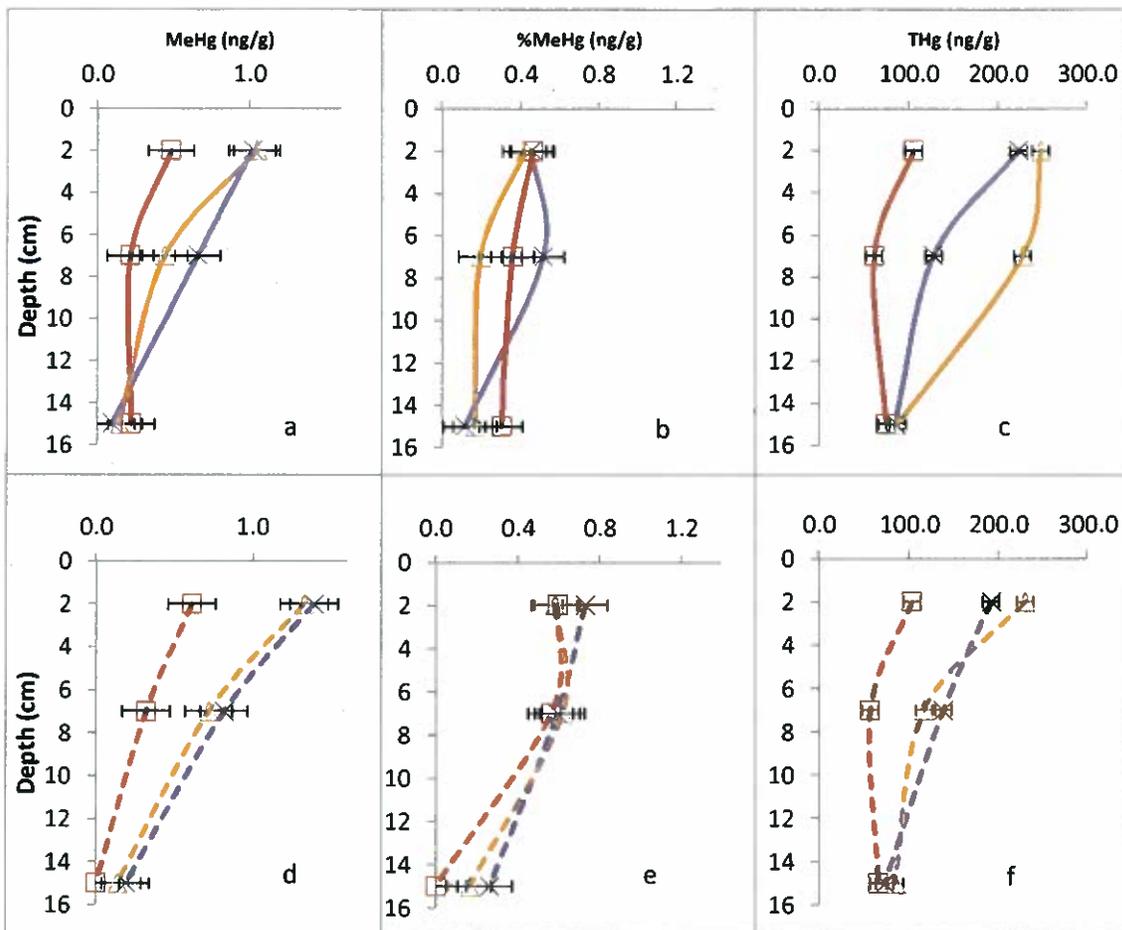


Figure 12. LEF sediment solid phase MeHg (a and d), %MeHg (b and e), and THg (c and f) for the LEF high (purple cross), medium (red square), and low (orange triangle) sulfate treatments at experimental initial conditions (dotted lines; d-f) and final conditions (solid lines; a-c). Error bars represent one standard deviation from the mean as calculated by the analysis of triplicate sub-cores from each habitat zone at the beginning of experiments.

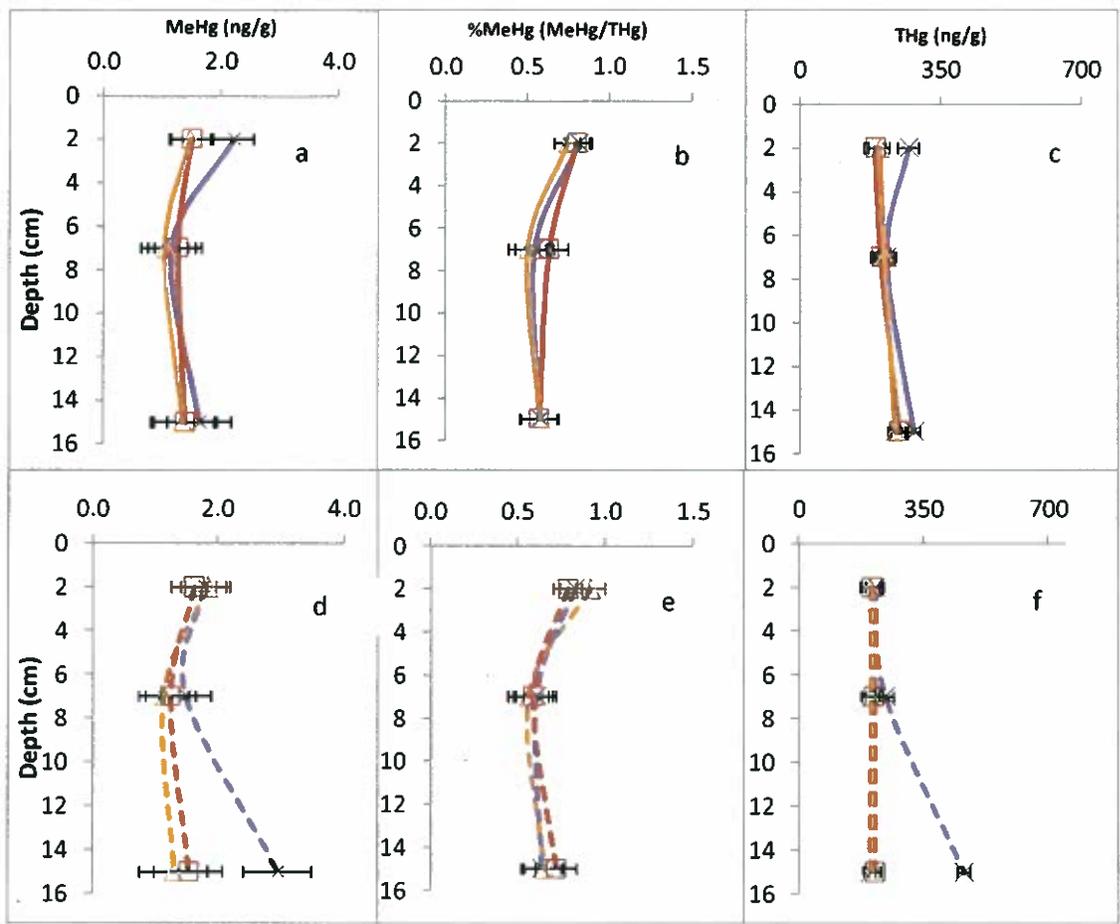


Figure 13. SB sediment solid phase MeHg (a and d), %MeHg (b and e), and THg (c and f) for the LEF high (purple cross), medium (red square), and low (orange triangle) sulfate treatments at experimental initial conditions (dotted lines; d-f) and final conditions (solid lines; a-c). Error bars represent one standard deviation from the mean as calculated by the analysis of triplicate sub-cores from each habitat zone at the beginning of experiments.

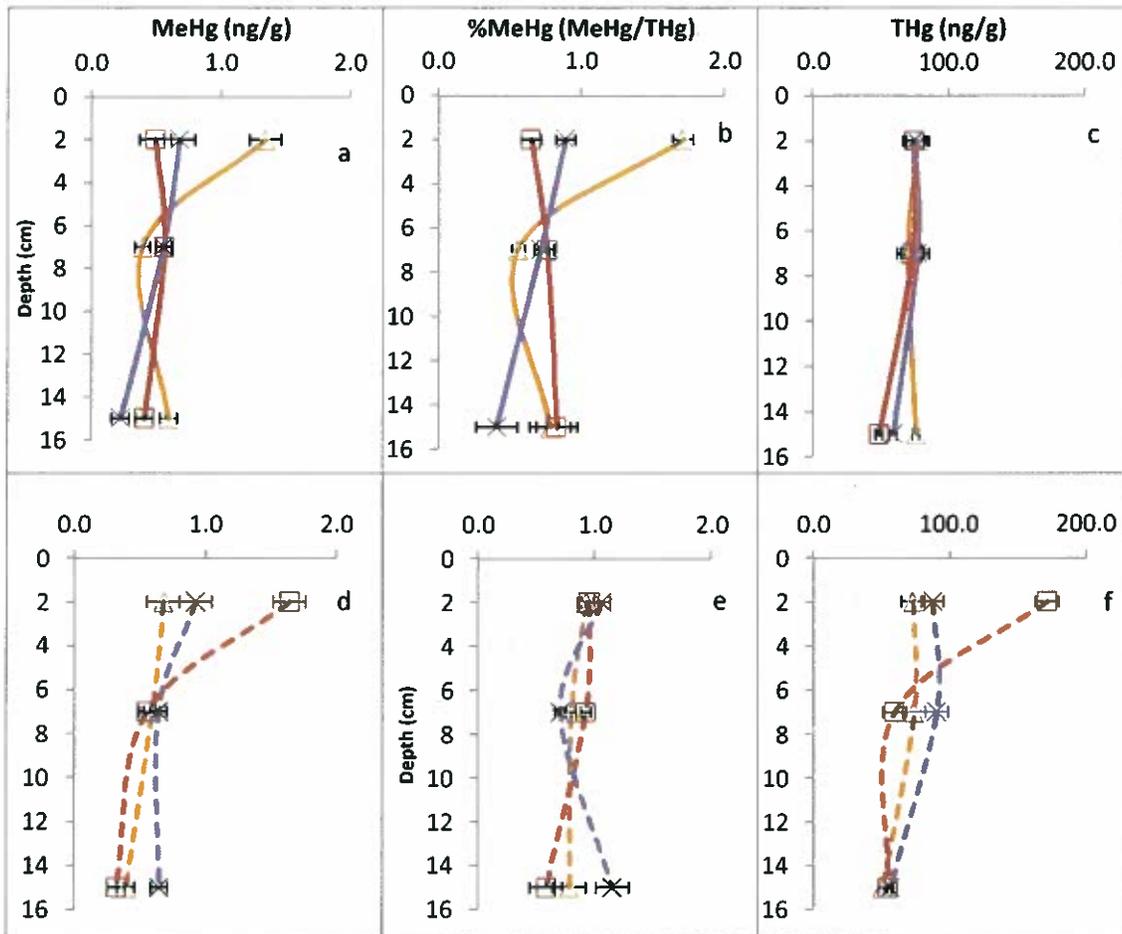


Figure 14. UEF sediment solid phase MeHg (a and d), %MeHg (b and e), and THg (c and f) for the LEF high (purple cross), medium (red square), and low (orange triangle) sulfate treatments at experimental initial conditions (dotted lines; d-f) and final conditions (solid lines; a-c). Error bars represent one standard deviation from the mean as calculated by the analysis of triplicate sub-cores from each habitat zone at the beginning of experiments.

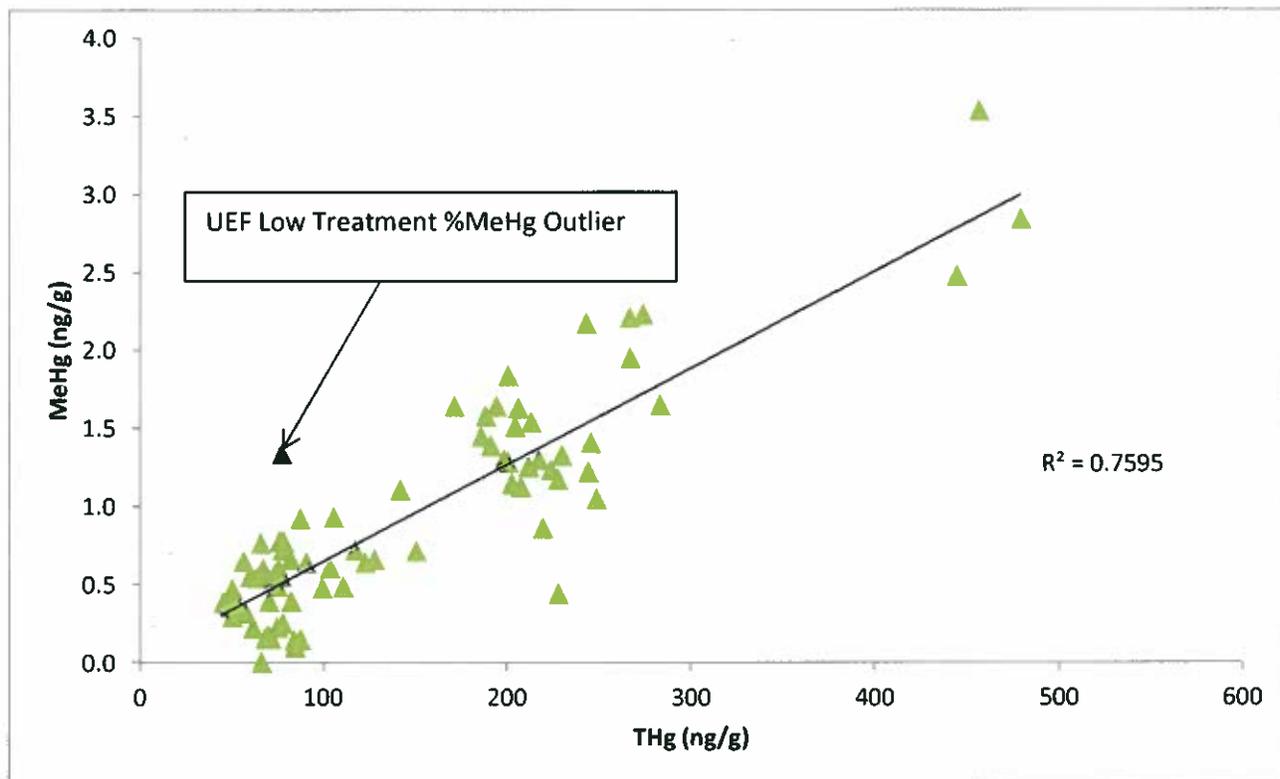


Figure 15. Relationship between THg and MeHg for all habitat zones sampled.  $R^2$  value is labeled next to regression line. THg and MeHg were significantly correlated ( $p < 0.05$ ) in the St. Louis River Estuary.

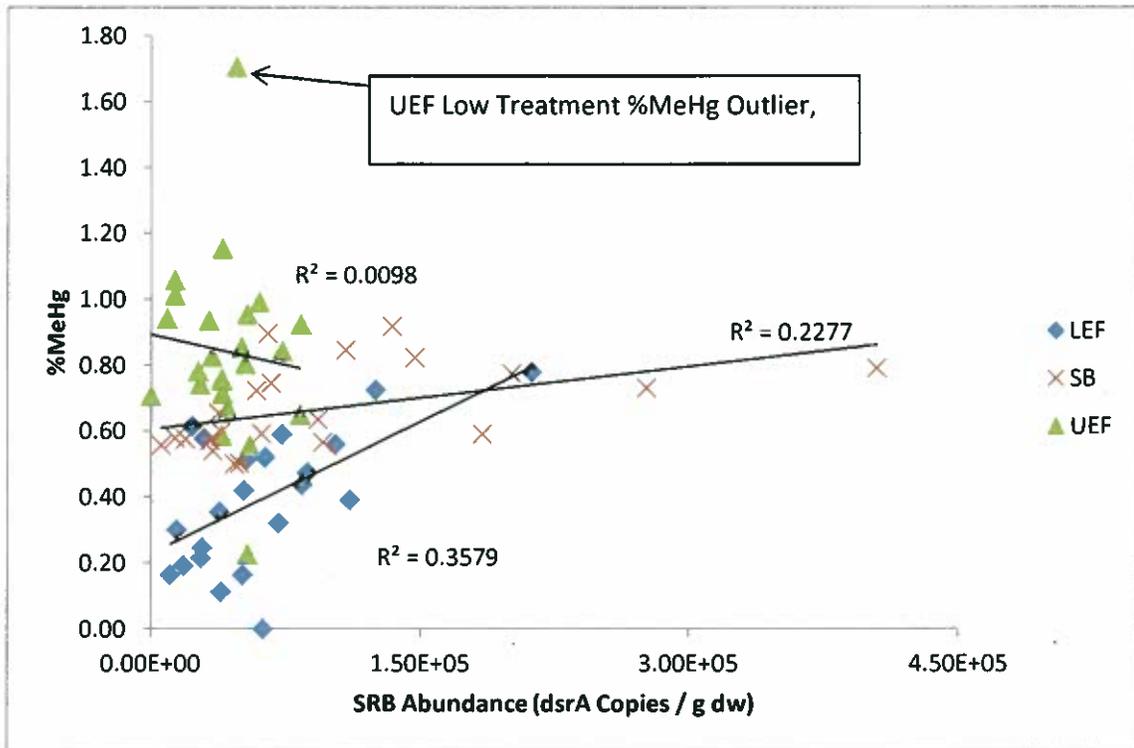


Figure 16. Relationship between % MeHg and SRB Abundance for LEF (blue), UEF (green), and SB (red) habitat zones.  $R^2$  values are labeled next to the regression line they represent.

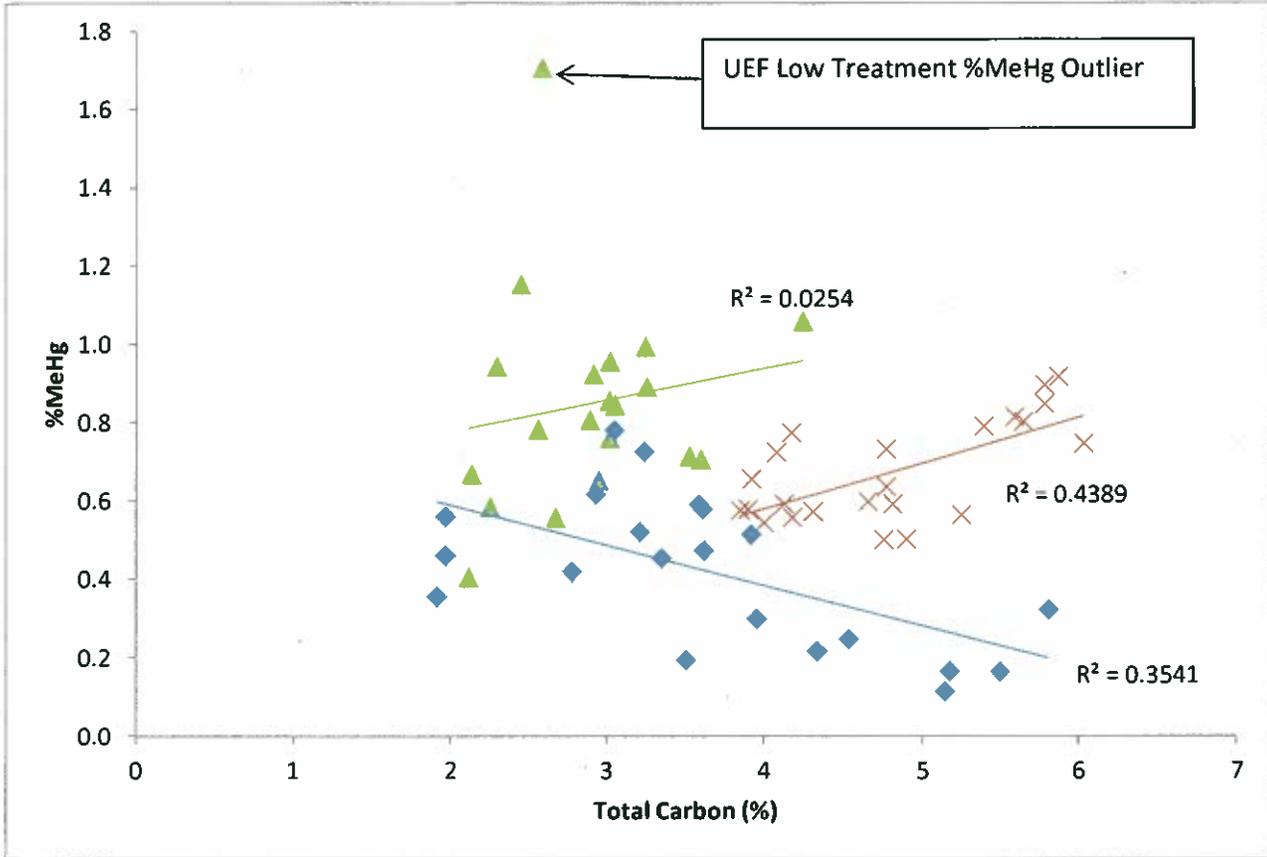


Figure 17. Relationship between % MeHg and TC Abundance for LEF (blue), UEF (green), and SB (red) habitat zones.  $R^2$  and p values are labeled next to the regression line they represent.

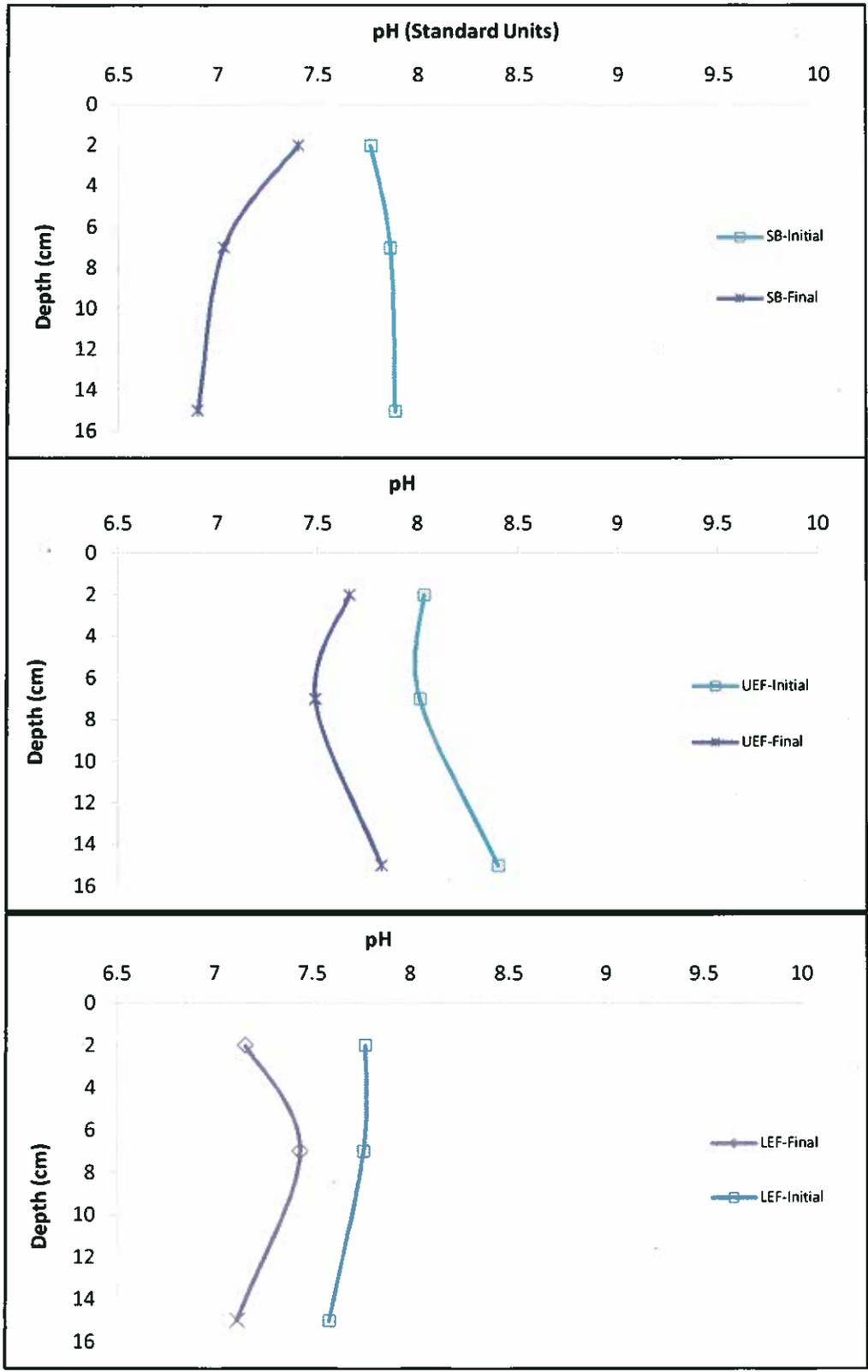


Figure 18 pH in sediment microcosms. pH was measured within 6hr of collecting sediment for final sampling, but 24hours later for initial sampling.

Table 1 Porewater MeHg and THg concentrations averaged in each habitat zone (n=3). Overlying water MeHg and THg concentrations from the flux experiments taken at the initial time point (t=0).

Habitat Zone	Porewater MeHg (ng L <sup>-1</sup> )	Overlying Water MeHg (ng L <sup>-1</sup> )	Porewater THg (ng L <sup>-1</sup> )	Overlying Water THg (ng L <sup>-1</sup> )	Measured THg Flux (ng m <sup>-2</sup> d <sup>-1</sup> )	Estimated MeHg Flux (ng m <sup>-2</sup> d <sup>-1</sup> )
LEF	0.46±0.55	0.18±0.18	26.29±21.63	1.45±0.10	40.23±19.71	0.72
UEF	0.33±0.07	0.25±0.37	12.27±5.37	1.43±0.15	18.50±5.98	0.15
SB	0.32±0.04	0.04±0.06	4.48±1.80	1.42±0.15	15.47±7.94	1.75

Table 2 K<sub>p</sub> values tabulated for MeHg in the top 10 cm of each habitat zone. Flux values for MeHg were below detection limits and not reported.

Habitat Zone	Porewater MeHg (ng L <sup>-1</sup> )	Solid Phase MeHg (ng g <sup>-1</sup> )	MeHg Log(K <sub>p</sub> )
UEF	0.33±0.07	0.52±0.02	3.20±0.51
SB	0.32±0.04	1.42±0.18	3.65±0.18
LEF	0.46±0.55	0.76±0.36	3.42±0.45

Table 3 MeHg and THg loads from upstream and sediment sources. Sediment MeHg flux calculations were scaled to the area of each habitat zones to obtain MeHg loads from sediment to the overlying water in the St. Louis River estuary. MeHg load calculated from USGS flow data that coincided with measured MeHg and THg concentrations in the St. Louis River at the USGS gauge station.

Site	Load MeHg (mg d <sup>-1</sup> )	Load THg (mg d <sup>-1</sup> )
Upstream Load (near median flow)	400.0	5333.5
Scanlon Dam (high flow)	7780.1	165975.4
Scanlon Dam (low flow)	173.3	4813.6
LEF Habitat Zone	7.6	423.2
UEF Habitat Zone	1.2	152.9
SB Habitat Zone	6.3	55.5

# Persistence of the Fecal Indicator Bacteroides in Sand and Sediment

## Basic Information

<b>Title:</b>	Persistence of the Fecal Indicator Bacteroides in Sand and Sediment
<b>Project Number:</b>	2011MN291B
<b>Start Date:</b>	7/1/2011
<b>End Date:</b>	2/28/2013
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	5
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Acid Deposition, Sediments
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	Michael Jay Sadowsky, Randall Hicks

## Publications

1. Eichmiller, J.J., R. E. Hicks and M. J. Sadowsky. 2012. Influence of Moisture and Temperature on the Persistence of Molecular Marker Genes to Detect Fecal Pollution in Sand and Sediment. American Society for Microbiology Annual Meeting, San Francisco, California. Eichmiller, J.J., R. E. Hicks and M. J. Sadowsky. 2011. The Distribution of Genetic Markers of Fecal Pollution on a Beach Receiving Consistent Inputs of Wastewater Effluent. University of Minnesota Biotechnology Institute Nara Institute of Science and Technology Joint Symposium, Nara, Japan.
2. Eichmiller, J.J., R. E. Hicks, M. J. Sadowsky. 2013. Distribution of Genetic Markers of Fecal Pollution on a Freshwater Sandy Shoreline in Proximity to Wastewater Effluent. Environmental Science & Technology. In press. DOI: <http://dx.doi.org/10.1021/es305116c>
3. Eichmiller, J.J.. 2012. The Distribution and Persistence of Genetic Markers of Fecal Pollution on Lake Superior Beaches (Doctoral Dissertation). University of Minnesota, Saint Paul, Minnesota.
4. Eichmiller, J.J., M. J. Sadowsky and R. E. Hicks. 2012. The Distribution and Persistence of Molecular Markers of Fecal Pollution in Sand and Sediment. Minnesota Water Resources Conference, St. Paul, Minnesota, October 16-17, 2012.
5. Eichmiller, J.J., M. J. Sadowsky and R. E. Hicks. 2012. Influence of Moisture and Temperature on the Persistence of Molecular Marker Genes to Detect Fecal Pollution in Sand and Sediment. American Society for Microbiology Annual Meeting, San Francisco, California, June 16-19, 2012.
6. Eichmiller, J.J., M. J. Sadowsky and R. E. Hicks. 2012. The Distribution of Genetic Markers of Fecal Pollution on a Beach Receiving Consistent Inputs of Wastewater Effluent. University of Minnesota Biotechnology Institute – Nara Institute of Science and Technology Joint Symposium, Nara, Japan, October 18, 2011.

**Persistence of the Fecal Indicator *Bacteroides* in Sand and Sediment**  
**Project Number 2011MN291B**

**Principal Investigator**

**Michael Sadowsky, Professor, Department of Soil, Water and Climate**

**1. Research:**

**SUMMARY**

Fecal contamination of surface waters is a widespread environmental problem and a public health concern. Advances in molecular methods has led to the development of several promising “real time” detection assays that quantify the abundance of genetic markers for fecal indicator bacteria (FIB), but their distribution and persistence in freshwater environments is not well-studied. This work examined the distribution of genetic markers of fecal pollution in sand and sediment of a Duluth-Superior Harbor beach near a wastewater outfall, measured the effects of temperature and moisture on the persistence of genetic markers in water, sand, and sediment, and compared the decay rates of genetic markers of fecal pollution and bacterial pathogens. Effluent loading likely controlled the abundance of molecular indicators of fecal pollution in the water column at a beach near a wastewater treatment plant outfall. Sand and sediment contained more enterococci and total *Bacteroides* genetic markers on a per mass basis than did water, whereas the concentration of human-specific *Bacteroides* was similar across sample types. In most instances, genetic markers were most abundant in the top 1 to 3 cm of sand and sediment. The decay of genetic markers of fecal pollution in sand and sediment was slow relative to the water column, and some genetic markers persisted or increased over time within sand and sediment. Molecular indicators decayed more rapidly at higher temperatures in all sample types and this decay was negatively correlated with sand moisture. The genetic marker for human-specific fecal contamination exhibited decay rates similar to markers for bacterial pathogens in sand, whereas non-source-specific markers decayed more slowly than bacterial pathogen markers under most conditions. Taken together, site-specific factors, such as the potential for resuspension of sand and sediment and pathogen abundance, should be considered in the choice of genetic markers for water quality monitoring on freshwater beaches.

**INTRODUCTION**

The degree of fecal contamination in surface waters is inferred by the presence and abundance of fecal indicator organisms. Fecal indicator bacteria can be enumerated by culture-based or molecular methods that target and quantify genus, species, or strain specific DNA sequences. Molecular methods such as quantitative PCR (qPCR) yield results in several hours, as opposed to 18 to 48 hr for culture-based methods. In addition, the development of source-specific primers allows for identification of fecal pollution sources (15, 20). Consequently, there is a shift in the use of culture-based methods to quantitative PCR (qPCR) for molecular markers for water quality monitoring. The use of qPCR to identify bacteria within the genus *Enterococcus* is a promising method for

detecting fecal contamination, as the abundance of the enterococci marker has been correlated to gastroenteritis disease risk in marine and freshwaters (24, 25). Similar to enterococci qPCR, qPCR for bacteria within the genus *Bacteroides* indicates the presence of fecal contamination, as it is abundant in fecal matter of both humans and animals (9, 26). *Bacteroides* is an obligate anaerobe, so its presence may indicate recent contamination. Additionally, *Bacteroides* markers have been developed that target source-specific strains, such as those that identify contamination from human sources (2).

The role of sand and sediment as a reservoir for molecular indicators is not well understood. Concentration of fecal bacteria in the water column can be influenced by sand and sediment bacterial concentrations. Sand and sediment can serve as reservoirs of fecal indicators in lake, river and ocean environments (10, 11, 27). Bacteria from the water column can be deposited in sand from wave action or settle out from the water column to the sediment (18). As some FIB are more abundant in shallow sand and sediment, resuspension of shallow sand and sediment may lead to water quality exceedances (6, 13, 18). Sands and sediments also offer protection from light, and nutrients may be more abundant in sand and sediment, potentially leading to indicator growth (3, 29). Sand can harbor pathogenic bacteria as well. *Campylobacter* spp., *Salmonella* spp., *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Vibrio vulnificus* have been detected in marine beach sand (4, 19, 28). *S. aureus* and MRSA have also been detected in sand at freshwater beaches (14). Taken together, there is high potential for indicators to accumulate in sand and sediment and sand can act as an exposure route to pathogenic bacteria (7, 8), so understanding indicator dynamics in these matrices is essential to accurately characterize the level of fecal contamination and associated health risk.

Although sand and sediment are integral to understanding the microbial loading of recreational beaches, the distribution, survival, and relationship to pathogen decay rates of genetic markers of FIB in sand and sediment is not well-studied (7). Molecular indicators for enterococci and *Bacteroides* markers have been detected in sand and sediment, but their distribution has not been well-characterized. The presence of *Bacteroides* markers in streambed sediments and enterococci markers in sand were found to vary with depth (5, 23), but the quantitative distribution has not been examined. In addition, the persistence of molecular indicators in sand and sediment is not known. Moreover, the persistence of genetic markers of FIB in sand and sediment cannot be directly inferred from the decay rate of culturable FIB, as the presence of viable but not culturable (VBNC) cells and extracellular DNA may result in slower decay of genetic markers of FIB relative to culturable cells (16, 17). For example, Yamahara et al. (28) and Klein et al. (12) found that enterococci measured by qPCR decayed more slowly than cultured enterococci in marine beach sand and manure.

This project had three objectives to further the understanding of FIB and pathogen dynamics in sand and sediment: 1) to examine the distribution of genetic markers of fecal pollution in sand and sediment, 2) to measure the effects of temperature and moisture on the persistence of genetic markers in water, sand, and sediment, and 3) to compare the decay rates of genetic markers of fecal pollution and bacterial pathogens.

## METHODOLOGY

**Study site and sample collection.** For objective one, the study site was non-recreational beach located on Duluth-Superior Harbor in Duluth, MN (47°13'37"N, 91°54'2"W) on the property of Western Lake Superior Sanitary District (WLSSD). The sampling site is located approximately 100 m from an outflow pipe that discharges treated effluent below the water's surface.

Samples were collected monthly from June to October in 2010 and from May to September in 2011. Three replicate water samples were collected 2 m from the shoreline below the water's surface. Three replicate cores of sand and sediment were taken one meter apart parallel to the shoreline. Three replicate treated effluent samples were taken from the effluent sampling station at WLSSD. Three times over the two-year study period, three replicate samples of raw influent were taken with the assistance from WLSSD staff. Cores were carefully removed from core tubes and sliced into 1 cm fractions for analysis.

**Microcosms.** Two types of microcosms were used: (1) sand only (Objectives 2 and 3); and (2) water with submerged sediment (Objective 2 only). Water, sand, and sediment for the experimental microcosms were collected from Duluth Boat Club Beach (DBC) in Duluth, MN, (46°46'10"N, 92°05'23"W) on June 20, 2011. Raw sewage inoculum was obtained from Western Lake Superior Sanitary District (WLSSD) treatment plant on June 20, 2011. Sand microcosms consisted of sterile 55 mL glass screw top test tubes filled with 40 g sand and 4 mL of inoculum. Water and submerged sediment microcosms consisted of 40 g of sediment overlaid with 112.5 mL of DBC beach water and 12.5 mL of inoculum, in 160 mL sterile glass milk dilution bottles. At each sampling time point, replicate microcosms were sacrificed and homogenized before further analysis.

Microcosms were inoculated with 4 mL raw sewage an, for Objective 3, pure cultures of *Campylobacter jejuni* ATTC 33560, *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain ATCC 14028, *Shigella flexneri* ATCC 20170, and methicillin-resistant *Staphylococcus aureus* COL strain. The amount of pathogenic bacteria inoculated was equivalent to  $1.03 \times 10^8$  *Campylobacter* cells,  $8.32 \times 10^8$  *Salmonella* cells,  $9.04 \times 10^8$  *Shigella* cells,  $1.20 \times 10^9$  MRSA cells.

For Objective 2, the effect of temperature on marker persistence in sand and sediment microcosms was tested at five temperatures. The effect of sand moisture on persistence was tested at three moisture levels (10, 20, and 30%) for Objective 2, and two levels (15 and 30%) for Objective 3.

**Bacterial enumeration.** The concentration of culturable *E. coli* and enterococci was determined by agitating 10 g subsamples of sand and sediment in 100 mL sterile ammonium phosphate solution with 0.01% gelatin in sterile milk dilution bottles. Supernatants and water, effluent, and influent samples were filtered onto 0.45  $\mu$ m nitrocellulose filters (Millipore, Billerica, MA) and placed on Modified mTEC (22) or mEI media (21) to enumerate *E. coli* and enterococci, respectively.

DNA for qPCR analysis was extracted from a 1 g subsample of sand or sediment by using the MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA). Liquid samples were filtered through 0.45  $\mu$ m cellulose filters (Millipore, Billerica, MA) and sliced into  $1 \times 4$

mm fragments with a sterile razor blade before DNA extraction with the MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA).

Genetic markers for FIB and pathogens were quantified by quantitative PCR (qPCR). Amplification was performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Carlsbad, CA), and quantification cycle ( $C_q$ ) values were automatically determined using the system software. Sample marker concentration was calculated on a per-run basis by comparison to plasmid standards with the corresponding insert. All sand and sediment values are reported per dry g of sand or sediment.

To distinguish between total genetic markers and markers from live cells for Objective 3, subsamples were treated with propidium monoazide (PMA) to bind free DNA (1). A 45 mL aliquot of supernatant or wastewater was centrifuged at  $7,650 \times g$  for 10 min, and the pellet was resuspended in 0.5 mL phosphate buffered saline. PMA (Biotium, Inc., Hayward, CA) was added to reach a final concentration of 100 nM PMA. Samples were incubated for 5 min in the dark. In order to inactivate remaining dye, samples were subjected to light treatment for 6 minutes 20 cm from a 1000W halogen light source (Osram, Germany) while on ice. Following light treatment, samples were centrifuged for 2 min at  $16,000 \times g$ , the supernatant was discarded, and samples were frozen at  $-20^\circ\text{C}$  until DNA extraction.

**Analysis.** First-order decay rates were calculated as the slope of the linear regression of ln-transformed genetic markers up to 28 d (Objective 2) or 14 d (Objective 3). First order decay is described by the following equation:

$$(1) \quad C = C_0 e^{-kt}$$

where  $C$  is the concentration of genetic markers at time ( $t$ ),  $C_0$  is the initial concentration of markers, and  $k$  is the decay rate constant.

Multiple comparisons tests were also used to determine the effect of temperature on the decay rate of marker genes in water. Test values were calculated by dividing the difference between two slopes by the pooled standard error.

$$(2) \quad q = \frac{(\bar{X}_i - \bar{X}_j)}{\sqrt{\frac{MS_w}{n}}}$$

As the critical value for Tukey's range statistic ( $q$ ) accounts for a factor of 2 (appears under the square root sign),  $q$  divided by the square root of 2 was used for the critical value for mean comparisons. Tukey's range test was done using Microsoft Office Excel 2007 (Microsoft, Redmond, WA) and critical values were determined using R (<http://www.r-project.org/>). All other statistical analyses were done using JMP<sup>®</sup> Pro version 9.0.2 (SAS Institute Inc., Cary, NC). All statistical analyses were done at a level of statistical significance of  $\alpha = 0.05$ .

## RESULTS

### **The distribution of genetic markers of fecal pollution in sand and sediment.**

Enterol, AllBac, and HF183 markers were detected in the water column at all sampling times. On average, AllBac markers were most abundant at  $6.3 \pm 0.5$  (mean  $\pm$  standard deviation)  $\log_{10}$  copies  $100 \text{ mL}^{-1}$ . The concentration of Enterol and HF183 markers was  $5.4 \pm 0.4$  and  $4.1 \pm 0.8 \log_{10}$  copies  $100 \text{ mL}^{-1}$ , respectively. The concentration of

culturable enterococci and *E. coli* measured in 2011 was 62 CFU 100 mL<sup>-1</sup>, 95% CI [32, 121], and *E. coli* averaged 368 CFU 100 mL<sup>-1</sup>, 95% CI [187, 722]. On individual sampling dates, few differences between effluent and water column indicator concentrations were observed.

The Entero1, AllBac, and HF183 genetic markers were more abundant in upper portions of sand and sediment. On average, there was no significant difference between the concentration of molecular indicators in sand and sediment. Sand and sediment marker concentrations were 5.6 ± 0.2 and 5.3 ± 0.6 for Entero1, 5.5 ± 0.2 and 5.4 ± 0.8 for AllBac, and 2.1 ± 0.1 for HF183 in both sand and sediment. HF183 fell below the limit of detection in 19% of sand samples, and 17% of sediment samples. In sand, all markers were most abundant in the upper 1 to 3 cm (Fig. 1). In sediment, Entero1 and AllBac were abundant in the upper cm, similar to the distribution in sand. In contrast, HF183 was most abundant in 5 and 7 cm portions of sediment. The culturable indicator bacteria enterococci and *E. coli* were most abundant in the upper centimeters of sand and sediment, similar to the distribution of molecular indicators. The concentration of culturable indicators was slightly higher in sand relative to sediment. The concentration of enterococci was 3.9 CFU g<sup>-1</sup>, 95% CI [0.8, 19] in sand and 1.7 CFU g<sup>-1</sup>, 95% CI [0.8, 3.8] in sediment. The concentration of *E. coli* was 4.0 CFU g<sup>-1</sup>, 95% CI [1.2, 13] in sand and 3.9 CFU g<sup>-1</sup>, 95% CI [2.5, 6.1] in sediment.

**The effects of temperature and moisture on the persistence of genetic markers in water, sand, and sediment.** The decay rates of the Entero1 genetic marker for sand at the 10 and 20% moisture levels had a negative, linear relationship with temperature ( $p = 0.01$  and  $0.001$ , respectively). When sand was at 30% moisture, there was no significant relationship between the decay rate and temperature ( $p = 0.56$ ). Although the decay rate of Entero1 in water was not linearly related to temperature, at 6°C the decay rate was significantly slower ( $p \leq 0.05$ ) than at 13, 21, and 37°C. The decay rates of the AllBac genetic marker in sand had a negatively linear relationship with temperature ( $p = 0.005$  to  $0.02$ ) for all sand moisture levels. In sediment, however, the regression was not significant ( $p = 0.53$ ). Similar to Entero1, the decay rate of AllBac in water was not correlated to temperature, but the decay was significantly slower at 6°C ( $p \leq 0.05$ ) compared to higher temperatures. The decay rate of the HF183 genetic marker was negative correlated with temperature ( $p = 0.01$  to  $0.02$ ) for all sample types.

Moisture also had a significant effect ( $\alpha = 0.05$ ) on the decay rate of the Entero1 and AllBac genetic markers. The decay rate of the Entero1 was not affected by moisture at 6°C (Fig. 2). At 6 and 13°C, the decay rate of AllBac was greater than zero, suggesting that there was likely growth of *Bacteroides* within the microcosm. There was no consistent pattern in the effect of moisture on the decay rate of HF183 genetic markers. For example, HF183 in sand at 30% moisture decayed more slowly than in sand at 20% moisture at 6°C, but HF183 decayed faster at 30% moisture relative to sand at 10% moisture at 30°C.

Matrix type influenced the decay rate of all the tested genetic markers. The decay rate of markers was often fastest in water relative to sand and sediment. However, the degree of difference decreased at higher temperatures (Fig. 2), especially for 10 and 20% moisture sand. The decay rates for the Entero1 and AllBac genetic markers were 92%

similar for all sample types based on Tukey's studentized range statistic (Fig. 2), and there was no significant difference between Entero1 and AllBac decay rates overall (paired t-test,  $p = 0.95$ ). By comparison, the decay rates for the AllBac and HF183 marker were only 32% similar between sample types, and HF183 decay rates were significantly faster than AllBac overall (paired t-test,  $p < 0.0001$ ).

**Comparison of the decay rates of genetic markers of fecal pollution and bacterial pathogens.** *Enterococcus* spp. decayed  $0.081 \text{ day}^{-1}$  slower at 28% moisture than at 14% moisture, whereas *E. coli* decayed  $0.099 \text{ day}^{-1}$  slower at 14% moisture relative to 28% moisture. For total genetic markers, decay was significantly slower at 28% moisture for AllBac and HF183 markers relative to 14% moisture ( $p \leq 0.05$ ), with decay rate decreasing by nearly half at higher moisture. For genetic markers from live cells only, Entero1, AllBac, HF183, qSalm, and qMRSA had slower decay rates at 28% moisture relative to 14% moisture. At 14% moisture, genetic markers from live cells decayed significantly faster than total genetic markers for all assays ( $p \leq 0.05$ ). At 28% moisture, however, there was no significant difference in decay rates of total markers and markers from live cells only for any assay ( $p > 0.05$ ).

The AllBac genetic marker increased over time at 28% moisture for both total and live markers, indicating growth of cells within the microcosm. Moreover, the total copies of AllBac on day 1 and from days 5 to 14 and copies of AllBac from live cells on day 1 and from days 7 to 14 was significantly greater than the initial copy number at the start of the experiment ( $p \leq 0.0009$ ). The qMRSA genetic marker also increased at several time points in the experiment relative to the initial conditions. Total copies of qMRSA at 14% moisture on day 3 was greater than the concentration at the start of the experiment ( $p = 0.0006$ ). At 28% moisture, copies of total qMRSA on days 1 through 5 were higher than the initial concentration ( $p \leq 0.05$ ), and copies of qMRSA from live cells was higher on day 1 relative to the initial concentration ( $p = 0.03$ ).

Decay rates of indicators were compared to the decay rates of total and live pathogens at 14% and 28% moisture (Table 1). At 14% moisture, the decay rate of total qCamp was similar only to the decay rate of total HF183. Similarly, at 14% moisture, the decay of live qCamp was similar only to the decay rate of HF183 from live cells. There was no indicator that exhibited a similar decay rate to qCamp at high moisture. Culturable indicator *Enterococcus* spp. and *E. coli* and total HF183 and HF183 from live cells had decay rates similar to qSalm, qShig, and qMRSA under several conditions. In contrast, the decay rates of Entero1 total and live cells were similar only to the decay rate of total qMRSA at 28% moisture. AllBac markers from live cells had a similar decay rate to total qSalm, qShig, and qMRSA at 14% moisture. The decay rate of total AllBac was not equivalent to the decay rate of any pathogen.

**TABLES**

**Table 1**

Decay rates and R<sup>2</sup> for culture-based and genetic marker-based indicators and pathogens.

Assay <sup>a</sup>	14% Moisture (wt/wt)				28% Moisture (wt/wt)			
	Control		PMA treated		Control		PMA treated	
	Decay rate <sup>b</sup> [std. err.]	R <sup>2</sup>	Decay rate [std. err.]	R <sup>2</sup>	Decay rate [std. err.]	R <sup>2</sup>	Decay rate [std. err.]	R <sup>2</sup>
<i>Enterococcus</i> spp.	0.361 [0.025]	0.88	–	–	0.280 [0.014]	0.93	–	–
<i>E. coli</i>	0.375 [0.025]	0.88	–	–	0.474 [0.026]	0.95	–	–
Enterol	0.062 [0.028]	0.14	0.178 [0.023]	0.67	0.035 [0.017]	0.12	0.050 [0.031]	0.08
AllBac	0.212 [0.020]	0.78	0.338 [0.034]	0.77	–0.112 [0.022]	0.47	–0.188 [0.038]	0.45
HF183	0.655[0.059]	0.87	1.52 [0.136]	0.93	0.342 [0.027]	0.85	0.473[0.059]	0.74
qCamp	0.798 [0.081]	0.84	1.21 [0.134]	0.85	0.767 [0.054]	0.90	1.01 [0.065]	0.93
qSalm	0.356 [0.020]	0.91	0.483 [0.025]	0.93	0.338 [0.015]	0.94	0.373 [0.028]	0.86
qShig	0.471 [0.029]	0.90	0.715 [0.041]	0.91	0.507 [0.046]	0.80	0.615 [0.054]	0.81
qMRSA <sup>c</sup>	0.369 [0.042]	0.81	0.596 [0.045]	0.89	0.208 [0.071]	0.38	0.356 [0.046]	0.73

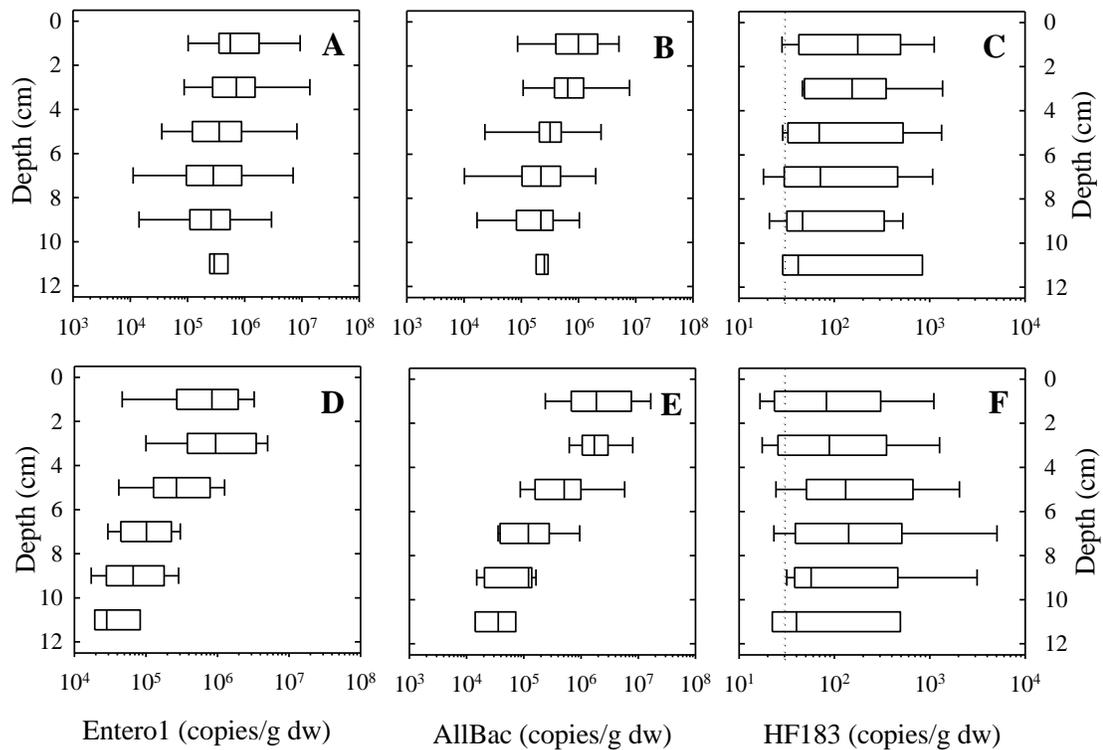
<sup>a</sup>*Enterococcus* spp. and *E. coli* are culture-based assays, whereas remaining indicators and pathogens are genetic marker-based assays.

<sup>b</sup>Decay rate units are in days<sup>-1</sup>. Negative decay rates indicate accumulation in the microcosm.

<sup>c</sup>qMRSA decay rate following plateau.

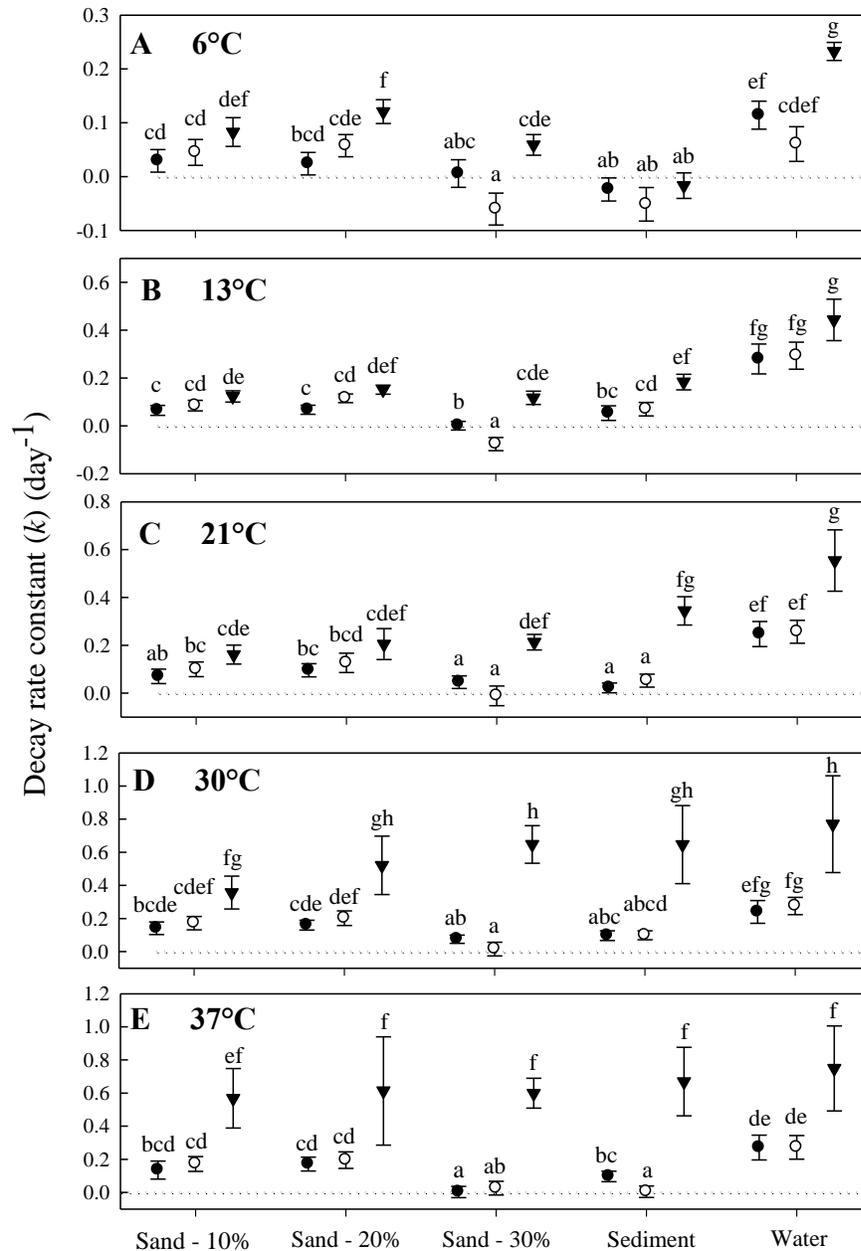
**FIGURES**

**Figure 1**



Box plots of Enterobacteriaceae (A, D), AllBac (B, E), and HF183 (C, D) with depth in sand (A, B, C) and sediment (D, E, F) across all sampling dates in 2010 and 2011. The left boundary of the box indicates the lower quartile of the data, the right boundary indicates the upper quartile, and the line within the box indicates the median. Whiskers show the 90th and 10th percentiles. The dotted line indicates the limit of detection for the HF183 assay.

**Figure 2**



The decay rate constant ( $k$ ) of Entero1 (filled circles), AllBac (open circles), and HF183 (filled, inverted triangles) at 6°C (A), 13°C (B), 21°C (C), 30°C (D), and 37°C (E). Error bars indicate 95% confidence interval of  $k$ . Samples that are not significantly different at  $\alpha = 0.05$  based on Tukey's studentized range test share the same letter. Dotted line indicates  $k$  of zero. Negative  $k$  values indicate the accumulation rate of genetic markers.

## **2. Publications:**

Eichmiller, J.J., Hicks, R.E., Sadowsky, M.J., 2013. Distribution of genetic markers of fecal pollution on a freshwater sandy shoreline in proximity to wastewater effluent. *Environ. Sci. Technol. In press*. DOI: <http://dx.doi.org/10.1021/es305116c>

Eichmiller, J.J., 2012. *The Distribution and Persistence of Genetic Markers of Fecal Pollution on Lake Superior Beaches* (Doctoral Dissertation). University of Minnesota, Saint Paul, MN.

Two additional manuscripts are currently in preparation.

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Eichmiller, J.J., Sadowsky, M.J., Hicks, R.E., The distribution and persistence of molecular markers of fecal pollution in sand and sediment. Minnesota Water Resources Conference, St. Paul, Minnesota, October 16-17, 2012.

Eichmiller, J.J., Hicks, R.E., Sadowsky, M.J., Influence of moisture and temperature on the persistence of molecular marker genes to detect fecal pollution in sand and sediment. American Society for Microbiology Annual Meeting, San Francisco, California, June 16-19, 2012.

Eichmiller, J.J., Hicks, R.E., Sadowsky, M.J., The distribution of genetic markers of fecal pollution on a beach receiving consistent inputs of wastewater effluent. University of Minnesota Biotechnology Institute – Nara Institute of Science and Technology Joint Symposium, Nara, Japan, October 18, 2011.

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# USGS Award No. G11AP20000 Identifying and Evaluating Best Practices for the Adaptive Management of Water Resources

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# **Implementation of Adaptive Management by the US Army Corp of Engineers**

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## EXECUTIVE SUMMARY

### Implementation of Adaptive Management by the US Army Corp of Engineers

#### INTRODUCTION

Within the United States Army Corps of Engineers (Corps), there has been a growing effort to utilize adaptive management (AM) strategies in a variety of ecosystem restoration projects. AM is a planning concept that falls under the broader umbrella of Integrated Water Resource Management (IWRM). AM is a type of natural resources management involving testing, monitoring, and evaluating applied strategies, and incorporating new knowledge into management decisions. The AM approach improves the flexibility and learning processes of managers. As managers address intersecting natural and human systems, AM provides a tool to identify, collect, and use the best available information and implement a plan that evolves over time. Since it is increasingly difficult and costly to predict what systems will look like in the future, AM allows managers to iteratively learn from outcomes of previous projects and select the most effective intervention. AM allows managers to become smarter about their systems and helps inform which interventions are achieving goals. However, AM is influenced by the institutional framework and how well staff buy-in to the approach, and the degree to which implementation is encouraged. Adaptive and flexible management plans and policies will be important as managers identify and face current and future challenges to manage water resources for sustainable uses.

***Objectives.*** The objectives were to: (1) identify and define the approaches that have been used for adaptive management of water resources; (2) describe the specific adaptive management practices that have been used by selected case projects of the Corps and evaluate their rigor and effectiveness; (3) assess these cases for opportunities, barriers, and lessons learned; and (4) make recommendations for best AM practices for the Corps.

***Methodology.*** AM projects were assessed and analyzed through interviews with Corps personnel. Project interviewees were selected by the Corps' Institute of Water Resources (IWR) and this research team. In broad terms, the interviews investigated how adaptive management was implemented across eight districts. More specifically the interviews focused on the criteria for success, outcomes, barriers, and unintended consequences of adaptive management. Finally, the interviews collected recommendations to improve adaptive management. The research team developed the research questions, ten project cases were identified, and project staff was interviewed using a standardized protocol. Phone interviews were conducted from January to May 2012.

#### KEY FINDINGS

The research team had difficulty identifying districts that were applying adaptive management techniques. The few case projects that were using AM were in the planning stage. Other interviews talked about the approaches their district has been using for decades which shared principles similar to AM. Also, if the district was not currently using AM, the interviewee was familiar with the principles of AM and could talk about the theoretical aspects and how it relates to the realities of their projects.

One consistent finding from all the interviews was a lack of a coherent message on implementation of AM. The interviews revealed that some districts were indeed following the guidance from U.S. Corps headquarters, but others were not doing AM at all, while others developed their own guidance. Those interviews that received clear guidance from Headquarters mentioned the main impetus for implementing AM was the Water Resource Development Act (WRDA) of 2007. Following the external pressure from WRDA 2007, these districts used the National Research Council's 2004 report and the U.S. Army Corps' Everglades studies to guide AM implementation.

The barriers identified in the interviews did not make AM impossible, but limited the degree to which AM was effective. This was captured in one interview response who mentioned AM "has such a grey area that it's hard to implement. One thing that I'm finding is that agencies don't have the budgeting authority to maintain active adaptive management over a long time". Another interview emphasized that districts face challenges applying active AM over long time periods because of budgetary limitations. Districts are aware of the differences between active and passive AM, and in most cases would like to apply active AM, but in reality what is most feasible is usually implementing passive AM. Budget constraints and preference for new projects rather than monitoring existing projects make it unlikely for a district to test multiple alternatives and monitor the results.

Regulations and laws increase coordination time and make it challenging to implement AM. For example, a requirement that calls for local cost sharing presents a challenge because there can be instances where the local partners are not as interested in adopting AM and it is difficult to get their buy-in and funding for AM approaches.

During the interviews, how AM was introduced to staff by their superiors and the staff's perception of AM influenced their buy-in and implementation of AM strategies. A skilled and experienced staff is crucial to implement AM in complex environments. One key finding was that absent this skilled staff, the district office uses external consultants. The skills are not retained at the district office level. This is problematic because high staff turnover could threaten the sustainability of AM as lessons and skills have to be re-learned.

The responses from the interviews showed a variety of opinions on stakeholder participation on AM projects. The positive responses emphasized that local stakeholders can improve the outcomes of AM projects because of their local knowledge and can assist with consistent monitoring, while providing useful and timely information back to the decision makers. Also, when stakeholders have a sense of ownership, the cost-sharing can help AM outcomes. The interviews mentioned that the most active stakeholders typically were from other U.S. agencies and non-governmental organizations (NGOs). The private sector was not specifically mentioned in the interviews.

Despite the positive reviews, there also were challenges with stakeholder participation in AM. The interviews shared insight into the realistic stakeholder challenges with implementing AM. For example, stakeholders would begin to resist a project as costs increased because some stakeholders held the view that the AM projects will crowd out implementation of other projects. Several of the interviews mentioned that the size and complexity of Corps' documents could be daunting for stakeholders to consume. The interviews recognized the importance of trying to

communicate the information in an accessible way. Although Corps staff listens to public comments, concerns over potential lawsuits prevent them from publicly responding to stakeholders. NEPA provides guidance to the Corps on stakeholder engagement, but there is no guidance for engaging stakeholders through adaptive management.

The respondents understood the importance of AM, and acknowledged the need for AM to meet the upcoming challenge of dealing with uncertainty. Several of the respondents discussed that AM provides a helpful and useful framework to responding to uncertainty, as “monitoring enables the Corps to learn from the outcomes and positions them to effectively address uncertainty in the future”. The interviews recognized that it would be “very difficult to do adaptive management without monitoring”, because monitoring is central to determine whether or not adjustments need to be made to a project. Therefore, long-term monitoring facilitates AM, and provides data to improve understanding and reduce uncertainty in projects.” The interviews mentioned that the requirement to monitor for ten years makes AM difficult to fund for the full time period, when it is challenging simply to find funding to construct a new project.

A potential challenge to implementing active AM in restoration projects. For example, “in these large ecosystem restoration programs in the real world rather than in a lab condition, active adaptive management seems to be very, very hard because we can’t control so many of the factors that need to be controlled.” The large natural variability leads to higher monitoring costs.

Another challenge was the size of AM teams and timescales. There is a tension between delivering short-term Congressional mandated progress, and completing long-term monitoring. Some of the interviews mentioned that ideally the team would be small and flexible to respond in a way necessary to implement AM. However, typically AM has resulted in larger teams, that make it difficult to implement the types of AM activities that make AM flexible. This underlines the importance of having a skilled and experienced AM staff that knows how to navigate and keep consistency in AM projects.

The interviews indicated the potential of AM for managing uncertainty and risk. One interview mentioned that AM helps build off of the concepts used in other projects on scenario planning and dealing with climate change. Several of the interviews showed a recognition that AM is catching on and is being incorporated in the planning stages of projects, and is addressing uncertainty and risk early on in the project.

The interviews also foreshadowed the possibility of learning that results from AM. The interviews referenced that AM provides internal learning, as teams incorporate feedback from the monitoring on their local specific projects. Also, the interviews mentioned that the communications have increased and managers are beginning to share best practices of AM. So there is the potential for intra and inter-District learning around AM.

## **RECOMMENDATIONS**

**(1) Develop common reference materials, disseminate Corps AM guidance widely, and have Headquarters reinforce their commitment to this guidance regularly.** The guidance from Headquarters is a good first step to communicate clear messaging on implementing AM. The principles of AM are not foreign to interviewees,

but using a common reference document, and then adjusting to meet local contexts, would help districts that experience high staff turnover to maintain some consistency by using common reference materials.

(2) **Secure long-term funding for the successful implementation of AM.** The interviews identified budgetary constraints as the top concern of long-term monitoring for AM projects. Increasing costs and decreasing funding streams threatens future AM implementation, especially in the current political environment. Where it is possible, work with other agencies to identify and leverage resources to secure funding that allows active AM approaches for a sustained period. Look to develop relationships with the private sector for cost-sharing opportunities, and improve the partner's understanding of adaptive management for the long-term.

(3) **Develop internal capacity.** External experts provide a short-term fix, but are problematic for sustainable use of AM. Along with the AM guidance, work with district offices on developing mentoring and training programs that would benefit personnel and address the effects of staff turnover. The goal is to retain the information on how to work with stakeholders and respond to the various context-specific challenges for each project.

(4) **Conduct Advocacy and Awareness Campaigns for district offices.** Due to the fact that the principles of AM closely resemble what some offices have been “doing for decades” there are likely many “grey areas” or assumptions that are specific to each office and result in variations of AM that might be inconsistent with the guidance from Headquarters.

(a) Promote inter-offices dialogue, workshop seminars, or webinar certifications on best-practices or “shared learnings” from peers;

(b) Develop a prize that recognizes and rewards district offices that have innovative AM programs. This would incentivize sharing of case-studies and accumulate a knowledge of best practices that could start to standardize terms and techniques.

(5) **Coordinate and collaborate with other agencies on approaches to AM,** in order to aim for solid understanding of terms and approaches. This would assist in knowledge-sharing as district offices work with other agencies. It also would likely help when communicating with Congress during appropriations when trying to demonstrate inter-agency approaches and solid “return on investments”.

In summary, adaptive management provides an effective toolkit that builds learning into projects, and could greatly benefit the Corps in its restoration projects. This learning can guide decisions to respond to uncertainty and changes that are difficult to predict. Climate change is likely to increase the level and scale of uncertainty, so AM offers a strong step forward for the Corps in the future.

## **I. Introduction**

Within the United States Army Corps of Engineers (Corps), there has been a growing effort to utilize adaptive management (AM) strategies in a variety of ecosystem restoration projects. AM is a planning concept that falls under the broader umbrella of Integrated Water Resource Management (IWRM). AM is a type of natural resources management involving testing, monitoring, and evaluating applied strategies, and incorporating new knowledge into management decisions. The AM approach improves the flexibility and learning processes of managers. As managers address intersecting natural and human systems, AM provides a tool to identify, collect, and use the best available information and implement a plan that evolves over time. Since it is increasingly difficult and costly to predict what systems will look like in the future, AM allows managers to iteratively learn from outcomes of previous projects and select the most effective intervention. AM allows managers to become smarter about their systems and helps inform which interventions are achieving goals. However, AM is influenced by the institutional framework and how well staff buy-in to the approach, and the degree to which implementation is encouraged. Adaptive and flexible management plans and policies will be important as managers identify and face current and future challenges to manage water resources for sustainable uses.

The Corps understands the complexities and uncertainties in its projects and recognizes that they require adaptive management approaches. Undoubtedly, such uncertainties make it difficult, if not impossible, for resource managers to accurately predict the outcomes and responses that will result from their management actions. However the end goal for resource managers is not to be good predictors, but to achieve

the best outcomes for resource management, and AM provides the tools to achieve that goal. AM gives decision-makers a structure that links scientific knowledge, learning, and collaboration into the decision-making process. As part of this process, AM allows resource managers to continually monitor and evaluate whether or not their management actions are producing outcomes that are consistent with goals and objectives. Through this consistent updating and accumulation of knowledge, it should be possible to modify existing actions in a manner that will more effectively achieve the project's desired outcomes.

Currently, AM is being used in the Corps as a mechanism for ecosystem management and restoration. In part, the use of adaptive management is due to the Water Resource Development Act (WRDA) of 2007. However, some districts used the approach before WRDA was implemented. Regardless of the impetus, AM is not currently used by all districts and in the districts where the approach is used, AM is not consistently applied. This study assessed the way AM is viewed, applied, implemented, and evaluated varied across districts. Summarizing the commonalities and differences informs the Corps, in terms of how AM is being implemented, and identifies possible areas for improvement. After an introduction into the background of AM, this paper discusses the methodology it used for investigating how AM is viewed and implemented in 8 districts and 2 non-Corp federal projects.

The objectives of this study were to: (1) identify and define the approaches that have been used for adaptive management of water resources; (2) describe the specific adaptive management practices that have been used by selected case projects of the Corps and evaluate their rigor and effectiveness; (3) assess these cases for

opportunities, barriers, and lessons learned; and (4) make recommendations for best AM practices for the Corps.

## **II. Literature Review**

Adaptive management is an approach for simultaneously managing and learning about natural resources, has been around since it was first introduced by Holling (1978). Holling and Walters (1986) provided the conceptual framework and a technical definition for adaptive management. Adaptive management highlights learning and uses experiments with alternatives, monitoring and feedback loops, to test hypothesis as ways to navigate uncertainty in natural resource management (Huitema et al.2009).

The literature identified complexity, variation, and uncertainty as universal concepts when linking natural and social systems for natural resource management (Medema 2008). AM recognizes that interactions between people and ecosystems are unpredictable and it offers a process based on learning, that allows managers to take action (Gunderson 1999, Johnson 1999).

AM is linked to concepts like resilience (Carpenter 2001), polycentric governance (Ostrom 2004 ), and social-ecological learning systems (Berkes 1998, Folke 2005). Although these concepts are helpful in exploring theoretical linkages and explanations for larger trends, they often struggle to be implemented into projects. Adaptive management takes the theory and works it into the policies, institutions, funding, and guidelines that make it a reality for managers.

Critics (Mclain and Lee 1996 ) argue that adaptive management can become a catch-all term and fashionable phrase, but lacks evidence of implementation, or that

even when it is implemented, it is only done in parts, such as just monitoring and evaluation, but not the hypothesis testing. However, in response many federal agencies have adopted AM into practice, and as experience and evidence builds, the transition from theory to practice will focus the attention towards how to make AM more efficient and effective.

The literature discussed common components to successful adaptive management techniques, and these categories included (Williams 2011, NRC 2004, Williams, et al. 2007)

1. *A real management choice* has to be made
2. *Stakeholders*- what is the level of trust among actors?
3. *Feedback Loops*: who, how, and when is it identified?
4. *Explicit and measurable* performance objectives
5. *Appropriate and actionable* monitoring data linked with management decisions
6. *Experimentation to test alternatives*
7. *Financial security* to ensure consistent monitoring and evaluation

Adaptive management relies on reliable, effective, and appropriate feedback loops.

These loops can easily be identified using organizational diagrams, but their implementation depends on several key factors. The first is the organizational structure of the management system. Case studies and literature provide mixed motivations for choosing centralized or decentralized management systems (Huitema et al. 2009, Dietz et al. 2003). Centralized organizations may decrease the democratic legitimacy but enhance the control of feedback loops to direct appropriate information to the relevant stakeholders. The benefit of a decentralized management is that local level organizations understand and develop the capability to apply information from the feedback loops at the appropriate scale and within reasonable time frames. The bureaucracy in centralized agencies may delay feedback loops or mismatch the

information due to lack of local knowledge. Alternatively, a management system that is too decentralized experiences information communication challenges. In sum, the appropriate institutional structure (centralized vs decentralized) must establish effective capabilities among staff to identify, understand, and communicate information from the feedback loops.

The second factor in effective feedback loops is appropriate and actionable information. If the monitoring system were incapable of providing useful or actionable feedback then using an adaptive management approach would not be effective. The frequency of monitoring must be sensitive to track the changes in the natural system to provide accurate information that informs the management decisions.

The literature also discussed barriers. Social or stakeholder resistance, financial costs, time scales of planning and management, low quality of information, and status quo behavior were identified as barriers to AM (McLain and Lee 1996, RECOVER 2010, Gunderson and Light 2006). The literature further highlighted other barriers such as, a lack of trust and power asymmetries among stakeholders, high transaction costs of information gathering and monitoring, managers who resist increased transparency and loss of control, and a organizational culture with fear of failure (Pahl-Wostl 2007).

The literature also discussed two types of adaptive management; active and passive (Bormann et al.1996). These two approaches are distinguished by how they address uncertainty (Williams 2011b). Active AM anticipates the effect of management actions on learning, and uses multiple alternatives to test the hypothesis. The feedback and monitoring mechanisms provide information to the decision-makers who then evaluate which alternative to pursue in the next round. Passive AM addresses

uncertainty by using models to assess the different alternatives, and then chooses one action. Passive does not test alternative hypothesis simultaneously as does active AM. Therefore, passive AM typically has a lower cost because it is not using multiple designs.

### **III. Methodology**

#### ***A. Interviews***

AM projects were discussed and analyzed through interviews with Corps personnel. Interviewees were identified by Army Corps IWR personnel and the research team. After talking to Corps personnel, the research team found that adaptive management was not used as often as expected. For example, one district replied that they were not even aware of the concept of “adaptive management” while other districts said that they were not using the approach at all. With this issue in mind, the research team had difficulties in identifying Corps districts that were using adaptive management in the planning process or implementing the approach to any degree.

Ultimately ten interviewees were identified, and phone interviews were conducted from January to May 2012. Interviews 1, 2, 3, 4, 6, 7, and 8 were conducted with interviewees from separate districts, and interviews 5a, 5b, and 5c were conducted with three individuals from one district. Interviews 9 and 10 were conducted with non-Corps agencies. However, the main findings in this report focus on interviews 1-8, with Corps staff.

The interviews investigated broadly how adaptive management was implemented across 8 districts. More specifically the interviews focused on the criteria for success,

outcomes, barriers, and unintended consequences of adaptive management. Finally, the interviews collected recommendations to improve adaptive management. The research questions were developed by the research team and are available in Appendix A.

Qualitative analysis coded the interview transcripts. Each line in the interview transcripts was descriptively coded to detect patterns of the way adaptive management was viewed, described, planned, funded, implemented, operationalized, and evaluated. These topics were then aggregated into categories inductively derived from the data (Bidwell and Ryan 2006; Jewell and Bero 2006). See Appendix B for the codebook.

### ***B. Project and District Background***

A description of the types of projects, who was involved, and how AM was applied for each of the districts is provided in Appendix C. In summary, there was a wide range of projects using AM including fish passage to ecosystem restoration. There also was a range of positions of the staff who were interviewed. There were high-level staff with expertise on using AM, and project managers who had no previous experience with AM.

## **IV. Findings**

### ***A. Adaptive Management Policies***

How AM is introduced can influence how districts view and implement AM into project designs and plans. Although there was no singular framework for how AM policies were introduced and adopted, there were some common themes in the interviews. One was that for the majority of interviews, AM was externally driven policy from the WRDA 2007 or agencies permits. Regardless of the external or internal origin,

as AM was introduced, the interviews emphasized that a committee or guidance plan often followed to help with implementation of AM.

The main external driver for including AM came from the Water Resource Development Act (WRDA) of 2007. Following the external pressure from WRDA 2007, the districts of interviews 5b and 5c used the NRC 2004 report and the Corps' Everglades studies to guide the way adaptive management was used. Interviewee 7 emphasized how WRDA 2007 was the main driver for including AM. This interview specified that WRDA 2007 requires AM for ecosystem restoration and mitigation projects. Interviewee 7's district has an implementation guidance memorandum that incorporates WRDA 2007 and specifies when adaptive management will be used. However, in order to create AM plans, interviewee 7 said that the district consults with other Corps personnel from places like the Everglades since the Everglades was one of the first places to implement adaptive management in the Corps.

Interviewee 3 reiterated an internal origin of AM policy and described that AM has been a "grass roots" movement among district personnel. As this interviewee notes, district personnel received the "blessing" of high-level leadership to begin using adaptive management strategies. Currently, this district has an executive steering committee for its ecosystem recovery program. This committee ultimately reviews and approves the district's AM policies. Similar to the grassroots or internal impetus, interviewee 8 explained that the impetus for AM came from lower levels of the international commission. The different groups involved in the commission agreed that AM would benefit stakeholders.

Another common response from the respondents was that district offices received instruction from headquarters to adopt AM, and then established interagency teams to implement AM. Interviewee 4 detailed his own district's effort to develop a technical guide that would describe their own approaches to adaptive management. The technical guide incorporates an interdisciplinary team comprised of scientists, engineers, and water managers. The comprehensive technical guide addresses the ways in which adaptive management can be used in project planning and design and operations. According to interviewee 1, there is an interagency team that monitors the use of AM within mitigation banks. This team, which is referred to as the "MBIT," includes a board that is responsible for reviewing projects and generating guidelines and policies for mitigation banks and how they should utilize adaptive management. Interviewees 1 and 2 discussed how AM policies were encouraged from Corps headquarters. Interviewee 1 notes that there is also an interagency team that oversees the AM policies that are applied to civil works projects.

During Interview 5a, the interviewee emphasized that district staff have been using AM for more than two decades. Although the district offices determined their AM policies, they have begun to incorporate guidance from headquarters. For Interviewees 5b and 5c, the main impetus for including AM was the Water Resource Development Act (WRDA) of 2007. Following the external pressure from WRDA 2007, these districts used the NRC 2004 report and the Corps' Everglades studies to guide how AM was implemented.

In addition to external drivers such as WRDA 2007, Interviewee 6 mentioned agencies' permits, biological opinions, and water quality certifications, required AM.

Interviewee 6 also was influenced to use AM in order to manage risk. Like the Everglades, Interviewee 6's district project was an early application of AM in the Corps. However, Interviewee 6's district was different from the Everglades because they were mandated whereas the Everglades was a joint agreement.

Frequently, interviewees noted the presence of various documents pertaining to AM that have been developed by the Corps at various points of time. As Interviewee 4 noted, there are several documents that are available to assist districts with the AM process. This interviewee discusses several published documents that guide the Corps' use of AM, including; The Institute for Water Resources' 1996 Planning guide, the National Research Council's (NRC) 2004 report, the Department of the Interior's guide.

As discussed in interview 5b, 6, 7, and 8, a team of Corps personnel has worked on agency guidance for AM. At this point, the guidance is not available to Corps personnel. At the district level, interviewee 5b discussed how there are publications that have been put together to assist teams in applying AM as it relates to ecosystem restoration and navigation projects. For example, an environmental science panel developed a publication to help guide AM for a navigation and ecosystem sustainability program in districts associated with interviewee 5b.

### ***B. Applications of Adaptive Management***

To briefly review the concepts of active and passive AM, active approaches involve multiple experiments to test competing hypotheses in ecosystem response. In passive AM, alternatives are assessed at the beginning, usually using prediction models, and then the best management action is designed and implemented. The key difference compared to active, is that passive AM only selects and implements one action. In active

AM, managers acknowledge they do not know which action will yield the best outcomes, and therefore tries multiple alternatives to design and implement. Monitoring and evaluation for each alternative informs the managers which action was most effective in meeting objectives. Adjustments to the next round of management decisions can be made based on feedback from the previous round. Passive adaptive management may initially be less expensive and require fewer people, but if managers are incorrect in their assumptions, it can take longer to try each alternative in a piecemeal fashion to learn which was the most effective. In comparison active AM tests multiple alternatives all at once. Active AM may require a larger initial investment of time, labor, and funds, but since several alternatives are tested there is usually higher confidence in the decision.

*i. Active Adaptive Management*

Interviews 1, 2, 4, 5b, 6, and 7 mentioned using active AM approaches. Interviewee 2 emphasized the importance of conducting scenario analysis during the plan formulation phase to reduce uncertainty. Interviewee 4 detailed using models to test alternative hypotheses about future events such as floods or droughts. This interviewee emphasized applying an extended “period of record” (approximately 41 years), when modeling future scenarios as this will assist in identifying the plans that will be most appropriate or flexible if such events actually occur. Interviewee 3 noted it’s district’s robust science program that applied multiple short-term research projects to determine uncertainties associated with alternative actions.

Interviews 5c and 5b, and 6, elaborated on experimenting with different alternatives when implementing active AM strategies. Specifically, interview 5b,

monitored different treatments for channel dredging and island construction projects, and evaluated which approach gave positive outcomes to inform future decisions on backwater restoration projects.

Some interviews mentioned difficulty in implementing active AM strategies. Interview 3 stated that adherence to strict master manual, restricted the district's ability to actively test alternative hypotheses. According to Interviewee 5a, districts face challenges applying active AM over long time periods because of budgetary limitations. This interviewee discussed it was extremely difficult for a district to commit to funding a project over many years. Additionally, interviewee 5a mentioned some personnel within the Corps preferred spending money for new projects, rather than funding research or scientific activities that supported decision making for AM. The budget concerns and personnel preferences complicate the district's prospects for implementing active AM.

*ii. Passive Adaptive Management*

Interviews 3, 5b, and 7 discussed passive AM approaches. Interviewee 7 used a barrier island restoration to highlight the differences between passive and active AM where the latter uses experiments. Interviewee 5b stated that passive AM strategies have already been in place for some time, but only recently has the district been using the term AM; however, the stakeholders in the district are increasing their interest in applying active strategies. Interview 3 emphasized the district experience difficulty implementing pilot projects to assess outcomes from potential actions, and could not test alternative hypotheses, so passive AM was by default more feasible. Based on the interviews, all districts are knowledgeable about the differences between active and

passive AM approaches, and despite both experience difficulty implementing, active approaches are preferred.

### ***C. AM Barriers***

#### *i. Laws, Regulations, and Army Corps Guidance*

Barriers are important because they increase the transaction costs to implement AM approaches. Identifying where these barriers arise, can guide strategies that will minimize the costs and make it more likely that AM will be implemented and effective in meeting project objectives.

One barrier identified by the interviews was laws, regulations, and the Corps' guidance documents. As regulations, laws, and guidance that apply to a project increase, so does the coordination and management costs for the district managers. Interviewee 6 said it was a balancing act to make sure all the laws and regulations were followed for the team members since everyone came from different agencies. Interviewee 1 discussed the challenge of implementing AM while also being in compliance with the Clean Water Act (CWA), the federal "no net loss" wetlands policy, and sustainability goals. On the other hand, for interviewee 2, a requirement that calls for local cost sharing presents one of the biggest challenges to AM. In this instance, if the local partners are not as interested in adopting AM then it will be difficult to get their buy-in and funding for AM approaches.

Similarly, interviewee 4 discussed the variety of environmental regulations, including laws pertaining to water quality and endangered species, which greatly impede the AM process. Interviewee 7 discussed the difficulties following the six-step planning process and adhering to regulations such as NEPA, when implementing AM

strategies. As interviewee 4 suggested, these laws and regulations not only constrain AM, but can transform a restoration effort into a “multi-purpose” project.

Interviewee 5c discussed how the Corps requirement of ten years of monitoring post-construction for a project would be difficult considering it is difficult to secure funding to even construct the project. Interviewee 7 gave an example of new guidance on sea level rise and how this guidance makes implementation expensive and complicated because of all the alternative scenarios that must be considered.

The interviews highlighted that additional legal issues or regulations increase the demand on personnel to apply AM standards across all stakeholders and it threatens the ability to keep attention to other aspects of AM.

*ii. Personnel Issues*

If the district office lacks the capacity among staff to understand and navigate the complexity of AM techniques, then the use of AM is limited. Two main themes emerged as barriers for personnel, perception or familiarity with AM, and turnover rates. During the interviews, how AM was introduced and the staff’s perception of AM, influenced their buy-in and implementation of AM strategies. As interview 4 noted, AM is still a relatively new concept for some Corps staff, so there can be a learning curve to improve overall understanding of AM. Perception and attitude towards AM plays important role for implementation. Interview 7 stated that some Corps personnel view AM as just one more thing that they have to do.

Whenever a new strategy is introduced, it is ideal to have an experienced staff member, introduce and walk the other members thru the implementation of the new strategy. Unfortunately reality does not imitate the ideal. Interviews 5c, 6, and 8

discussed how AM was not defined for their projects and so staff operated without a good understanding of the strategies. They were left to muddle through to achieve outcomes. Interviewee 7 discussed that the district relies a lot on older employees' experience, but that guidelines and procedures are needed for implementation of AM.

Team management skills are key to any project, and AM is no different. However, because it is a new approach and involves multiple stakeholders, trust plays integral role among team members. Interviewee 6 discussed how lack of trust on the team hindered AM at the beginning of the project. Leading from a lack of trust, several of the interviews emphasized issues with cooperation among staff members. Interviewee 7 highlighted uncooperative behaviors that resulted in a team leader deliberately not including the AM team, even though the person knew the AM team should have been involved.

Similarly, Interviewee 6 emphasized that managing strong personalities was difficult during AM projects. At times, interviewees discussed how disagreements and conflicts between Corps personnel impact the planning process. Interviewee 7 said that to convince other Corps personnel that adaptive management is needed, sometimes the argument must be reframed in terms of the risk associated with the course of action.

Turnover was strongly emphasized as an issue of concern among several interviews. High turnover is disruptive to ongoing projects, challenges consistency and relationship building, and the re-education of new staff take up staff time and resources. Since AM relies on an iterative process of feedback loops, experienced staff are invaluable because they accumulate the skills and knowledge on how to effectively implement AM approaches. However, according to interviewee 3, it was often difficult

for a district to accumulate that staff expertise. Absent that experience, the district turned to external support to assist personnel with the AM strategies. External experts provide a short-term fix, but are problematic for sustainable use of AM. Interview 2 suggested mentoring and training programs would benefit personnel to more effectively deal with the effects of staff turnover.

Interviewee 5a discussed personnel turnover at great length. In particular, this interviewee noted the Corps' partners at the state level are more likely to be more knowledgeable about ongoing projects due to the fact that these individuals remain in their positions for longer periods of time. As interviewee 5a suggests, it is somewhat rare for Corps staff to remain involved with a project for an extended period of time.

### *iii. Issues of Funding and Cost*

Even the most-qualified personnel can struggle to implement AM if the financial resources are not there to support the lifecycle of the project. Interview 2 identified budget challenges as the top concern of long-term monitoring for AM projects. As interviewee 2 explained, the requirements for local cost sharing pose an enormous barrier because it can be difficult to convince local partners to provide funds for monitoring. Investing in shovel-ready projects is more appealing than data collection, because the new projects are examples of taxpayers money "at work". Interview 4 noted that members of Congress often do not fully understand the process and costs associated with ecosystem restoration, and flexible and robust projects will inevitably increase costs.

Likewise, interviewee 5a emphasized that agencies rarely have the budgetary authority to maintain active AM for extended periods of time. Additionally, this

interviewee discussed a navigation study that would have involved adaptive management that ultimately did not receive funding. Furthermore, Interview 5b discussed how one project was far along in the design process, but the project has been put on hold because it was not in the President's budget.

Interviewee 5b also discussed concerns about cost. For example, the interviewee discussed having to balance what proportion of your budget to be used for monitoring and adaptive management versus building the next project. In another example, the interviewee discussed how stakeholders will sometimes argue that adaptive management is too expensive. Interviewee 5b also discussed how under section 2039 of the 2007 WRDA guidance, if there is a need for monitoring beyond 10 years to support the adaptive management, then the full cost becomes responsibility of project sponsor. In addition, the first 10 years is actually cost-shared with the partner and that can be a bit of a challenge for the local cost-sharing partner.

Interviewee 5c stated that adaptive management has caused the project to expand in scope, which has caused the costs to increase. Increasing costs and decreasing funding streams makes it challenging to implement AM. In addition, interviewee 5c discussed how stakeholders resist a project as costs increase because they see that other projects will not be constructed as a result of the adaptive management project. Lastly, Interviewee 5c also stated that funding the Corps requirement of ten years of monitoring post-construction for a project would be difficult considering they cannot get funding to even construct the project.

Interviewee 7 and 8 argued that they are expected to do more with fewer resources, and that the current fiscal climate of reduced funding challenges the use of

adaptive management. Adaptive management is just one of many considerations the district must make, and they must think of the costs involved in every action.

Interviewee 6 discussed the problems with knowing who will continue paying for the project now that it is in the operation and maintenance phase. In some cases, agencies are looking to pool resources and seek solutions to implement adaptive management within their current operating budgets.

*iv. Institutional Structure*

Quite frequently, interviewees discussed various institutional barriers that impede the adaptive management process. Whether it was national or agency level, the institutional structure influences the use of adaptive management.

According to interviewee 4, there are inherent limitations to the Corps process that complicate the process of getting projects “authorized, approved, budgeted, and constructed.” In particular, this interviewee notes, that budgetary priorities often shift “depending on who is in office,” which can have a clear impact on the Army Corps’ ability to carry-out certain activities. For example, interviewee 4 believes that as of recently, there has been political pressure to cut costs, which has resulted in less emphasis on monitoring. On the other hand, Interviewee 5c discussed how the district’s fish passage project is a victim of being a large program in an environment where the government cannot support a large program. Other interviewees also touched on institutional barriers that exist at within the agency itself. Specifically, interviewee 7 discussed how upper management could override decision-making that was informed by AM information and so it was important to get buy-in from upper management

Likewise, interview 5a highlighted that “cumbersome bureaucracy” threatens the desired flexible structure for AM, and will likely delay construction of twelve new islands at least five years. Furthermore, as interviewee 5b, 5c, 7, and 8 discussed, adaptive management projects are in the planning and design phase, but nothing has been constructed or implemented yet. Perhaps, these delays can be traced back to the multitude of issues that are currently demanding the attention of federal agencies. For example, Interviewee 8 argued that climate change is getting more attention than adaptive management at the moment. While according to interviewee 5a, new construction is consistently prioritized over monitoring for adaptive management.

Furthermore, several interviewees described instances in which Congressional oversight provided a barrier to adaptive management, and interviewee 2 noted there are always decisions made in Congress that have the potential to “change the way [the Corps] does business.”

Interviewee 5c discussed how Congress is one of the biggest barriers to implementing AM. Interviewee 5b discussed the difficulties of reporting expenditures to Congress in order to do the necessary monitoring and to make the project adaptable. Interviewee 5c discussed how the Corps must do whatever Congress wants them to do. Oftentimes, Congress will task projects to the Corps without providing the necessary funds. Yet the Corps is unable to lobby directly to Congress for increased funding to support AM implementation. Interviewee 1 offered supporting opinion, as he noted that it was virtually impossible to implement adaptive management measures if Congress did not supply appropriate funds. However, this interviewee notes that it is

sometimes possible to find partners or other entities that can provide the financial backing to complete a project.

Additionally, interviewee 5a stressed a flexible management structure would support AM implementation, but in practice, project delivery teams are large and any activity involving a large team becomes costly, and impossible to do “a little AM study”. Interviewee 4 suggested that large project delivery teams were a burden to AM. Other interviewees also touched on the various institutional barriers that exist at the district level. For example, interviewee 7 discussed the need to have staff completely dedicated to adaptive management full-time (not part-time as occurs at present). While Interviewee 8 discussed how all the different regulations and practices for each initiative and agency can be complicated to handle.

Additionally, some interviewees discussed how institutional structures complicate decision-making processes and ultimately restrict adaptive management. For example, interviewee 5b stated that when collecting information, it is difficult to know which manager is going to use the information and make a decision that would change a project. In addition, depending on the institutional structure, interviewee 5b said it can be challenging to organize who plays what role in these structures. Interviewee 5c discussed how implementation and interpretation of adaptive management can occur differently depending on the level of Corps personnel involved (i.e., district-level vs. headquarters).

*v. Time Scales*

Frequently, interviewees discussed the relationship between project time frames and adaptive management. The interviews discussed a tension between

pressure to complete tasks quickly and long delays in project designs. According to interviewee 2, project time frames complicate the implementation of AM with pressure to “do things faster,” and “get projects off the books”. Hence, there is often a limited time frame in which monitoring and potential adjustments may occur. On the other hand, interviewee 4 discussed the fact that it often takes an extremely long time to get a project authorized, budgeted, and constructed. As a result of this, interviewee 4 believes that it is difficult to keep a local sponsor engaged through this long process, and often times, partners become disinterested in the project, or begin to believe that it has become too expensive.

Interviewee 5a discussed the difficulty in conducting rigorous peer review for AM projects. Similarly, interviewee 5a suggests that the long-time frame of most AM projects makes it difficult to maintain “consistency” over time. There is a mismatch between the institutional time frames and the changing environment. The interviewee explained time consuming planning and pre-project monitoring efforts delayed the construction of a lake-dredging project. As these delays were taking place, the natural systems underwent several changes that drastically impacted the project. Consequently, the entire experimental design “fell apart very rapidly,” as a result of these changes and the long-time frame that was given to pre-construction activities.

Interviewee 5c discussed the difficulties in knowing when to apply adaptive management. Interviewee 7 identified that trying to implement AM after a project already was started does not work because, “it was difficult to play catch-up with adaptive management – if you know anything about adaptive management.”

Interviewee 6 discussed how the team could not agree at what point adaptive

management would need to be used in the project. Interview 7 reiterated that some projects had unrealistic deadlines and argued that it is a balancing act in trying to not let studies drag on for years but also trying to allow themselves enough time to understand the changes that will be made to large ecosystems. This requires a skilled staff with experience in AM.

Some interviewees also discussed problems with how projects are transitioned. For example, interviewee 5a discussed how the Corps often “hands off” projects to their partners when they’re done. As interviewee 5a notes, the Corps often does not “check up” on these projects after they’ve been handed off to their partners, which makes it difficult to gain insight into whether or not their actions have been effective in the long-term. Likewise, interviewee 6 discussed how ending adaptive management needs to be included in the plan. Often the Corps continues to pay for some of the studies, and the agencies are continuing to require adaptive management as part of their biological opinions and water quality certifications. However, the Corps has agreed that all the data that needs to be gathered for decision-making has been acquired. For example, Interviewee 6 stated, “When you’ve dealt with the risks and uncertainties, you’ve been able to scientifically prove that there is no impact, that it’s time to let it go.”

The time component of AM projects reflects the complexity and tension between the short term pressure to complete projects, and the long-term project design. The difficulty this creates were mentioned above in the interviews, but also underlines the importance of having a skilled and experienced AM staff that knows how to navigate and keep consistency in AM projects. It is important to identify and track the trend of barriers across projects. This helps an assessment of whether or not the barriers are

prohibiting successful implementation of AM, or if they are making implementation more difficulty and costly in terms of time and funding. If it is the former, then a prioritization and narrowing of the key barriers is necessary, and if it the latter then solutions will likely improve buy-in as projects become less costly.

#### **D. AM Facilitations**

##### *i. Cooperation and Collaboration*

Frequently, interviewees discussed the benefits of forming local and state partnerships and fostering collaboration within the Corps. Interviewee 5a, 5b, 7, 2 noted that the Corps has a very good relationship with a variety of state and federal partners, including (the EPA, Fish and Wildlife, the National Park Service, USGS, state Department of Natural Resources, and the state Governor's office). Interviewee 8 emphasized that relationships improve work effectiveness by leveraging resources and expertise, and not duplicating efforts with state and local partners.

Several interviewees discussed how AM can benefit from collaboration and cooperation within the Corps. In particular, interviewee 1 described how his district's organizational culture supports teamwork and fosters an organizational culture that is highly supportive of AM. For example, interviewee 1 detailed how the district offers awards to teams of personnel who demonstrate positive teamwork through the AM process. Similarly, interviewee 3 discussed how teamwork takes place among different districts. In this instance, the interviewee detailed how personnel from multiple districts formed a team that regularly comes together to discuss a particular restoration project. Interviewee 6 discussed how the team was able to remain close and maintain relationships despite staff changes. Interviewee 7 discussed how members of the

adaptive management team and the project development team work together. In general, interviewee 7 believed that people worked well together in the Corps.

According to some of the interviewees, AM has been greatly facilitated by the “buy-in” and support of Corps personnel. Interviewee 1 emphasized that district personnel try to be extremely supportive of the AM process, even when projects may be experiencing difficulties. This interviewee emphasized that district managers encourage personnel to work through difficulties in order to get projects “back on track.” Likewise, interviewee 2 noted that AM is now embraced “at all levels of the Corps of Engineers.” Interviewees 7 and 8 anticipate buy-in from Corps personnel to support adaptive management.

On several occasions, interviewees discussed the ways in which AM can benefit from stakeholder involvement. According to interview 1, stakeholder involvement ensures that Corps personnel have not overlooked any aspect of the project. Although stakeholder involvement can be time consuming, interviewee 1 believes that it is highly necessary and beneficial to the AM process. Similarly, interviewee 2 believes that it is highly “risky” to restrict stakeholder involvement. This interviewee emphasizes that public input can be highly useful. Interviewee 5c discussed how NGOs lobby to Congress on behalf of the Corps.

*ii. Army Corps Personnel*

According to some interviewees, the AM process has greatly benefited from the knowledge and expertise of Corps personnel. As interviewee 1 discussed, this particular district has several personnel with extensive experience in designing and implementing AM projects. Additionally, interviewee 1 notes that these individuals are highly

experienced at communicating AM-related information to both upper level management and project delivery teams. At times, the Corps' expertise with AM promoted other agencies as well. For example, Interviewee 6 stated that adaptive management allowed the Corps to educate other agencies and improve credibility among agencies.

Skilled and experienced staff is a key facilitator. Interviewee 7 discussed how employees' experience is useful, but even with their experience; they need to document what they are doing. When new employees start working on AM, tools and a guidance documents based from experienced personnel serves as a great source as each develops their skill-set. Interview 6 provided an example of using an expert consultant to help with the AM process. This particular consultant worked on the first AM Everglades project, knew how Corps projects operated, and also had experience on channel improvement, which was the specific context for Interview 6.

Although many interviewees discussed problems related to personnel turnover, others indicated that it was not a problem. For example, interviewee 1 did not identify any problems related to staff turnover, and interviewee 2 discussed how turnover can be dealt with by instituting mentoring programs. Similarly, Interviewee 6 discussed how the lack of turnover with Corps personnel helped even though turnover in other agencies occurred.

### *iii. Miscellaneous*

At times, interviewees emphasized that their budgetary resources were actually adequate for facilitating AM. In particular, interviewee 1 notes that budgetary issues have not led to delays or constraints. Interviewee 1 noted that there are costs

associated with AM, but with adequate cost projections and budgeting, these costs can be properly accounted for. Similarly, interviewee 3 noted that budgetary issues have not constrained AM, as the Corps has largely embraced AM and has ensured that adequate funding is available for both science and monitoring. Interviewee 5b discussed how they were limited to three percent for monitoring on our projects, but the new 2039 guidance under WRDA 2007 removes that absolute three percent limit.

Other interviewees discussed how AM has been facilitated by the timing of projects, or the time frames that are available to plan and complete projects. According to interviewee 1, there has not been an instance in which time constraints has impeded the AM process. As interviewee 1 suggests, AM should be incorporated into the normal planning process, which means that it should not cause any timing delays. Likewise, interviewee 2 indicated that there seems to be adequate time for implementing and completing AM.

#### **E. Institutional Structure and Adaptive Management**

The interviewees discussed several district-level institutional factors that affected adaptive management. According to interviewee 1, specific details related to AM plans are generally left to the district's department of environmental quality. For the most part, the plans that this department is responsible for tend to be more science-based. Interviewee 1 also discussed how this particular district underwent a significant reorganization in order to better facilitate the AM process. For example, this district formed a "water resources division," which includes a regulatory branch, an operations branch, and a planning and policy branch.

Prior to this reorganization, each of these branches had been under separate divisions. As interviewee 1 states, this reorganization helped streamline the AM process. Overall, Interviewee 1 believes that having a positive and supportive organizational culture has helped to facilitate the success of AM within this district. For example, this district gives out awards to project teams that successfully utilize AM.

For interviewee 2, the project delivery team is ultimately most responsible for the success of AM. At the district level, interviewee 3 believes that it is necessary to “institutionalize” the process of AM. As interviewee 3 states, it is important to make sure that all personnel fully understand the AM process in order to ensure that projects are successfully carried out.

As interviewee 4 suggests, there has been more of an effort to include AM earlier on in the planning process. In part, interviewee believes that this has been a direct result of the CERP guidance memorandum, which emphasized that AM is required by the Army Corps’ 2009 guidance.

There was a trend in the responses among interviewees 2, 5a, 5b, 5c, and 7, that discussed how they had been applying AM principles for years, but they were just not calling the process, “adaptive management.” For example, Interviewee 5b discussed how a number of programs where the principles of adaptive management have been applied over the last 20 years; however, formal integration of adaptive management has only occurred in the last year. This interview further described a project that adhered to many of the principles of AM; the project that was set up as an experiment, coordinated with diverse groups of stakeholders, and identified triggers at which they would change the management strategy. For example, Interviewee 5a noted that past environmental

management programs have utilized scientific research and monitoring, so to some extent, this district has used AM strategies for decades.

Interviewee 5c stated “adaptive management is also kind of a term that was coined for things that we’ve been doing for a long time – we just assigned a name to it.” Interviewee 7 reiterated this by saying, “AM has been part of the Corps planning process from back in the 1970s, although they didn’t call it AM.”

Similarly, interviewee 2 seemed somewhat fascinated by the Corps’ emphasis on AM, as this individual suggested that AM is “just the way of doing business.” Specifically, this interviewee believes that AM and the process of long-term monitoring are “catch phrases” that are becoming increasingly popular. However, as interviewee 2 notes, the growing popularity of AM and long-term monitoring will not be helpful so long as Congress does not provide adequate funding for these activities.

Due to the large size of the Army Corps, interviewee 4 shared it was often difficult to facilitate change. Interviewee 4 suggested districts look to headquarters to lead the way with respect to the development of AM policies. Interviewee 1 also mentioned that the policies and guidelines for AM should originate from headquarters. Although there is an interagency team that is responsible for developing the guidelines for each project, interviewee 1 believed that headquarters ultimately formulates most of the AM policies that are followed at the district-level.

With respect to ecosystem restoration, interviewee 4 emphasizes the need to conduct modeling before a system is ever tested. However, this interviewee suggests that the Army Corps has been reluctant to embrace this strategy.

To provide context for adaptive management, Interviewee 7 discussed the six-step planning process the Army Corps uses. The interviewee also discussed the fact that adaptive management is only used for ecosystem restoration and mitigation as per Army Corps rules.

In addition, several interviewees worked on interagency initiatives that allowed the Corps to discuss AM initiatives. For example, Interviewee 5b discussed playing a part in White House Council on Environmental Quality's (CEQ) report on freshwater systems and climate change. An interagency group, which included Department of Interior, Department of Agriculture, NOAA, and the Corps of Engineers, were all asked to benchmark adaptive management activities in each agency. The second step will be discussing what obstacles are there to more formally integrate adaptive management as it relates to climate change on freshwater systems. However, adaptive management is only one of 17 actions that the CEQ is studying.

#### **F. The Planning Process and Adaptive Management**

The majority of the interviews discussed how adaptive management fit within the planning process. Interviewee 5b discussed how the districts are in the planning and design phase, but nothing has been constructed yet. In general, planning involves choosing the type of adaptive management to be used and estimating the monitoring costs. According to the interviewee, planning in this way makes everyone look closely at expenditures and justify them. As far as the planning process is concerned, a project development team in the district develops goals and objectives and then targets the monitoring plans. The team also develops the full adaptive management plan for that

project. The project development team must also follow the goals and objectives that the three districts outlined at larger geographic scales.

In one program, interviewee 5b said a panel made up of independent scientists provides direction for adaptive management. In addition, two Corps scientists act as a liaison between the science panel and the project development team. Interviewee 5b said that the districts are looking at a similar science panel structure for another program.

However, interviewee 5b also said that one of the district's programs has a coordinating committee made up of members from the Corps and other agencies. The committee has been in place for 25 years, and the Corps is trying to work with the committee to accommodate adaptive management. Interviewee 5b also said that the three districts each have interagency teams that help manage certain activities. The districts currently need to modify this nested hierarchy of governance to support adaptive management.

In addition, interviewee 5b stated that in some situations, Congress can play a part in the planning process. For example, when a certain invasive species became a threat, Congress directed the districts to create barriers to stop the exotics' spread.

Interviewee 5c provided a good example of AM in the planning process for a fish passage project. The key components in the planning process were creating a science panel, identifying a goal, and including variety of stakeholders. It was important to establish the goal in the planning process because it gave direction and informed the planning for other components to achieve the goal. A second key component was creating a science panel that analyzed AM and drafted a strategy. The science panel

consisted of Corps personnel, academic professors, and other agencies' personnel. Even though the science panel made recommendations for AM, the project delivery team is primarily responsible for the implementation. The project delivery team consisted primarily of Corps personnel who were chosen based on their area of expertise. Where the team lacked expertise, the Corps would seek external support from other agencies.

Interviewee 5c said the design of adaptive management structure is to learn from early projects and apply knowledge to later ones, which will eventually allow the agency to save money and do things smarter, faster, and cheaper. Interviewee 5c stated that because this is the first of many fish passage projects, AM is critical to this first project. Adaptive management allowed the project development team to determine the size and location of projects, which then informs how the project will be constructed. Since context is deterministic for the outcomes of a project, location becomes a key component.

Because a fish passage project has not been completed at this scale, the Corps needs to determine its costs before replicating in other places. This particular project was identified through an interdisciplinary and interagency group. The AM process allowed the team to examine similar projects to create an effective proposal, which involved four different AM studies.

In addition, Interviewee 5c's district has guidance for ecosystem restoration projects and adaptive management. Interviewee 5c also explained how there are vertical teams created with staff from different levels in the Corps. Also, the project must get approval at each level in the Corps.

The planning phase for the channel improvement project in interview 6 came from an interagency team based on a biological opinion study. A charter helped the team understand roles, operating procedures, meetings, and engagement with upper management. The charter assisted with interagency team management and allowed for constructive planning of the AM strategy. Interview 6 planning phase considered the budget and included triggers and decision points for the implementers. When disagreement occurred on setting these triggers, a facilitator was used to build trust between team members and resolve disputes.

Interviewee 7 discussed how the district formed a team specifically for adaptive management. They designed a conceptual ecological model (CEM) that explained the relationships between different variables and helped the team determine the course of action. This model guided the planning development team establish goals and objectives and uncover uncertainties. The CEM tool also helped the team devise hypothesis and determine triggers which when activated would initiate AM actions to test the hypothesis.

Interviewee 7 discussed their adaptive management team assisted the project development team during AM implementation. The AM team created plans for a series of projects, and currently is drafting the implementation guidance for the district. Now, the district is moving into the pre-construction, engineering, and design (PED) phase, which is a much more detailed design. However, since the district has never done adaptive management in the PED phase, one to two years of pre-construction monitoring will be needed.

Interviewee 8 represents the Corps on a commission that oversees water plans between the U.S. and Canada. The commission is made up of several agencies. Two projects involve the creation of adaptive management programs to better manage water resources. The purpose of using adaptive management is to improve the environment with a focus given to particular species and how they are impacted by human actions such as changing lake levels. During the time of the interview, AM was still in the planning phase and no implementation had occurred.

During the planning phase, AM projects are included in the broader water resource management plan that the commission must approve and then recommends to the U.S. State Department and the Canadian Department of Foreign Affairs. Adaptive management is just one aspect of these international water plans.

As interviewee 8 explained, one of the projects already had adaptive management included, but stakeholder opposition of the entire project caused the two countries to reconsider the overall plan. At this point, a working group is trying to reformulate a whole new plan and the group also wants an agency for oversight of adaptive management. Part of the adaptive management proposal is for monitoring, however questions remain who will lead the monitoring and where it will be done?

In addition to planning and design, some interviewees discussed AM implementation (however, some interviewees have not moved past planning and design at this point). Interviewee 5a believes that AM is “structured to do the right thing.” However, with respect to actually implementing AM, this interviewee states that he doesn’t know how much faith he has in the bureaucracy to “actually pull it off.”

Interviewee 6 discussed how active adaptive management was implemented during the first phases of construction. During implementation, the group would meet quarterly to discuss decision points and data collection. They used a decision matrix to guide decision-making. Overall, Interviewee 6 discussed how the adaptive management plan got implemented, finalized, and is now in the operation and maintenance phase.

In addition to implementation, some interviewees also discussed evaluation. Interviewee 5b said that currently, third party reviews of the process are done with other agencies and interested NGOs. Interviewee 5c discussed how the district used outside reviewers to analyze the adaptive management planning process. However, the third-party reviews did not change any of the plans. Interviewee 6 said that the Corps would do their own evaluation and then presents the results to the adaptive management team.

Lastly, some interviewees discussed how using adaptive management will change the way districts operate in the future. Interviewee 5c discussed how they implement adaptive management at this fish passage project will impact future fish passage projects. In addition, interviewee 6 recommended that ending adaptive management needs to be discussed earlier in the process instead of letting the project continue for longer than needed. Interviewee 7 discussed how the district had always done monitoring, but now they are also doing monitoring from an adaptive management standpoint.

## **G. Stakeholder Engagement**

### *i. Communication with and Participation of Stakeholders*

Before any engagement with stakeholders, most of the interviews (Interviews 2, 5a, 8) emphasized transparency. These interviews mentioned that the default was to be fully transparent with sharing information and communicating with stakeholders, especially cost-share partners. However, interview 8 mentioned that sometimes the stakeholders' perceptions on transparency differed.

Several ways of identifying and engaging stakeholders were discussed, including using websites, public meetings, comments, and hearings. Interviews 1 and 2 seek out voluntary stakeholders but if they do not develop organically then they will send notices, visit businesses, churches, and other organizations. Interviewee 2 discussed how public meetings are beneficial in establishing some consensus among stakeholders. According to the interviews 1, 2, 5c, and 8, public meetings give the Corps the opportunity to explain the rationale behind their actions, while also providing stakeholders the opportunity to discuss and debate issues amongst themselves. Through this process, it has become somewhat common for many stakeholders to broaden their views or opinions with respect to a proposed action.

The interviews showed a range in usefulness of public feedback. One interview noted that stakeholder comments were very influential, but at other times as interview 3 noted, the public comments lacked substantive value for the project teams. Interviews 3 and 4 discussed the challenges associated with stakeholder comments, especially when opinions differed. For example, this interviewee describes a situation in which some stakeholders wanted the Corps to move quickly and attempt to complete a restoration as soon as possible, while others may be concerned about flooding and will

be more likely to favor an incremental approach. In sum, the interviews displayed a variety of methods for stakeholder engagement as part of AM procedures.

*ii. Challenges with Stakeholder Participation*

In some instances, interviewees indicated there are areas for improvement with stakeholder engagement. Interviewee 4 noted that two-way dialogue between the Corps and stakeholders has been severely restricted at public meetings. Although staff listens to public comments, the district's concerns over potential lawsuits prevent them from publically responding to stakeholders.

When asked about engaging stakeholders, interviewee 7 stated, "we have done a very poor job in adaptive management of engaging stakeholders. Extremely poor. Almost non-existent." However, interviewee 7 acknowledged the importance of engaging stakeholders and provided examples through experience with NEPA. NEPA provides guidance for stakeholders, but there is no guidance for engaging stakeholders through adaptive management.

In addition to doing a poor job engaging stakeholders, interviewees also discussed the problems associated with stakeholders opposing plans. Interviews 4 and 8 gave examples of stakeholders who completely halted the project. Interviewee 3 discussed the challenges associated with stakeholders who are highly critical of the Corps' proposed plans. In particular, interviewee 4 notes that some stakeholders can be critical of AM because they do not understand why the Corps would undertake actions in light of the uncertainties that may exist.

Interviewee 5c stated that some stakeholders oppose plans because they believe the project is too large or too expensive. In addition, interviewee 5c discussed how

stakeholders would begin to resist a project as costs increase because they see that the AM projects will crowd out implementation of other projects. Interviewee 6 and 8 discussed how the process can stall if there is no agreement among stakeholders and how crucial stakeholder buy-in was.

At times, stakeholders have unrealistic expectations for projects. Interviewee 5a notes that NGOs cost-share partners can have unrealistic expectations, especially with respect to funding. According to this interviewee, it is common for NGOs to be unhappy with the amount of money that the Corps is ultimately able to provide. Interviewee 5b discussed how difficult it was to justify spending money to stakeholders in order to do the necessary monitoring.

In addition, reports can be too technical for stakeholders to understand. Interviewee 2 voiced concerns over the size and complexity of many Corps documents. Consequently, this interviewee discussed the importance of holding public workshops that assist stakeholders comprehend these reports. Interviewee 3 emphasized that it is necessary for Corps personnel to be able to communicate information in an accessible manner to stakeholders who do not have a great deal of technical or scientific knowledge.

### *iii. Positive Aspects of Stakeholder Participation*

Interviewee 5c discussed how, over a period of 15 years, relationships between stakeholders were built. Having more good experiences than bad ones led to establishing sustainable stakeholder relationships. Stakeholder participation can enhance the work of AM implementation, and can be used to troubleshoot uncertainty by tapping into a good understanding of local context. Interviewee 1 strongly believes

there are benefits associated with stakeholder participation. Not only can stakeholders provide the Corps with access to new perspectives, it can also help the team manage uncertainty because the stakeholders share unique local knowledge with Corps personnel. Interviewee 5b discussed how stakeholder groups helped design one of the programs. Stakeholder involvement can also have the potential for cost-sharing. Interviewee 5a and 5b noted that the district attempts to make NGOs eligible to become cost-sharing partners.

NGOs and agencies are the actively engaged stakeholders. Interviewee 5b stated that it is difficult at the regional and systems scale for the general public to stay involved. Interviewee 5b stated that interagency groups are the predominate stakeholders, but NGOs have strong participation as well. Interviewee 5b also stated that NEPA is the main impetus for public involvement and engagement. Interviewee 5c also stated that they get more participation from NGOs.

#### **H. Monitoring and Reducing Uncertainty**

Interviewee 7 stressed, “the primary thing you want to think about with adaptive management is uncertainties.” It is highly likely that uncertainty will challenge future Corps projects, and AM is one tool to navigate uncertainty and achieve project goals. Being able to make adjustments and undertake alternative actions allows a project to respond to numerous threats and improves its sustainability. Interviewee 3 spoke to the role of monitoring in AM very well, and said, “if you read our implementation guidance for adaptive management, so that implementation guidance of Section 29, Section 2036, it doesn’t really talk about that traditional definition of adaptive management of testing multiple hypotheses and selecting the best model. It’s

really focused more at a level of...being clear about the expected outcomes of our management actions and undertaking monitoring in order to determine whether or not those things are being realized, and then making potential adjustments if they aren't, and being upfront about what those potential adjustments might be.”

Monitoring improves understanding of current conditions and as the AM team accumulates knowledge and experience, this reduces but does not eliminate the uncertainty. According to interviewee 3, monitoring is extremely necessary for dealing with and addressing uncertainty. Not only does monitoring provide the Corps with insight into the expected outcomes associated with projects, but it also generates information pertaining to the biological responses that occur in response to certain actions. If it is clear that the project's objectives are not being met, then adjustments must be made. Interview 4 reiterates that monitoring enables the Corps to learn from the outcomes and positions them to effectively address uncertainty in the future. However, monitoring runs into budgetary constraints

Interviewee 5c discussed how AM allows the team to ask questions about the uncertainties that arise during the project. Interviewee 6 also stated that adaptive management allowed the Corps to demonstrate through science that the Corps would not damage the environment.

At times, interviewees discussed the relationship between uncertainty and funding. Interviewee 5c discussed how there are uncertainties with adaptive management associated with costs and timing. Interviewee 7 discussed how you must answer the question, “How certain are you of that?” Interviewee 7 also discussed how experimentation with natural systems could be difficult because of the costs.

In addition, interviewees discussed some of the risks and uncertainties that play a part in ecosystem restoration projects. For example, Interviewee 5b and 6 stated that one of the most difficult challenges with monitoring is distinguishing natural variability from actual ecological responses for management actions.

Interviewee 5b mentioned that that climate change and invasive species influenced how projects use experimental designs and used monitoring to address uncertainty in future scenarios.

Each project is context specific and covers a range of uncertainty. Between Interviewee 6 and 7, the range of areas in a changing ecosystem that dealt with uncertainty included salinity intrusion, depth, temperature, habitat, dredging, creating diversions, and marsh restoration. The process of how the district performed the restoration depended on the timing of the implementation schedule. If the diversion occurred first, then you would wait to see the impacts of it before doing the marsh restoration.

Interviewee 7 also discussed recognizing the uncertainties associated with changes in natural systems. In particular, interviewee 7 discussed the need to combine expertise with statistical analysis when applying adaptive management: “When you go to any of the other places around the country, you’re going to have a different context for the adaptive management.”

Interview 6 stated that the Corps should not continue monitoring if there is no benefit, so what is the benefit? Interviewee 2 believes that it is important to monitor in order to determine whether or not the project is meeting its goals and objectives. According to this interviewee, monitoring is one of the only ways to determine whether

or not adjustments need to be made to a project. Therefore, long-term monitoring facilitates AM, and provides data to improve understanding and reduce uncertainty in projects.

Interviews discussed the importance of different types of monitoring. Interviewee 5a described some of the benefits that have been associated with the biological monitoring of fish populations. This monitoring assisted this district in their efforts to design an “attraction-production” hypothesis about this particular fish population and helped them to quantitatively document the benefits associated with their actions. Interviewee 5c discussed how baseline monitoring is done to measure change. In addition, there is a requirement to do monitoring for 10 years after construction. Interviewee 5c also discussed how monitoring will be done while the project is constructed.

Interviewee 6 discussed how benchmarks were used for monitoring. Then, the team would meet quarterly to review the results, analyze the criteria, and make adjustments based on the monitoring information. Interviewee 7 discussed how the district had always done monitoring, but now they are also doing monitoring from an adaptive management standpoint.

Interviewee 5b stated that more physical monitoring (example: water clarity, water depth, etc.) is done more than biological monitoring (example: plant response, fish response, etc.), but their goal is to do more cause and effect monitoring with adaptive management. Interviewee 5b stated that because distinguishing natural variability from actual ecological responses for management actions is so difficult, trend monitoring over time is important.

Interviewee 2 discussed the use of continuous monitoring in an island restoration project. In this instance, sturgeons were electronically monitored in order to continuously track their movement. However, interviewee 2 notes that in some instances, it is more appropriate to conduct periodic monitoring, as not all environmental or ecological conditions change quickly. Interviewee 5c discussed how it is very difficult to do adaptive management without monitoring.

## **V. Non-Corp Interviews**

Appendix D refers offers a more detailed account of how AM was used in two other federal agencies. Even with a small sample size, there were some common findings between the two, and some differences between the non-Corp findings and the Corp findings. Both agencies had an interest to use AM to improve effectiveness of projects. The main barriers included regulations, costs, and uncooperative partners. The interviews cited a shift towards a less risk-averse organizational culture, support from upper management, and using monitoring and evaluation as reasons for increased use of AM techniques.

## **VI. Recommendations**

**(1) Develop common reference materials, disseminate Corps AM guidance widely, and have Headquarters reinforce their commitment to this guidance regularly.** The guidance from Headquarters is a good first step to communicate clear messaging on implementing AM. The principles of AM are not foreign to interviewees, but using a common reference document, and then adjusting to meet local contexts,

would help districts that experience high staff turnover to maintain some consistency by using common reference materials.

(2) **Secure long-term funding for the successful implementation of AM.** The interviews identified budgetary constraints as the top concern of long-term monitoring for AM projects. Increasing costs and decreasing funding streams threatens future AM implementation, especially in the current political environment. Where it is possible, work with other agencies to identify and leverage resources to secure funding that allows active AM approaches for a sustained period. Look to develop relationships with the private sector for cost-sharing opportunities, and improve the partner's understanding of adaptive management for the long-term.

(3) **Develop internal capacity.** External experts provide a short-term fix, but are problematic for sustainable use of AM. Along with the AM guidance, work with district offices on developing mentoring and training programs that would benefit personnel and address the effects of staff turnover. The goal is to retain the information on how to work with stakeholders and respond to the various context-specific challenges for each project.

(4) **Conduct Advocacy and Awareness Campaigns for district offices.** Due to the fact that the principles of AM closely resemble what some offices have been “doing for decades” there are likely many “grey areas” or assumptions that are specific to each office and result in variations of AM that might be inconsistent with the guidance from Headquarters.

- (a) Promote inter-offices dialogue, workshop seminars, or webinar certifications on best-practices or “shared learnings” from peers;

(b) Develop a prize that recognizes and rewards district offices that have innovative AM programs. This would incentivize sharing of case-studies and accumulate a knowledge of best practices that could start to standardize terms and techniques.

(5) **Coordinate and collaborate with other agencies on approaches to AM**, in order to aim for solid understanding of terms and approaches. This would assist in knowledge-sharing as district offices work with other agencies. It also would likely help when communicating with Congress during appropriations when trying to demonstrate inter-agency approaches and solid “return on investments”.

In summary, adaptive management provides an effective toolkit that builds learning into projects, and could greatly benefit the Corps in its restoration projects. This learning can guide decisions to respond to uncertainty and changes that are difficult to predict. Climate change is likely to increase the level and scale of uncertainty, so AM offers a strong step forward for the Corps in the future.

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## **Appendix A: Questions for Interviews**

### **I. General Overview**

- General discussion of the project (if needed)
- Discussion of AM policy/guidelines, if any, and application to this project
  - Who promulgated the policy? Where in organization? When? History of policy?
  - How do they define AM for this project? Who determined this definition?
  - Was AM included from the beginning or added later?
  - Ask questions to determine whether the AM was active or passive
    - Did your design include testing of alternatives, experiments to reduce uncertainty or optimize project outcomes? (e.g. hypotheses; to determine if active)
    - How were alternatives determined/selected?
- Goals and objectives of AM for this project
- AM participants: decision-makers, experts, and stakeholders
  - Include organizational location, titles, AM responsibilities (project organizational chart would be helpful)
  - Discuss communication and coordination and management lines
  - Discuss continuity in management (versus turnover)
- Was the project considered successful? Define success (does it include project goals and objectives, etc.). Explain.
- Was the AM process considered successful? Why or why not? How did the project benefit from AM? (did AM add value to the project?)
- How did the project suffer from AM? Could the project goals have been achieved w/o AM?
- What obstacles arose in the conduct of AM and how were these overcome (if they were overcome)?
- What opportunities presented themselves to make AM successful?
- If you had to do this project again using AM, what should have been done differently?
- How should AM be implemented in future projects?

### **II. Constraints and Facilitations**

- Discuss how the time frame of project constrained/facilitated AM
- Discuss how the budget for project constrained/facilitated AM
- Discuss how staffing and staff competence constrained/facilitated AM
- Staffing turnover, management turnover
- Discuss how the organizational hierarchy and organization culture constrained/facilitated AM
- Describe what statutes, regulations, laws affect the implementation of AM and of project?

### **III. Process – Institutional**

- Describe the organizational culture (risk averse, vertical command and control, collegial, conservative, etc.)

- Describe the organizational decision-making processes (approvals, locus of control, authorities, timing, formalities, etc.)
- How is AM supported (or not) by organizational leadership?
- Describe flexibility in project planning and implementation
- Describe partnerships with other organizations
- Describe relationships with public stakeholders
- Describe the institutional processes that mitigate conflict among stakeholders
- What incentives are provided, if any, to encourage effective use of AM?

#### **IV. Stakeholder Participation**

- Discuss how stakeholders were identified
- Discuss how stakeholders were selected
- Discuss how stakeholders were involved
- Discuss the influence that stakeholders were given in AM
- Discuss benefits, costs, and risks of stakeholder involvement
- Discuss how decisions were made in stakeholder groups
- Discuss process in terms of fairness, inclusiveness, empowerment, transparency (did they get training, documents, were documents accessible to audience in tone, were they informed of meeting times with advance notice)

#### **V. Process – Evaluation of Outcomes**

- Describe how AM was used to monitor outcomes (against goals and objectives)
- Discuss how these results were used to adjust project implementation
- Where were the decision points in the AM process
- How responsive was the plan – how often did you gather data, and evaluate the data for purposes of evaluating plan
- What tools did you use to evaluate data on AM monitoring?
- Who is accountable for management of adaptation?
- Are there third-party reviews built into AM process
- Are there program reviews of AM in the organization?

#### **VI. Process – Reduction of Uncertainty**

- Discuss what types of uncertainty were associated with the project outcomes?
- Discuss whether and how AM was used to reduce uncertainty (e.g., field experiments, hypothesis testing, etc.)
- Discuss how these results were used to adjust project implementation
- Discuss the processes to communicate the uncertainty in the science, social, and management data across models and to the relevant decision-makers?
- How does the project differentiate failure from uncertainty?

#### **VII. Process – Institutional Change**

- Discuss how AM changed how the institution conducts similar projects elsewhere

#### **VIII. Lessons learned**

- What would they do differently in this project, what would they carry forward to future projects

**Who else should we talk to in your organization or project management?**

## **Appendix B: Codebook**

### **General Overview**

Interviewee background: (examples: scientist, facilitator, etc)

Project background

District background

Natural systems background

Army Corps background

### **Discussion of AM policy/guidelines/plans**

AM impetus: (examples: Water Resources Development Act [WRDA], legislation, etc)

AM: been applying principles for years, just not calling it AM

AM: Florida influence

AM: active AM example

AM plan (Army Corps): (example: some interviewees discussed agency-wide plan being made)

AM Definition:

### **Constraints and Facilitations**

AM barrier: conflicts and complicates implementation of other Army Corps guidance

AM barrier: conflicts with planning process

AM barrier: lack of knowledge about AM

AM barrier: uncertainties

AM barrier: timing and timeframes

AM barrier: funding/costs

AM barrier: disagreement over when AM needed

AM barrier: Planning process vs AM timing

AM barrier: only used for environmental restoration and mitigation

AM barrier: implementing after project has started

AM barrier: AM just another check on list in planning process

AM barrier: some personnel difficult to work with

AM barrier: bosses can override AM decisions or do not approve of using AM

AM barrier: choosing between AM and monitoring versus building next project

AM barrier: stakeholders

AM barrier: Congress

AM barrier: finding local cost-sharing partner

AM barrier: national government and institutional arrangements

AM barrier: institutional structure for decision-making

AM barrier: getting science and management to talk to each other

AM barrier: a lot of parties involved

AM barrier: no agreement by stakeholders

AM barrier: lots of proposed studies but nothing is on the ground and running

AM barrier: other issues getting more attention

AM barrier: developing plan, decision-points, and triggers

AM barrier: no Corps guidance

AM barrier: at some point AM needs to end

AM barrier: staff turnover (in other agencies or Army Corps)  
AM Barrier: legal restrictions.  
AM Barrier: AM is not a priority  
AM Barrier: AM works better @ program level than @ project level  
AM Barrier: Not following up with projects once their done/handed off  
AM Barrier: Balancing Stakeholder needs  
AM Barrier: Lack of quantitative data  
AM Barrier: long-term monitoring is costly  
AM barrier: trust  
AM barrier: balancing strong personalities  
AM barrier: AM caused project to expand in scope  
AM Support: Adequate monitoring budget.  
AM Support: Adequate construction budget.  
AM support: collaboration and pooling resources  
AM support: most personnel willing to work together  
AM support: anticipates buy-in from personnel or partnering agencies  
AM support: WRDA 2007 – 2039 guidance got rid of three percent limit for monitoring  
AM support: newer employees open to new ideas  
AM support: older employees' experience helpful  
AM consideration: relationship between costs and natural systems  
AM Support: stakeholders support  
AM Support: Proactive monitoring and research program  
AM Support: AM is embraced throughout the Corps  
AM Support: Good communication among decision-makers.  
AM Support: Always apply lessons learned  
AM Support: Staff understands when AM is necessary  
AM Support: Adequate staffing and expertise  
AM Support: timing/time frames aren't a constraint  
AM Support: lack of turnover  
AM support: AM smarter way of doing business

### **Process – Institutional structure and Institutional changes**

Institutional change:  
Planning process structure:  
AM process structure:  
AM (planning):  
AM (implementation):  
AM (operation and maintenance phase):  
AM (evaluation):  
Institutional structure (Army Corps)  
Institutional structure (district):  
Institutional Structure:  
AM (future changes):  
Organizational Culture:  
AM Incentives: Awards given for successful projects

### **Stakeholder Participation**

Stakeholders: poor job engaging stakeholders  
Stakeholders: no implementation guidance for engaging stakeholders  
Stakeholders: engaged through NEPA  
Stakeholders: importance of stakeholder participation  
Stakeholders: public comments  
Stakeholders: public meetings  
Stakeholders: interagency groups  
Stakeholders: potential cost-share partners  
Stakeholders: NGOs  
Stakeholders: difficult at regional and systems scale for general public involvement  
Stakeholders: updates website for them  
Stakeholders: oppose plans  
Stakeholders: Engaging Stakeholders  
Stakeholders: Forming Groups  
Stakeholders: Public Workshops  
Stakeholders: Unrealistic expectations  
Stakeholders: Full transparency  
Stakeholders: beneficial to AM process  
Stakeholders: public hearings  
Stakeholders: Reports are too technical  
Stakeholders: Facilitating discussion  
Stakeholders: influential in AM process  
Stakeholders: identifying stakeholders

### **Process – Monitoring and Reduction of Uncertainty**

AM: helps in dealing with uncertainty  
AM: relationship between uncertainty and funding  
AM: Baseline monitoring needed  
Monitoring: AM monitoring can be different than monitoring for other actions  
Monitoring: AM monitoring can overlap with monitoring for other actions  
AM: now using best professional judgment to deal with uncertainties  
Monitoring: physical monitoring  
Monitoring: biological monitoring  
AM: cause and effect monitoring  
Monitoring: distinguishing natural variability from actual ecological responses for management actions  
AM risks and uncertainty: natural variability  
AM risks and uncertainty: climate change  
AM risks and uncertainty: invasive species  
Monitoring: benchmarks  
Monitoring: some monitoring techniques did not work  
Uncertainty: (specific example)  
Monitoring: trend monitoring  
Monitoring: helps to achieve desired results/outcomes  
Monitoring: Continuous Monitoring

Monitoring: Frequency of monitoring depends on project.  
Monitoring: review results

**APPENDIX C: Table of Background Information for District Interviews**

Interview	Role	Location and Types of Projects AM used	Personal involvement in AM
1	Technical Support Specialist	Wetland restoration, sediment remediation, oyster reef construction, and oyster restoration, predator and invasive species management	Technical and environmental support during AM process
2	Program Manager	Large scale ecosystem restoration, coastal activity, storm damage, risk reduction, navigation, and saltwater intrusion	Involved in plan formulation, construction, operation, and monitoring of AM
3	Environmental Resource Specialist	Ecosystem recovery, endangered species management, shallow water habitat management, fish passage projects, and mitigation projects.	Implementation stages
4	Manager	Large scale restoration	Developed AM strategies and system-wide monitoring
5a	Ecologist	25 year habitat rehabilitation and enhancement project in 3 districts that face challenges sedimentation, dams, loss of depth, and the diversity of aquatic habitat. 50 ecosystem restoration projects occurring in 3 districts, but formal adaptive management application has only occurred in the last year. 5b and 5c work on fish passage projects	Developed AM plans for several regional ecosystem restoration projects
5b	Advisor		Created a technical guide for agency on AM, Lead on investigating how AM is used across federal agencies in response to climate change
5c	Biologist- Team Lead		Program implementation
6	Project manager, mediator, facilitator	Channel improvement between states	
7	Wildlife biologist, environmental manager, environmental planner, and adaptive management team leader	Coast-wide ecosystem restoration, just in the planning phase, but no implementation of AM	
8	AM subject matter expert	No ongoing AM projects, one in proposal stage	US government freshwater initiatives and National Oceans Council

## **APPENDIX D: Non-Corps Interview Findings**

In addition to the eight interviews conducted with Army Corps personnel, two interviews with personnel from other federal agencies were also conducted. These interviews are labeled as interviews 9 and 10.

*Background.* Interviewee 9 works with an agency that oversees activities such as hydroelectric power generation, flood control, municipal water use, and recreation. Interviewee 9 oversees the adaptive management process for the agency. In many ways, interviewee 9's agency is similar to the Army Corps. The agency associated with interviewee 9, uses adaptive management to manage water levels to accommodate drought. In the limited circumstances that the agency has used adaptive management, interviewee 9 argued that the technique was successful and that the agency benefited from using AM.

Interview 10 was conducted with a water supply manager and a source water specialist for a metropolitan utility authority.. These individuals detailed the ways in which their agency utilizes AM in the process of monitoring source water lakes and the tributaries that feed into them. The utility used AM in order to ensure that discharge monitoring requirements are being met. In particular, AM was used as a means of evaluating a variety of options that could increase the effectiveness of the agency's treatment processes. Overall, the interviewees felt that AM was a highly beneficial process that increased efficiency and improved productivity.

*AM Barriers: Laws, Regulations, and Agency Guidance.* Similar to the Corps interviews, Interviewee 9's agency was constrained in using adaptive management because of conflict with other regulations. Interviewee 9 also indicated institutional

barriers complicate using adaptive management. The governing agency, the Federal Energy Regulatory Commission [FERC], placed certain requirements in license for Interviewee 9's agency that prevented it from being able to operate adaptively. Interviewee 9 stated in order to implement AM, the license would need to consider specific contingencies as defined in the license.

Similarly, the participants in interview 10 noted that the Clean Water Act (CWA) prevents them from effectively mitigating the presence of phosphorous in streams, as they are legally restricted as to the actions that can be taken. However, the interviewees noted that regulators have often been very flexible with their agency and their desire to use AM. One of the participants in interview 10 noted that the presence of strong science and cost comparisons could persuade regulators to be somewhat more agreeable.

*AM Barrier: Collaboration and Coordination.* Unlike several of the Corps interviewees, interviewee 9 discussed how some partnering agencies are not flexible in allowing the agency to try adaptive management techniques. Interviewee 9 indicated that approval from other agencies presents a barrier to adaptive management use.

*AM Barrier: Bosses do not approve of AM.* According to the participants in interview 10, it is often difficult to convince decision-makers or other high-ranking agency officials to be supportive of AM. Specifically, the interviewees discussed a tendency for agency officials to "only listen to engineers." These interviewees also emphasized that many individuals within their organization can be somewhat "narrow-minded" or fearful when it comes to trying something different, such as AM. However, both interviewees did acknowledge that both their utility director and the board that

oversees the agency have been increasingly supportive and open-minded with respect to their recent use of AM.

*AM Barrier: Funding/Costs.* Interview 10 also included a discussion of several cost-related barriers to AM. As with many of the Army Corps interviews, representatives from the utility authority felt that issues pertaining to the cost of AM made it difficult to implement. Similarly, the interviewees felt that agency employees were constantly trying to adapt to changes taking place in their watersheds or treatment facilities, which could be both time consuming and costly.

*Adaptive Management Facilitations.* Overall, interviewee 9 stated that the agency's CEO is supportive of adaptive management. In addition, interviewee 9 indicated that some (but not all) partnering agencies are willing to work with them to utilize adaptive management.

*Institutional Structure and Adaptive Management.* When adaptive management is used, interviewee 9 indicated that the agency does not incorporate the practice from the start. In terms of institutional structure, the personnel involved with making adaptive management decisions would be agency staff, the CEO, and at times, partnering resource agencies..

*Organizational Culture.* The participants in interview 10 noted that their organization has become less risk-adverse. The interviewees emphasized that they are allowed to take part in risky decision-making in the context of AM as long as they have adequate science to back up their actions. In particular, the interviewees noted that their agency's utility director is not a "micro-manager" and is supportive of their efforts to try various courses of action with respect to watershed management.

*Monitoring and Reduction of Uncertainty.* In one example, interviewee 9 discussed how monitoring allowed for feedback to address a problem.

*AM Future Changes.* Interview 10 included a discussion of the various ways in which the utility agency has changed their institutional processes in response to AM. The interviewees discussed how AM has encouraged agency officials to partake in more complete evaluations of the water and sewer department and to conduct studies that can assist the organization in its efforts to become more efficient and productive.

# Tracing nutrient sources at the land-water interface in urban environments

## Basic Information

<b>Title:</b>	Tracing nutrient sources at the land-water interface in urban environments
<b>Project Number:</b>	2012MN314B
<b>Start Date:</b>	2/1/2012
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	4th
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Hydrogeochemistry, Management and Planning
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	Jacques C. Finlay, Sarah Hobbie

## Publication

1. Importance of Hydrologic Pathways to Urban Nutrient Loading and Implications for Current Stormwater Management Practices. 2012. Minnesota Water Conference, St. Paul, MN. October 16, 2012.

## *Annual Progress Report*

2012MN314B Tracing nutrient sources at the land-water interface in urban environments

**Jacques Finlay & Sarah Hobbie**

### **1) RESEARCH**

#### **Introduction and study objectives**

In urban landscapes, excess nutrients from human activities combined with high impervious surface cover accelerate transport of water and nutrients into waterways, leading to high nutrient loading and eutrophication of urban and downstream aquatic ecosystems. Little is known, however, about the importance of different sources of nutrients as water moves from urban landscapes to streets and storm sewers, and ultimately to surface waters. This project examines tracer techniques toward improved understanding of contributions of specific nutrient sources in urban ecosystems to aquatic ecosystems. Stable isotopes of carbon (C), nitrogen (N), and phosphorus (P; as  $\text{H}_2\text{PO}_4^-$ ) are used in conjunction with other indirect tracers of water sources and element ratios to assess the relative contribution of these sources to storm water nutrient loads across a range of sites and seasonal conditions in urban watersheds in St. Paul, Minnesota (MN) (Figure 1). Because many of these sites have permanent baseflow that contributes substantially to watershed nutrient yields (Janke et al. in review) we have expanded studies to include tracers of baseflow nutrients. The project relies on collaborations with a local watershed district which supports instrumentation for flow monitoring and water quality monitoring via automated storm samplers. Information generated in this project could be used by managers to prioritize efforts to control specific nutrient sources (e.g. organic debris vs. soil erosion) and can contribute to long term decisions such as selection of tree species to plant on boulevards and how to change management in response to climate variations.

#### **Research Activities**

Our study sites are contained within the Capitol Region watershed (CRW), which is located in southeastern Minnesota, USA, encompassing sub-watersheds primarily in the city of Saint Paul and in parts of the surrounding cities of Roseville, Maplewood, Lauderdale, and Falcon Heights. The highly urbanized watershed has an area of 106 km<sup>2</sup>, with a total imperviousness of approximately 45% (CRWD 2010). A large variety of land cover types are

present, including parks and several natural lakes, as well as dense residential, commercial, and industrial development. Most of the land surface is connected to a storm sewer system draining to the Mississippi River at 55 locations along the southern boundary of the watershed (CRWD 2010). The Capitol Region Watershed District (CRWD; <http://capitolregionwd.org>) has conducted extensive monitoring in the CRW since 2006.

Our approach has been to combine intensive monitoring of a small number of sites with more spatially extensive surveys of a wider array of streams and storm drains in the TC area. CRWD monitoring sites located at the outlet of seven sub-watersheds serve as primary study sites for

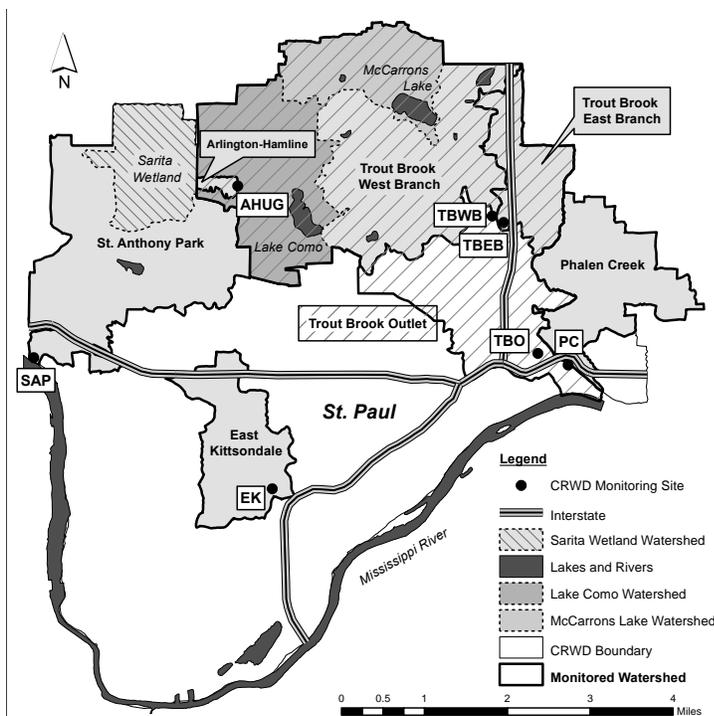


Figure 1 Map of major watersheds and study sites in CRWD.

combined storm water and baseflow studies (Figure. 1). A small watershed, Arlington-Hamline Underground (AHUG), located at the inlet to an underground storm water vault, is the site of intensive studies that link dynamics of terrestrial areas to storm water runoff. The AHUG site lacks surface water and has a sewer system that lies above the water table and therefore receives no baseflow. During 2012, we conducted several surveys during baseflow conditions that included 30 sites spread out around the Twin Cities. Sample analyses from

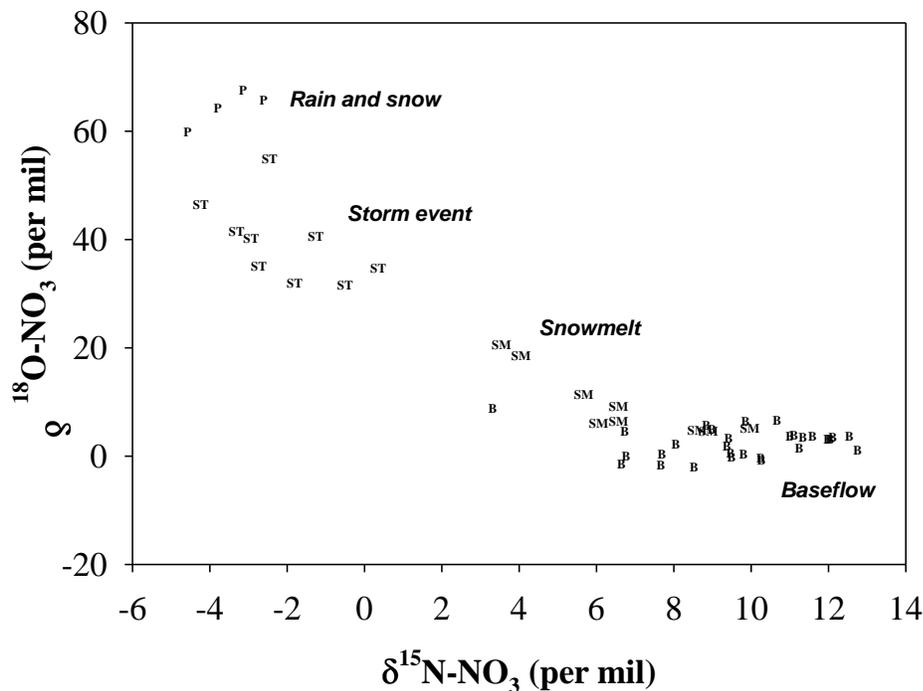
these efforts is ongoing. Our preliminary results, described below, combined with analyses to determine the specific form of nutrients in storm water (as part of a complementary project on urban vegetation; see <http://environment.umn.edu/urbanvegetation/>) will inform more efficient application of tracers during the upcoming summer.

## Preliminary Results and Ongoing Research

Our preliminary results are organized with the questions we proposed to address in this project.

**1) What are the sources of N, P, and C that enter storm drain systems in urban residential areas?** To address this question, we are evaluating and applying tracers of specific nutrient sources including atmospheric deposition, fertilizer, soils, vegetation, pet waste, and throughfall across an annual cycle at two small residential watersheds, and for a larger number of sites from select storm events and baseflow conditions from May to November.

Samples collected in 2011 and 2012 have been analyzed for C and N isotope ratios in particulate organic matter.  $\delta^{13}\text{C}$  values from most sites show values consistent with vegetation sources of organic matter with little  $^{15}\text{N}$  enrichment of  $\delta^{15}\text{N}$ , suggesting little influence of denitrification (data not shown). Nitrate stable isotopes show that storm water  $\text{NO}_3$  is derived from precipitation, as expected, while at baseflow, where concentrations are often elevated relative to stormwater and surface water, all  $\text{NO}_3$  is derived from urban soils (Figure 2).



**Figure 2 Nitrate stable isotope data for precipitation (P), stormwater (ST), snowmelt (SM), and baseflow (B) conditions. Data are from sites shown in Figure 1. Storm event and snowmelt represent mixture of precipitation and soil derived nitrate, indicating that nitrate isotopes effectively separate sources of nitrate within streams and drains in the watershed.**

Sampling efforts in one of the small urban watersheds (AHUG) involved collection of runoff temperature and conductivity data, the latter of which may be used to determine the presence of ions in runoff, and particularly to distinguish “first flush” conditions from samples

collected later within individual events. During snowmelt and early spring rains, winter road salt applications wash into storm drains and provide a tracer for impervious surfaces. An example is shown in Figure 3 for a snowmelt and rainfall event on March 8-10, 2013. A small flow peak due entirely to snowmelt occurred on March 8, with a corresponding large peak in conductivity presumably due to road salt, likely due to a large contribution of runoff from the major roads in this watershed. Rainfall on the morning of March 9 produced a brief peak in conductivity before the flow peak arrived, diluting the conductivity source, suggesting that portions of the watershed with little or no road salt (side streets, alleys, rooftops) were contributing runoff at this point. A second example using temperature and conductivity is shown for a rainfall event occurring on May 23, 2012 (Figure 4). Both runoff temperature and conductivity peak at the onset of runoff due to the influx of runoff from directly-connected streets, which are warmer than vegetated

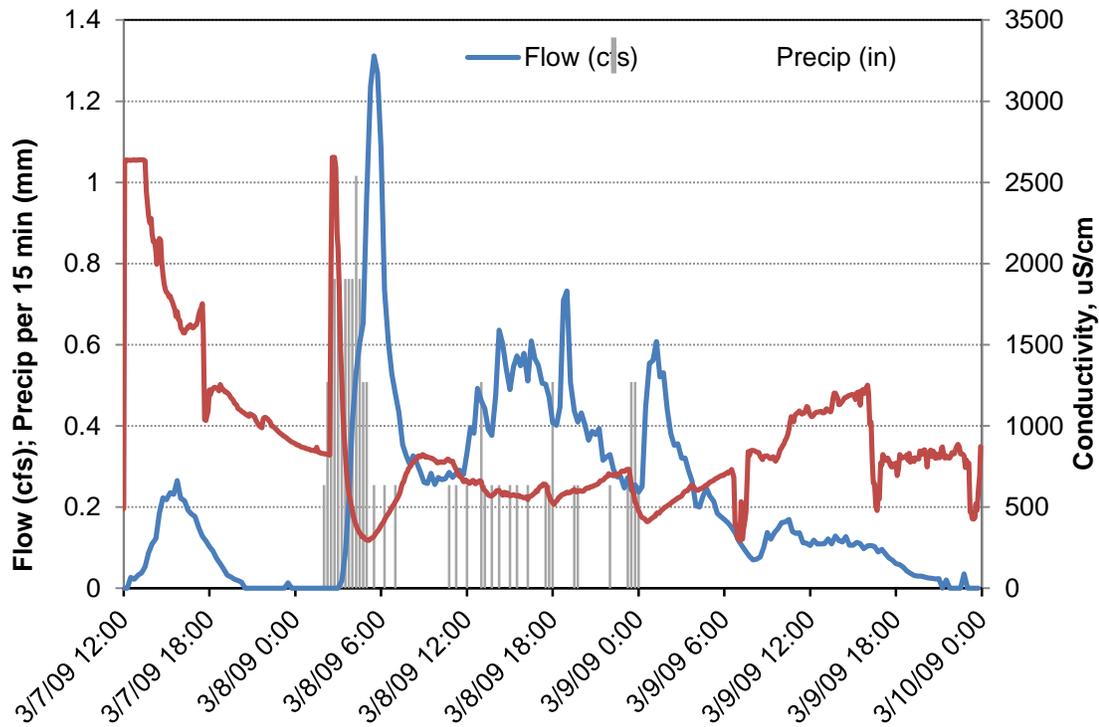


Figure 3 Runoff flow rate (cfs) and conductivity (uS/cm) measured at the outlet of a small (42-ac) watershed for a snowmelt and rainfall-runoff event on March 8-10, 2013. Precipitation (mm) shown on left axis. Peaks in conductivity are associated with road salt dissolved in the first flush of snowmelt and stormwater.

surfaces and also serve as collectors for atmospheric deposition (which likely explains the increase in conductivity, as no road salt would be present at this time). A rapid decrease in conductivity (less pronounced in

runoff temperature) suggests runoff contribution from vegetated surfaces. Samples collected during rainfall and snowmelt events, both at the watershed outlet and within the watershed, will be analyzed for C and NO<sub>3</sub> isotopes and particulate nutrients to determine more specific source

areas within the watershed, e.g., vegetation vs. soil or streets vs. lawns. The relative importance of these source areas for different types of storms (i.e. low-intensity vs. high-intensity) or within storms may also be investigated with nutrient tracer data.

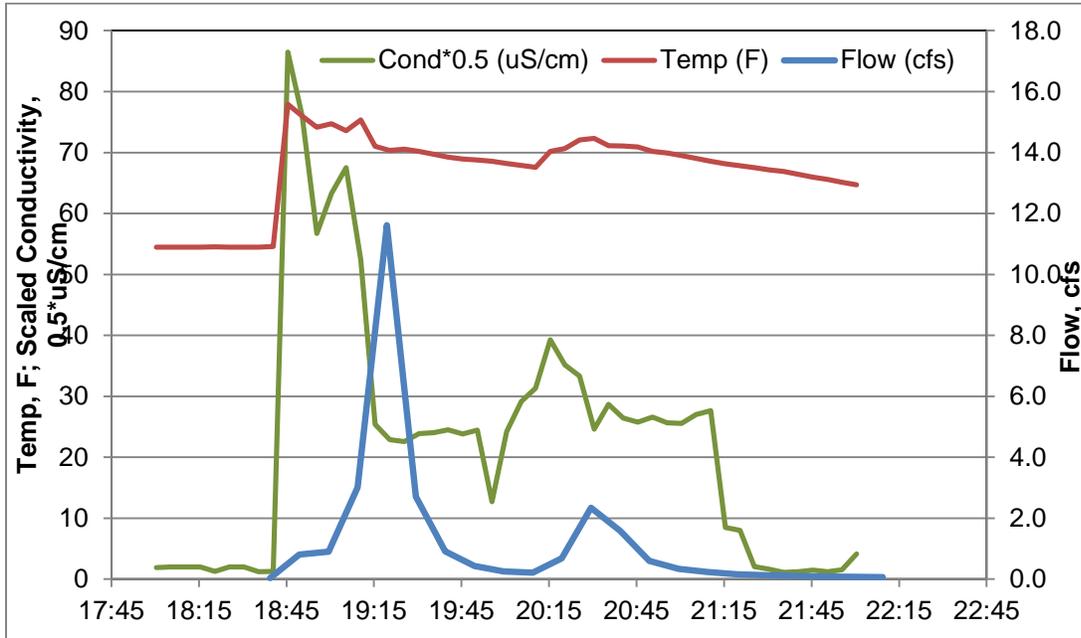


Figure 4 Runoff flow rate (cfs), runoff temperature (°F), and conductivity (0.5 \* uS/cm) measured at the outlet of the AHUG site for a storm event on May 23, 2012. Peaks in temperature and conductivity are associated with first flush runoff from road areas.

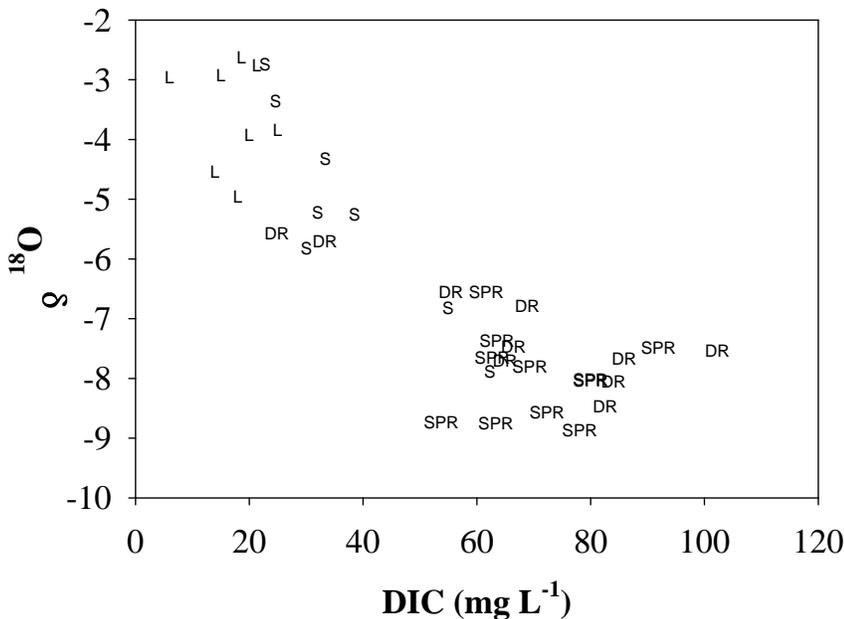


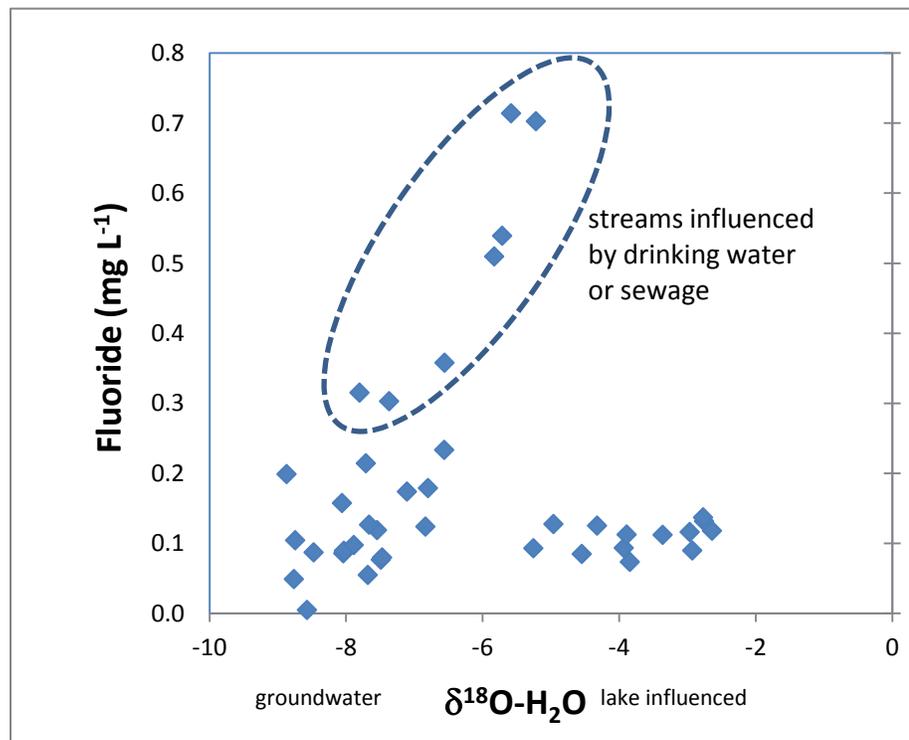
Figure 4 Oxygen stable isotopes ( $\delta^{18}\text{O}$ ) and dissolved inorganic carbon (DIC) effectively separate water sources in urban streams. L=lake outlet, S=stream, SPR=spring, DR= storm drain

(2) *What are the sources of N and P entering urban streams at base flow?* Water sources for urban streams and storm drained channels in our study system include surface waters such as wetlands and lakes, groundwater and storm runoff. Contributions of base flow and storm flow to nutrient loading during the 6 month warm season are variable among sites. For example, baseflow carries 8% to 34% of

total P loading, and 33% to 68% of N loads (Janke et al. in review). We have explored geochemical and stable isotope tracers to help understand the origin of water and nutrients in baseflow at our sites.

We are using tracers to distinguish among lake, groundwater, and stream derived water sources and as tracers of nutrient sources during base flow conditions. Figure 5 shows mean values of DIC concentration and oxygen stable isotope ratios of oxygen ( $\delta^{18}\text{O}$ ) as measured in 2011 and early 2012 in a variety of water sources and at the main CRWD monitoring sites during baseflow periods. A clear distinction is present between surface water sources (lake and pond outlets, streams, and wetlands) and groundwater sources (springs and groundwater flowing in shallow storm drains). Baseflow at several main monitoring sites (TBEB, EK, PC) appears to be primarily groundwater, while the remaining sites (TBWB in particular) are influenced to some extent by surface

water, a sensible result given that TBWB, TBO, and SAP have upstream lakes and wetlands connected to the storm drains. This information is proving useful in understanding variation in both nitrogen and phosphorus in these watersheds because lakes can substantially modify the concentration of both nutrients (Janke et al. In review;



**Figure 6 Baseflow concentrations of fluoride (Fl) and water stable isotopes in streams and storm drains in St. Paul. The drinking water plant releases high Fl water to Trout Brook as seen by concentrations declining with distance downstream (i.e. 0.7, 0.5, 0.5, 0.2 mg L<sup>-1</sup>). The other high Fl concentration sites (between 0.3 to 0.7 mg L<sup>-1</sup>) are storm drains that apparently are affected by leaking drinking or domestic sewer pipes**

unpublished data).

Fluoride (F1) is a potentially useful trace of domestic treated water sources, and analyses of F1 concentrations in a survey of streams and drains in 2012 shows that some storm drain sites are influenced by leaking pipes (Figure 6).

(3) *How well do management activities (street sweeping, catch basin clearing) perform in reducing sources of urban nutrient runoff?* In the AHUG watershed, we are examining stormwater nutrient concentration and yields before and after these activities. We are measuring the amount of material on streets and will assess fluxes observed in runoff in relation to street

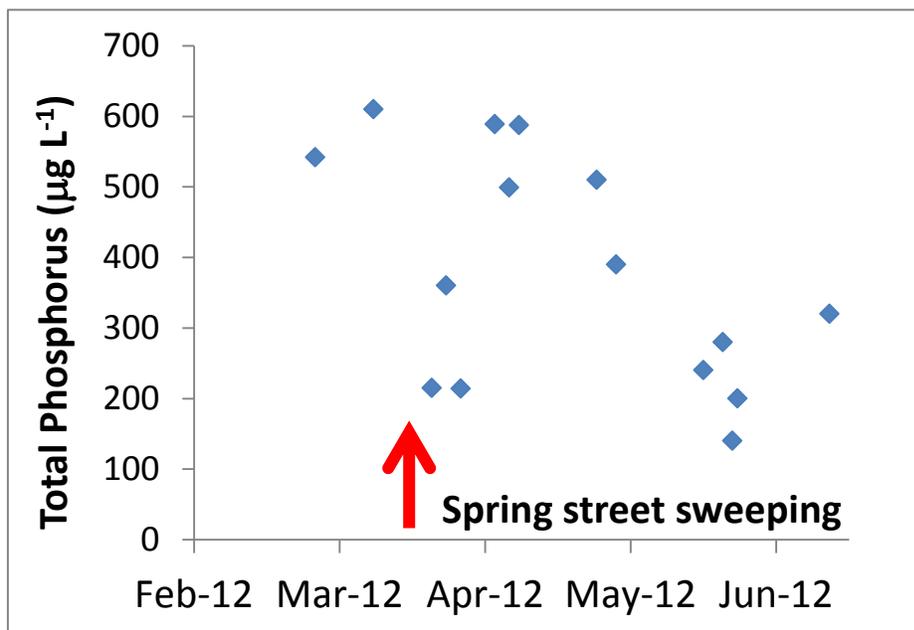


Figure 5 Stormwater total phosphorus concentrations in the AHUG watershed outlet for a series of storms prior to and after city street sweeping. TP concentrations decreased by more than 50% after sweeping but increased after two weeks to levels measured before sweeping.

material present before and after management activities.

During 2012 we successfully sampled stormwater runoff before and immediately after spring city sweeping. We

observed a sharp decrease in stormwater TP

concentrations for three rain events after sweeping, followed by an increase in TP (Figure 7). We hypothesize that this increase is related to new inputs of nutrients from surrounding vegetation during springtime. (In the fall, the extensive drought prevented useful comparisons of pre and post sweeping nutrient concentrations.) We are preparing to sample around city sweeping events in the AHUG watershed during 2013.

During the past year we have leveraged support from a concurrent project and collaborations with CRWD to collect samples and understand patterns of nutrient loading and form across our study sites. We have analyzed small subsets of samples to assess the performance of candidate tracers for identifying water and nutrient sources. During 2013 we will use this information to focus our efforts on application of tracers in urban watersheds using archived samples, and samples collected by CRWD.

## **2) PUBLICATIONS**

None

## **3) STUDENT SUPPORT**

**Anika Bratt**- Graduate student in the Ecology, Evolution and Behavior Graduate Program. One chapter of her dissertation will investigate use of DOC tracers to understand urban organic matter sources.

**Adam Worm**- Directed undergraduate research (spring 2013). His project examines the role of litter decomposition on release of soluble nutrients to impervious surfaces, and this project is helping to support his analyses.

## **4) PRESENTATIONS**

“Stormwater Management and Water Quality Issues in St. Paul.” [class lecture] *University of St. Thomas*, Feb 13, 2013.

“Importance of Hydrologic Pathways to Urban Nutrient Loading and Implications for Current Stormwater Management Practices.” [presentation] *Minnesota Water Conference*, Oct 16, 2012.

## **5) AWARDS**

None

## **6) RELATED FUNDING**

Anika Bratt received a 3-year EPA STAR graduate fellowship to work on urban nutrient cycling.

## References

Capitol Region Watershed District (CRWD). 2010. Capitol Region Watershed District 2010 monitoring report. April 2011. 191 pp.

Janke, B.D., Finlay, J.C., Hobbie, S.E., Baker, L.A., Sterner, R.W., Nidzgorski, D., and Wilson, B.N. 2013. "Contrasting Influences of Stormflow and Baseflow Pathways on Nitrogen and Phosphorus Export from an Urban Watershed." Submitted to *Biogeochemistry*, Jan 2013.

# Identification of Municipal Wastewater as a Key Reservoir of Antibiotic Resistance: Itasca State Park as a Model System

## Basic Information

<b>Title:</b>	Identification of Municipal Wastewater as a Key Reservoir of Antibiotic Resistance: Itasca State Park as a Model System
<b>Project Number:</b>	2012MN316B
<b>Start Date:</b>	3/1/2012
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	Minnesota fifth
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Treatment, Ecology, Wastewater
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Timothy Michael LaPara

## Publications

There are no publications.

## **1. Research**

### **Identification of municipal wastewater as a key reservoir of antibiotic resistance: Itasca State Park as a Model System**

#### **Principal investigator**

Timothy M. LaPara, Department of Civil Engineering, University of Minnesota

#### **Research assistant**

Kyle Sandberg, Department of Civil Engineering, University of Minnesota

**Start date: 3/1/2012**

**End date: 2/28/2014**

**Research Project Title:** Identification of municipal wastewater as a key reservoir of antibiotic resistance: Itasca State Park as a Model System

#### **Abstract**

Antibiotics and antibacterials are critically important drugs for the protection of public health. Historically, concerns about antibiotic resistance have been virtually disregarded, as it was assumed that new antibiotics would be discovered or that, similarly, existing drugs could be structurally modified to extend their effective lifetime. However, this assumption has been horribly wrong, as antibiotic resistance has developed at an alarming rate and the development of new antibiotics has almost completely stopped. New and complementary initiatives are therefore needed to help resolve this critically important problem.

Over the past decade or so, a new paradigm has developed with respect to the evolution and ecology of antibiotic resistance. The foundation of this theory is that antibiotic resistant bacteria are common in the environment but that pathogenic bacteria, which live inside the human body, are typically antibiotic-sensitive. Under the umbrella of this “antibiotic resistance paradigm”, this research project tests the theory that municipal wastewater and its treatment are critically important in the proliferation of antibiotic resistance. Treated municipal wastewater still contains substantial quantities of antibiotic resistant bacteria and antibiotic resistant genes, which are then released to the environment where they can intermix with environmental organisms and potentially further exchange resistance genes to the detriment of public health.

The goal of the research described herein is to unequivocally identify human sewage as a statistically significant source of antibiotic resistance and antibiotic resistance genes in the environment. This goal will be achieved by determining the quantities of several antibiotic resistance genes in the wastewater treatment lagoon and four lakes within Itasca State Park. Itasca State Park provides an ideal opportunity for this research because it produces a substantial quantity of domestic sewage (i.e., there are no industrial or agricultural inputs). Itasca State Park also has numerous lakes, with different levels of human use, which can serve as experimental controls (likely negative) for surface waters without an input of sewage.

#### **Introduction**

Antibiotics and antibacterials are critically important drugs for the protection of public health. These compounds target specific features of bacterial physiology (e.g., the bacterial cell

wall) to suppress activity (bacteriostatic) or to kill (bacteriocidal) these organisms. Because the target site is unique to bacteria, antibiotics and antibacterials have great medical value because they can be used to treat bacterial infections without a direct effect on the patient. Unfortunately, after decades of indiscriminate antibiotic use by the medical profession as well as a host of other frivolous uses (e.g., subtherapeutic antibiotic use in agriculture), antibiotic resistant bacteria are now pervasive, threatening the efficacy of virtually all applications of antibiotic chemotherapy. Indeed, many scientists fear that the “antibiotic era” will soon end.

Historically, concerns about antibiotic resistance have been virtually disregarded, as it was assumed that new antibiotics would be discovered or that, similarly, existing drugs could be structurally modified to extend their effective lifetime. However, this assumption has been horribly wrong, as antibiotic resistance has developed at an alarming rate and the development of new antibiotics has almost completely stopped.

The primary focus of the medical community to thwart the development of antibiotic resistance is to limit inappropriate use and to improve hygiene within the hospital setting. The latter efforts are intended to limit nosocomial infections – secondary infections, which are often resistant to antibiotic treatment, that develop during hospital visits (hospitals are viewed as hotspots of antibiotic resistance). The effort to reduce inappropriate use has been much more challenging (and sadly, less effective), but includes initiatives to: (1) reduce inappropriate antibiotic prescriptions (i.e., viral infections, like the common cold, are unaffected by antibiotics), (2) eliminate antibiotic use in agriculture for growth promotion and prophylaxis (this practice continues in the USA; it has been banned in the European Union), and (3) reduce the superfluous use of antibacterial use in soaps and other personal care products (antibacterials in most of these cases are redundant and unnecessary; this practice also continues). While each of these initiatives by the medical community is an excellent idea, they are difficult to implement and they are likely to be insufficient to indefinitely extend the antibiotic era. New and complementary initiatives are therefore needed to help resolve this critically important problem.

Over the past decade or so, a new paradigm has developed with respect to the evolution and ecology of antibiotic resistance. The foundation of this theory is that antibiotic resistant bacteria are common in the environment but that pathogenic bacteria, which live inside the human body, are typically antibiotic-sensitive (D’Costa *et al.* 2007). The proliferation of antibiotic resistant bacteria, therefore, stems from the genetic exchange that inevitably occurs when these two types of organisms are intermixed as well as the selective pressure imposed by the heavy antibiotic use that has occurred since World War II. The key feature of this new paradigm – dubbed the antibiotic resistome (D’Costa *et al.* 2007) – is that environmental bacteria are critically important, as they are the most prominent source of the genes that are observed among medically-relevant pathogens (Allen *et al.* 2010).

Under the umbrella of this “antibiotic resistome paradigm”, this research project tests the theory that municipal wastewater and its treatment are critically important in the proliferation of antibiotic resistance. Municipal wastewater (a.k.a., sewage) contains the fecal material of a substantial fraction of the population, which has been long been known to contain substantial quantities of antibiotic resistant bacteria. In contrast, wastewater treatment improves the water quality of the sewage such that it can be released to the environment without detrimental impact. It is critical to note that an explicit goal of municipal wastewater treatment is to merely limit direct exposure to pathogens such that people accidentally ingesting surface waters do not become fatally sick. Treated municipal wastewater still contains substantial quantities of antibiotic resistant bacteria and antibiotic resistant genes, which are then released to the

environment where they can intermix with environmental organisms and potentially further exchange resistance genes to the detriment of public health.

**The goal of the research described herein is to unequivocally identify human sewage as a statistically significant source of antibiotic resistance and antibiotic resistance genes in the environment.** This goal will be achieved by determining the quantities of several antibiotic resistance genes in the wastewater treatment lagoon and four lakes within Itasca State Park. Itasca State Park provides an ideal opportunity for this research because produces a substantial quantity of domestic sewage (i.e., there are no industrial or agricultural inputs). Itasca State Park also has numerous lakes, with different levels of human use, which can serve as experimental controls (likely negative) for surface waters without an input of sewage.

## Methods

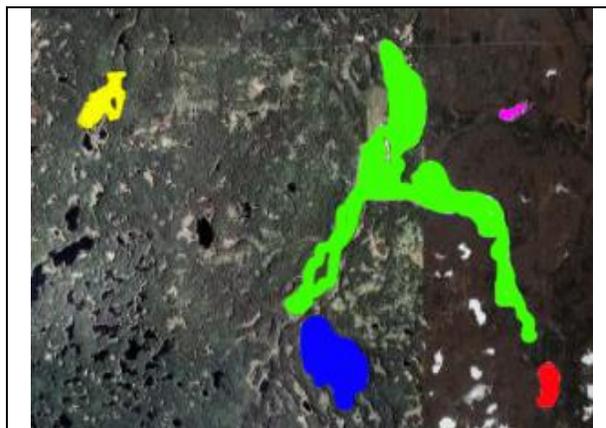
### Sample Collection.

Surface water samples (sample volume = 250 mL) are being collected from the wastewater treatment lagoon, Lake Itasca, Mary Lake, Elk Lake, and Lake Ozawindib within Itasca State Park (Fig. 1). These surface water samples are manually collected from one location within each lake (or wastewater lagoon) at a distance of 0.5 m below the water surface using sterile polystyrene bottles. As soon as possible after collection (less than 6 hours), surface water samples are passed through a 47 mm-diameter nitrocellulose filter (pore size = 0.22  $\mu\text{m}$ ) to concentrate microbial biomass. Filters are then immersed in 0.5 mL of lysis buffer (120 mM phosphate buffer, pH = 8.0, 5% sodium dodecyl sulfate) to preserve the sample until genomic DNA can be extracted and purified.

Similarly, triplicate sediment samples will be collected from each lake (or wastewater lagoon) using a gravity-corer (HTH Teknik; Luleå, Sweden) during one of the sample collecting trips (probably in June or July 2013). Sediment samples will be sliced into approximately 2.5 cm sub-sections to a depth of about 15 cm (i.e., about 6 sub-samples per sediment core).

Additional samples are being collected from numerous other locations to help test the hypothesis that manure and fecal material are pertinent sources of ARGs. These samples consist of numerous untreated municipal wastewaters (to date, we have collected samples from Marshfield, WI, Rochester, MN, Baxter, MN, and Brainerd, MN) as well as animal manure from various farming operations (some of these animals are grown without non-veterinary use of antibiotics, other animals are grown with substantial subtherapeutic antibiotic use).

All samples are stored on ice while they are transported to the University of Minnesota (within 1 day), after which they are stored at  $-20^{\circ}\text{C}$ .



**Fig.1.** Map showing the relative locations of the wastewater treatment lagoon (magenta), Lake Itasca (green), Lake Ozawindib (yellow), Elk Lake (blue), and Mary Lake (red) within Itasca State Park.

### Genomic DNA extraction.

Water samples (preserved in lysis buffer) undergo three consecutive freeze-thaw cycles and an incubation of 90 minutes at 70°C to lyse cells. Genomic DNA is then extracted and purified from these samples using the FastDNA Spin Kit (MP Biomedicals; Solon, OH) according to manufacturer's instructions. Genomic DNA is also extracted from sediment samples and manure samples (~ 500 mg of wet weight per sample) using a bead beater to lyse cells. All genomic DNA extractions are performed in triplicate and stored at -20°C until needed.

### Quantitative PCR

Quantitative real-time PCR (qPCR) is used to quantify 16S rRNA genes (a measure of total bacterial biomass) as well as three genes encoding tetracycline resistance (*tet(A)*, *tet(W)* and *tet(X)*) and the integrase gene of class 1 integrons (*intI1*) as described previously (Diehl and LaPara, 2010). These genes will be targeted in this study because these genes encode proteins that confer tetracycline resistance via each of the three known mechanisms of resistance. Furthermore, our prior work has demonstrated that *tet(A)* and *tet(X)* are detectable when there is significant influence of wastewater; in contrast, *tet(W)* was detectable in all of our previous surface water samples. qPCR is also used to quantify the 16S rRNA genes of all members of the domain *Bacteria* as well as total and human-specific *Bacteroides* spp. as described previously (LaPara *et al.*, 2011).

The qPCR analysis is conducted using an Eppendorf Mastercycler ep *realplex* thermal cycler (Eppendorf; Westbury, NY). Each qPCR run consists of an initial denaturation for 10 min at 95°C, followed by forty cycles of denaturation at 95°C for 15 s, and anneal and extension at 60°C (most targets) or at 56°C (human-specific *Bacteroides*) for 1 min. A typical 25 µL reaction mixture contains 12.5 µL of iTaq SYBR Green Supermix with ROX (Bio-Rad; Hercules, Calif.), 25 µg bovine serum albumin (Roche Applied Science; Indianapolis, Ind.), optimized quantities of forward and reverse primers, and a specified volume of template DNA (usually 0.5 µL). The precise volume and concentration of template DNA is empirically optimized for each sample to generate the lowest detection limit while minimizing inhibition of PCR.

The quantity of target DNA in unknown samples is calculated based on a standard curve generated using known quantities of template DNA. Standards for qPCR have already been prepared by PCR amplification of genes from positive controls, followed by ligation into pGEM-T Easy (Promega; Madison, Wisc.) as described previously (Diehl and LaPara, 2010). Ten-fold serial dilutions of plasmid DNA are prepared and run on the thermal cycler to generate standard curves ( $r^2 > 0.99$ ). Following qPCR, melting curves are generated and analyzed to verify that non-specific amplification does not occur.

**Table 1.** Description of target genes and PCR primers that are targeted by quantitative real-time PCR in this study. Detection limits are based on prior work and are expected to be similar in the this study.

Gene Target	PCR primer sequence (5'→3')	Detection limit (genes/mL water)	Detection limit (gene/ g sediment)
<i>tet(A)</i>	GCT ACA TCC TGC TTG CCT TC CAT AGA TCG CCG TGA AGA GG	$1.2 \times 10^1$	$6.2 \times 10^4$
<i>tet(W)</i>	GAG AGC CTG CTA TAT GCC AGC GGG CGT ATC CAC AAT GTT AAC	$2.0 \times 10^1$	$2.0 \times 10^4$
<i>tet(X)</i>	AGC CTT ACC AAT GGG TGT AAA TTC TTA CCT TGG ACA TCC CG	$2.6 \times 10^2$	$1.3 \times 10^4$
<i>intI1</i>	CCT CCC GCA CGA TGA TC TCC ACG CAT CGT CAG GC	$2.0 \times 10^2$	$6.0 \times 10^4$
All <i>Bacteroides</i>	AAC GCT AGC TAC AGG CTT CAA TCG GAG TTC TTC GTG	$1.2 \times 10^0$	$6.0 \times 10^2$
Human <i>Bacteroides</i>	ATC ATG AGT TCA CAT GTC CG CCA TCG GAG TTC TTC GTG	$1.2 \times 10^0$	$6.0 \times 10^2$
16S rRNA	CCT ACG GGA GGC AGC AG ATT ACC GCG GCT GCT GG	$3.0 \times 10^3$	$1.6 \times 10^5$

The quantity of target DNA in unknown samples is calculated based on a standard curve generated using known quantities of template DNA. Standards for qPCR have already been prepared by PCR amplification of genes from positive controls, followed by ligation into pGEM-T Easy (Promega; Madison, Wisc.) as described previously (Diehl and LaPara, 2010). Ten-fold serial dilutions of plasmid DNA were prepared and run on the thermal cycler to generate standard curves ( $r^2 > 0.99$ ). Following qPCR, melting curves will be generated and analyzed to verify that non-specific amplification does not occur.

### Data Analysis

Non-metric multidimensional scaling (nMDS) will be statistically compare the qPCR profiles from each of the lake and wastewater lagoon samples. Each sample will be scored with respect to the concentration of each of the genes tested.

One way analysis of variance (ANOVA) will also be performed to compare the concentrations between lakes for all gene targets. Tukey's Honestly Significant Difference (HSD) test will be conducted for each gene target to determine the difference in mean gene concentrations between each possible pair of surface water samples sites. Pearson correlation coefficients of gene concentrations will also be calculated for all possible pairs of gene targets. An F-test will be conducted to determine if results from a surface water sample exhibited gene concentrations that are significantly different from results at the other sample locations.

### **Progress to Date**

#### Sample Collection

Although this project was formally initiated on March 1, 2012, activity did not commence until late July 2012 because a research permit was needed from the Minnesota Department of Natural Resources to collect samples from within a State Park and because the graduate student working on the project did not matriculate onto the University of Minnesota campus until August 2012. Once this permit was obtained and the student arrived, numerous samples have been collected from Itasca State Park (August 2012; November 2012), from other

untreated municipal wastewaters, and from various agriculturally-related animal manures. We anticipate collecting samples from Itasca State Park on two more occasions (likely May 2013; June 2013) as well as other sample locations.

#### Genomic DNA Extraction and quantitative real-time PCR

Genomic DNA has been extracted and preserved from all samples collected to date. No samples have been analyzed by quantitative real-time PCR because this assay is a high-throughput technique in which 96-well plates are used. We are waiting, therefore, for a sufficient number of samples before we initiate qPCR.

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#### **2. Publications**

None to date

#### **3. Student Support**

Kyle Sandberg, Department of Civil Engineering, University of Minnesota (Ph. D. Student; Anticipated Graduation: May 2016). Kyle was awarded a fellowship from the Department of Civil Engineering for the 2012-2013 academic year; Kyle will be supported on this project beginning in Fall 2013.

#### **4. Presentations**

None to date

#### **5. Awards**

None to date

#### **6. Additional Funds**

None to date

# Predicting Erosional hotspots in North Shore streams from high-resolution spatial data

## Basic Information

<b>Title:</b>	Predicting Erosional hotspots in North Shore streams from high-resolution spatial data
<b>Project Number:</b>	2012MN320B
<b>Start Date:</b>	5/1/2012
<b>End Date:</b>	6/30/2013
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	MN 8
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Geomorphological Processes, Sediments, Surface Water
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Karen Bobbitt Gran

## Publications

1. Wick, M. J. and K. B. Gran, 2012. Identifying Riverine Erosional Hotspots Using Airborne Lidar. Abstract G23A-0897, 2012 Fall Meeting, American Geophysical Union, San Francisco, California, December 3-7, 2012.
2. Gran, K. and M. J. Wick, Duluth Flood of June 2012: Stream Visual Assessments, Living with Uncertainty: Duluth Streams in the Aftermath of the 2012 Floods. Minnesota Pollution Control Agency Offices, Duluth, Minnesota, October 31, 2012.

## **Predicting erosional hotspots in North Shore streams from high-resolution datasets**

### **Principal Investigator**

**Karen Gran, Assistant Professor, Department of Geological Services**

**Funding Sources: USGS-WRRI 104B/CAIWQ Grants Program**

**Reporting Period: 3/1/2012-2/28/2013**

### *1) Research:*

#### Introduction

This research focuses on predicting erosional hotspots from remote data along the North Shore of Lake Superior in Minnesota. Many of these streams are listed as impaired for turbidity according to section 303d of the U.S. Environmental Protection Agency's Clean Water Act. Although previous studies have hypothesized that land use is the central driver in water quality impairments in Lake Superior streams (Detenbeck et al., 2003, Detenbeck et al., 2004; Crouse, 2013), correlations between land use measures of sediment loading are poor (Crouse, 2013). Instead, we hypothesize that much of the fine sediment that contributes to turbidity comes from natural erosional hotspots. If erosional hotspots arise naturally due to local geomorphology and surficial geology, they should be predictable given high-resolution topography and soils data. This project focuses specifically on identifying near-channel erosional hotspots based on newly-available high-resolution remote datasets for streams along the North Shore of Lake Superior. These natural hotspots represent areas that would contribute a disproportionate volume of sediment to the channel under current conditions, and may be exacerbated by changes in land use or climate.

Both high-resolution topography and soils data were recently released for northeastern Minnesota. High-resolution lidar-derived DEMs (digital elevation models) are now available for the entire region at 3m-resolution from the Minnesota Geospatial Information Office. Lidar data were acquired May 3 - June 2, 2011, and tested to meet a vertical accuracy of 5.0 cm Root Mean Squared Error (RMSE). In addition, the high-resolution Soil Survey Geographic Database (SSURGO) dataset for St. Louis County was recently released by the Natural Resource Conservation Service (NRCS), and should be released in the near future for Lake and Cook Counties. Our original goal was to construct an erosional hotspot model using solely these two datasets as the data will be available throughout the entire North Shore. However, we found that additional information was required on the locations of bedrock outcrops. This is discussed below.

We constructed a model in ArcGIS for predicting hotspots using five main factors: stream power, bluff location, angle of impingement, soil erodibility, and bedrock exposure. We conducted these five analyses and tested our predictive model on three target watersheds: Amity Creek, the Talmadge River, and the French River (Figure 1). The lidar data have been prepped for ten additional North Shore watersheds including Lester, Sucker, Knife, Split Rock, Beaver, Baptism, Poplar, Cascade, Brule, and the Flute Reed, but the erosional model has not yet been run on these watersheds.

In order to validate our erosion potential predictions, we conducted field surveys over the summer of 2012. First, we conducted modified Bank Erosion Hazard Index (BEHI) surveys at sites in the Amity, Talmadge, and French watersheds. The BEHI surveys are a pre-established protocol for assessing erosion potential, giving a rating of very low to extreme (Pfankuch, 1975). We also completed what we call Field Erosion Index surveys. The Duluth area experienced a 500-year flood event on June 19 – 20<sup>th</sup>, 2012. Areas in the region received 6 – 10 inches of rain within a 24 hour period (Huttner, 2012). Duluth streams are very flashy due to their bedrock channels, so water levels in Duluth streams rose very quickly and then fell very rapidly after the event. Stream gages were lost during the event, so peak

discharge rates in individual streams have not been calculated (J. Jasperson, pers. comm.). The flood resulted in substantial geomorphic changes to Duluth streams, and the historic flood event offered us the opportunity to collect post-storm data and essentially compare our predicted erosion hotspots to where erosion actually occurred. We completed Field Erosion Index surveys (FEI) in which we walked the lower portions of Amity Creek and the Talmadge River in order to locate areas where extensive erosion occurred in order to assess the validity of our predictive model.

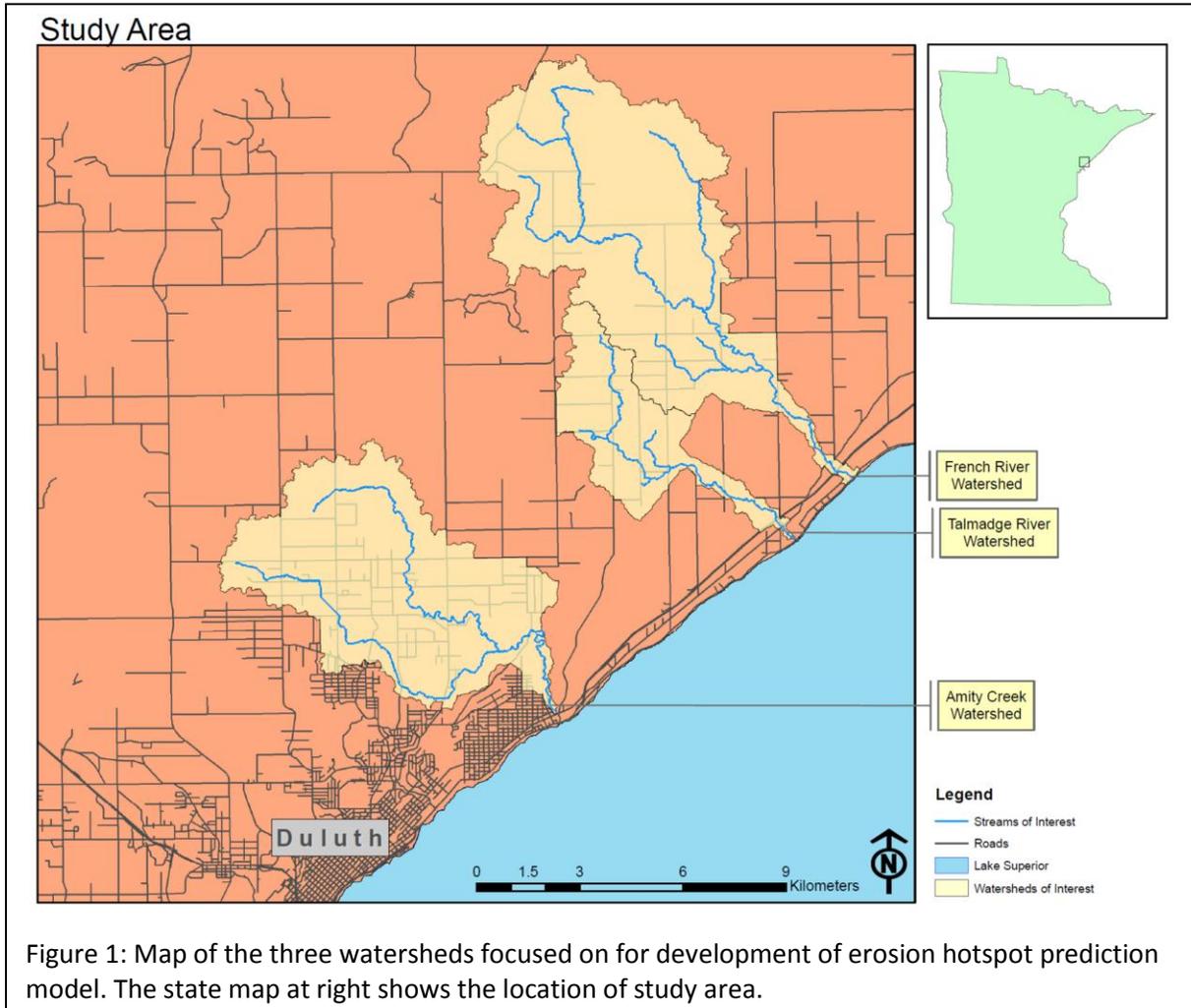
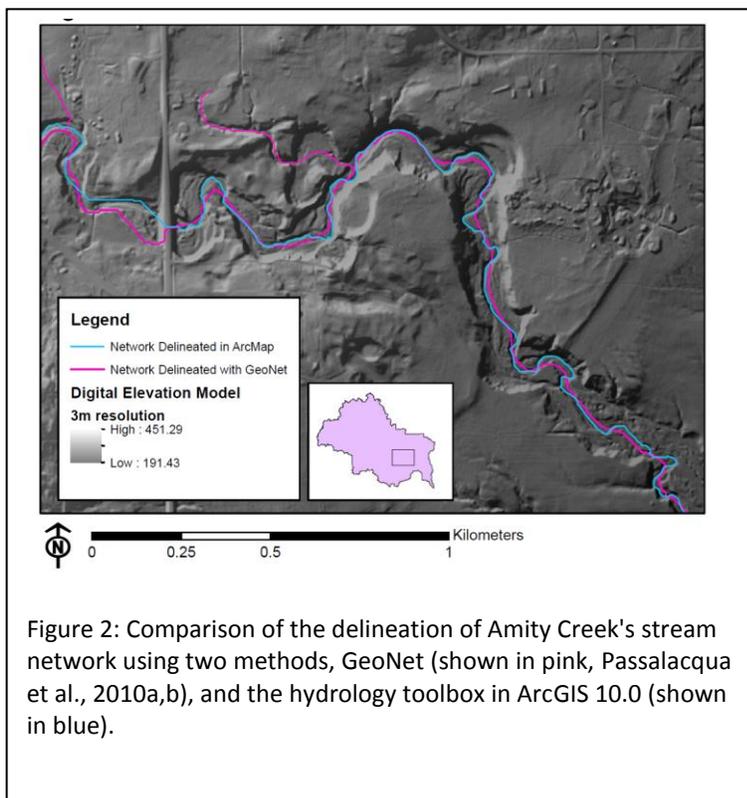


Figure 1: Map of the three watersheds focused on for development of erosion hotspot prediction model. The state map at right shows the location of study area.

#### Defining stream networks and delineating watersheds from lidar data

We delineated stream networks based on the lidar-derived DEMs. We used two methods, a program called GeoNet (Passalacqua et al., 2010a,b), and the Hydrology toolbox in ArcGIS to delineate Amity Creek. The benefit of GeoNet is that it is designed to deal with “data dams” that arise when trying to delineate channel networks with very high-resolution topographic data. Essentially, the data resolution is so high that road crossings become topographic barriers to flow. Geonet is an automated routine that can delineate channels across these topographic barriers. ArcGIS routines can also be used, but they require manual removal of bridges and other blockages that become topographic barriers to flow in a sometimes time-consuming iterative process.

Errors in delineated networks were identified by comparing the networks using both techniques to DEM and hillshade layers and to high-resolution air photos. Both networks were significantly more



accurate than the existing Minnesota Department of Natural Resources stream files for most of the stream length, but both networks contained errors in the very flat upper reaches of the stream networks where topographic variability is low. Figure 2 shows an area along the East Branch of Amity Creek that illustrates the difference between the two networks. The ArcGIS network follows meanders very closely while the GeoNet network cuts off meanders. We found the ArcGIS Hydrology toolbox to be more user-friendly. GeoNet required a significant amount of computing power and time to run. Therefore we delineated all further networks using the ArcGIS hydrology toolbox, using an accumulation threshold of 10,000 m<sup>3</sup> to define the limits of network delineation. Errors in the network were corrected only if essential for the identification of erosion hotspots. For

example, if errors were located upper reaches and wetlands where erosion potential is known to be low or where the stream is intermittent, they were disregarded. Hydrologic processing has been completed on fourteen North Shore streams (Amity, Talmadge, French, Lester, Sucker, Knife, Split Rock, Beaver, Baptism, Poplar, Cascade, Cross, Brule, and Flute Reed) and will be made available to the public through the Lake Superior Streams website ([www.lakesuperiorstreams.org](http://www.lakesuperiorstreams.org)).

### GIS Analysis Methods

We used ArcGIS to analyze five potential predictor variables as described below: stream power, bluff proximity, angle of impingement, soil erodibility, and bedrock exposure. We created an addressing system with 25m reaches along which the predictor values were calculated. The erosion model we generated from these predictor variables was developed initially using data from Amity Creek, where we have the most dense field dataset for validation. We are in the process of testing it on the Talmadge and French Rivers. We have not yet applied the erosion model to other North Shore streams.

Erosion potential in bedrock streams is a function of stream power. We used a stream power-based erosion index to predict the fluvial erosion potential along mainstem streams (e.g. Whipple and Tucker, 1999). Unit stream power ( $\omega$ ) is a function of the specific weight of water (density times gravity, or ( $\rho \cdot g$ )), slope ( $S$ ) and unit discharge (total discharge divided by channel width, or ( $Q/w$ )):

$$\omega \propto \rho g (Q/w) S \quad (1)$$

However, channel width varies as a function of discharge,  $w = c_1 Q^b$ , and discharge varies as a function of area ( $A$ ),  $Q = c_2 A$ , so we can rearrange equation 1 to form a stream power-based erosion index (SP) in terms of upstream drainage area and slope:

$$SP = kA^{(1-b)}S \quad (2)$$

where  $k$  is a coefficient accounting for the specific weight of water and the coefficients above ( $c_1$  and  $c_2$ ), which incorporate the effects of varying bedrock and substrate erodibility. Although we have both till and bedrock in these channels, we assign  $k$  a constant value here, and account for differences in erodibility separately using the SSURGO dataset and bedrock exposure mapping. The parameter  $b$ , the exponent in the width-discharge relation, was assigned a value of 0.5. Width-discharge relationships in North Shore streams are poor, but Leopold & Maddock (1953) found that 0.5 was appropriate in alluvial channels, and Montgomery & Gran (2001) found values of 0.3 – 0.5 are appropriate for bedrock channels. To calculate the stream power-based erosion index using ArcGIS, we extracted elevation data at points every 25 meters along the main stem channel and used them to calculate the slope at each point over a 100 m reach (50 meters upstream to 50 meters downstream). The upstream area at each point was extracted from the flow accumulation raster created using the Hydrology toolbox in ArcGIS.

Bluffs were delineated using topographic data to identify high bluffs along streams. Bluffs represent potential point sources of sediment, and locations where channels interact with bluffs can be erosional hotspots, particularly if those bluffs are composed of till or glaciolacustrine sediments rather than bedrock. We delineated bluffs using the focal statistics tool in ArcGIS to identify areas with relief > 2m over a 12 m by 12 m window. We also tracked areas with relief > 4 m to potentially separate out valley walls from in-valley terraces. Only bluffs adjacent to the stream were used in the erosion prediction model. Bluffs were defined as adjacent if they intersected a 14 m buffer established around the channel centerline. Most channels in Amity Creek are < 7m wide, so this analysis selects all bluffs a full channel width away from the stream on either side.

Secondary flows in rivers often drive erosion along the outside bend, with tighter bends resulting in higher shear stresses. To capture the effects of bend geometry on potential erosion, we calculated the angle of impingement for the channel centerline every 5 m. We used the Planform Statistics Toolbox (Lauer, 2006) to calculate a value for the angle of impingement every 5 m along the channel centerline. The angle of impingement here is defined as the difference between the stream direction vectors in two adjacent points along the stream centerline (5 m apart). Thus, a bend that is changing rapidly will have a higher angle of impingement than a more gradual bend.

To determine the role of substrate erodibility on erosion, we used two different approaches. The first measured soil erodibility using a “K factor”, which is the erodibility factor from the Revised Universal Soil Loss Equation. The K factor incorporates characteristics such as texture, structure, organic matter, and permeability of the soil and rates the soil based on the susceptibility of soil particles to be removed and transported away by water (Renard et al., 1991). We extracted K factor values at the prediction points every 25 m along stream networks from the SSURGO dataset, using the dominant K value for all soil horizons.

We quickly realized that there is little variability in K factors in our study area, and what is most important is the presence or absence of bedrock in the channel. Unfortunately, the SSURGO dataset does not include this information. We thus defined an additional layer that identified bedrock outcrop locations. This proved to be a challenging layer to create solely from remote data. One method we are working on uses the Feature Analyst program distributed by Overwatch Systems, LTD, to extract bedrock outcrop from air photos and lidar data. Feature Analyst is an extension for ArcGIS that allows the user to create “training polygons” which the tool then uses to identify similar polygons based on the input datasets. Input datasets included 4-band air photos (0.3m resolution, obtained from the USGS); lidar first returns (vegetation height), last returns (bare earth), and intensity (all 1m resolution) (all calculated from the lidar point cloud data, obtained from the Minnesota Geospatial Information Office); and the

Normalized Difference Vegetation Index (NDVI, used to visualize green vegetation, calculated as  $(\text{Band 4} - \text{Band 3}) / (\text{Band 4} + \text{Band 3})$  from the air photos). After the Feature Analyst identifies similar polygons to the training polygons, the user then inputs correctly and incorrectly identified polygons and reiterates the program, until a satisfactory map is produced.

We used a corridor of 300 meters wide to be sure to include the valley walls, and ran the program only on Amity Creek below Jean Duluth Road, as we know that bedrock outcrop interaction with the creek is very limited along the creek upstream of Jean Duluth Road. Typically, features are mapped in Feature Analyst solely based on training polygons defined by the user and based solely on visual inspection of remote data. However, because of the limits of our datasets, we used records of outcrop exposure from our field data as well as outcrop maps from the Minnesota Geological Survey to verify outcrop locations for our training polygons.

### Field Surveys

Field work was completed during the summer of 2012. We completed Field Erosion Index (FEI) surveys and modified Bank Erosion Hazard Index (BEHI) surveys in order to validate our erosion potential predictions. Our initial plan was to spread surveys out across different North Shore streams. Instead, we decided to focus on a more dense data set in only a few streams. Field Erosion Index surveys were conducted on Amity Creek and the Talmadge River on a range of different channel types on approximately the lower third of the main stem channels in each watershed. BEHI surveys were along Amity, Talmadge and French River main stems throughout the stream network.

BEHI surveys utilized a pre-established protocol for assessing erosion potential, giving a rating of very low to extreme bank erosion hazard for each bank (Pfankuch, 1975). The BEHI survey is based on field observations of the near-channel zone, including bank height, material, angle, channel area, and signs of erosion. We used a modified BEHI survey, adding a component to account for stream interaction with till valley walls. We completed 28 sites on Amity's main stem, 10 sites on Talmadge's main stem, and 12 sites on French's main stem.

In the middle of the field season, Duluth experienced a very large flood event. We took advantage of this opportunity to not just predict erosion, but instead to actually measure it. The FEI surveys focused on documenting the erosion that occurred during the June 2012 flood. We assumed that the degree of erosion that occurred during this flood should be proportional to the erosion potential along the streams during a typical annual flood. A rating system was created based on field observations, from 1 (no erosion) to 7 (complete scour on both banks). A value of 0 denoted bedrock exposure and indicates erosion potential is very low. We used this rating system to create a running assessment of field erosion potential based on locations that were highly eroded compared to areas that were not eroded in the June flood on Amity Creek and the Talmadge River.

### Preliminary Results: Erosion Potential Predictions

We predicted erosion potential based on five predictor variables: stream power-based erosion index, bluff proximity, angle of impingement, soils, and bedrock exposure, for Amity Creek, the Talmadge River, and the French River. The results of our erosion predictors for a portion of Amity Creek are shown in Figure 3. We then compared the results of our GIS predictors to our sets of field data from Amity Creek and the Talmadge River. Here we focus on our FEI data because we have significantly more observations in that dataset (Figure 4). The last step of the analysis involves combining predictor variables to develop an erosion hotspot index. This work is still on-going.

Stream power is lowest in the upper reaches of the stream network where drainage area is small and slopes are very low, with a rapid increase towards the outlet as both slope and drainage area increase. Because the erosion index assumes a constant erodibility, the stream power-based index varies only with upstream area and slope. For both streams, the correlations with stream power are

very poor because we did not account for substrate variability. Bedrock exposure is restricted to areas near the outlet in these watersheds. These areas typically have high stream power values (high drainage area and steep slope) but low erodibility due to the presence of bedrock. Erosion predictability should improve when combined with soil erodibility data and information on bedrock outcrop locations.

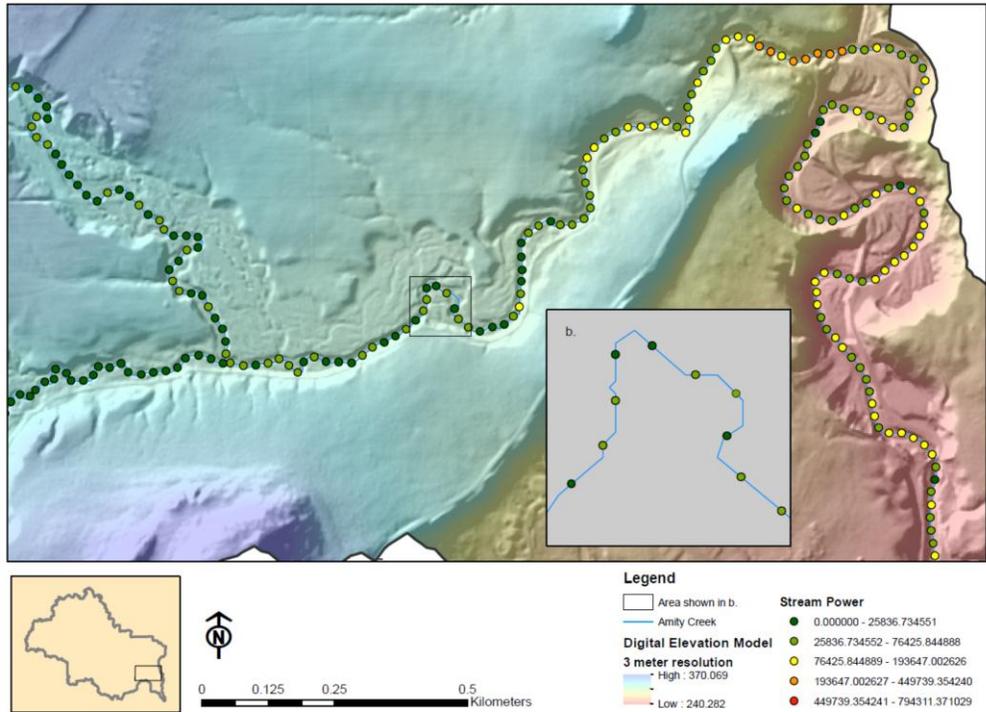
Soil erodibility was extracted from the SSURGO soils dataset. Despite the vast improvement in resolution over the STATSGO (State Soil Geographic) database, soil K factor values along the stream network varied minimally in all three watersheds. Bedrock exposure was a much more useful parameter for determining erosion potential than mapped soil K factors. Bedrock exposure for a 300m corridor along the channel, from Jean Duluth to the outlet, was mapped using feature extraction methods for Amity Creek. Along Amity Creek, most bedrock outcrops are located along Seven Bridges Road, especially in the vicinity of the uppermost three bridges, and near the first bridge (area shown in Figures 3 and 4 and downstream). This method resulted in identification of the large obvious outcrops which were visually confirmed on the air photos, but also small polygons (~1 to 10m<sup>2</sup>) along the creek that may be erroneous identification of bedrock. The bedrock exposure maps derived using Feature Analyst were more accurate than the Minnesota Geological Survey maps (Hobbs, 2002; Hobbs, 2009), which contain very large, generalized polygons. Unfortunately, the feature extraction method relied upon high-resolution air photos which are not available throughout the entire North Shore. We also used field notes on the locations of bedrock outcrops to help “train” the polygons prior to automating the procedure, so the results were not completely derived from remote datasets alone.

The delineation of steep bluffs adjacent to the stream is a very simple calculation that yielded the most promising results when compared to field surveys. We saw positive correlations of percent of points near bluffs with FEI surveys. On the Talmadge,  $r^2$  values were 0.2 and 0.25 for 2m and 4m bluffs, respectively. On Amity,  $r^2$  values were 0.9 and 0.7 for 2m and 4m bluffs, respectively. Bluff delineation may be used as a starting place to identify areas that may be actively eroding. The major limitation of this analysis is the presence of different substrate materials. If a bluff that was delineated is made of bedrock, the erosion potential is likely very low, while if the delineated bluff consists of glacial till, then erosion potential may be quite high. Therefore, this analysis is most useful with either prior knowledge of the watershed or bedrock outcrop maps.

The angle of impingement is calculated along the stream network, so by nature it is dependent on accurate network delineation. It is also highly dependent on using an applicable “ruler”, or distance along which the value is calculated. We used a ruler of 5m, which captured most sharp turns, but may have been too short of a distance for large-amplitude bends along Amity Creek. Possible values for the angle of impingement range in radians from 0 to 6.28 (straight to curved), with the highest observed values for each creek equal to 1.57 rad along Amity and French Creeks, and 1.18 rad along Talmadge Creek. For Amity Creek, there is a positive correlation between angle of impingement and FEI, with an  $r^2$  value of 0.8. However, in Talmadge Creek, we do not see a positive correlation, and we see a large spread in the data at moderate FEI values. This is likely due to the limited number of data points on Talmadge (137 points) compared to Amity (341 points).

Overall, the most useful predictor variables were bluff proximity and angle of impingement, combined with locations of bedrock outcrops. Stream power in the absence of information on bedrock outcrop locations was not useful, and soil K factor data were simply too low of a resolution with too little variability to be useful.

A. Stream Power



B. Bluffs

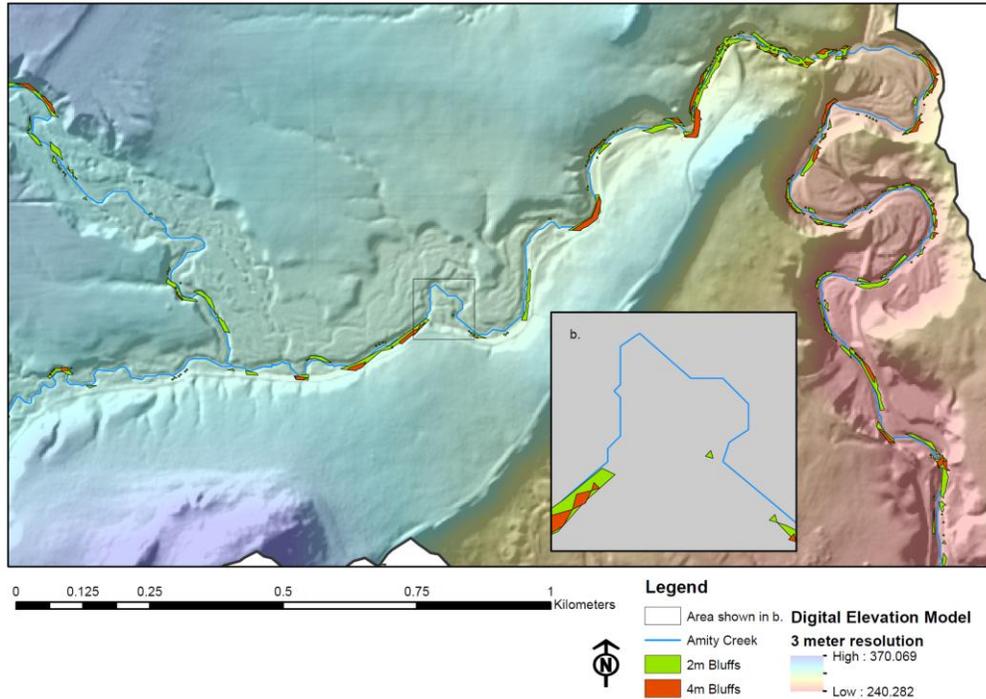
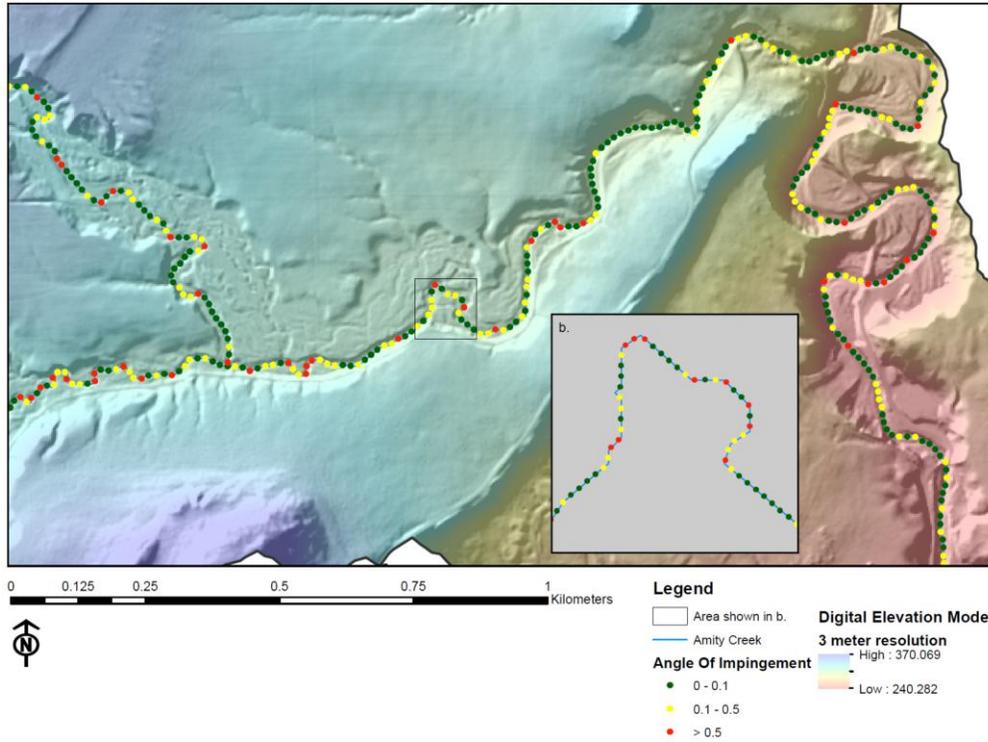


Figure 3: Results from erosion predictor analyses. The portion of the watershed shown in all tiles is outlined in the watershed map in A. Tile A shows stream power. Tile B shows delineated bluffs, with >2 meter bluffs in green and >4 meter bluffs in orange.

C. Angle of Impingement



D. Bedrock Exposure

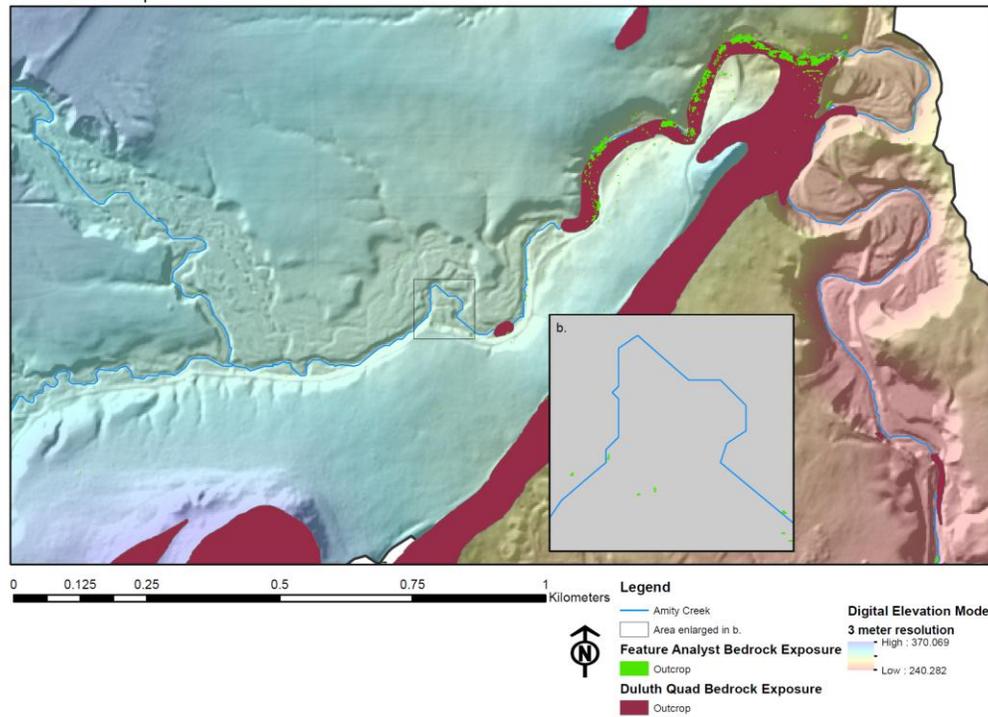
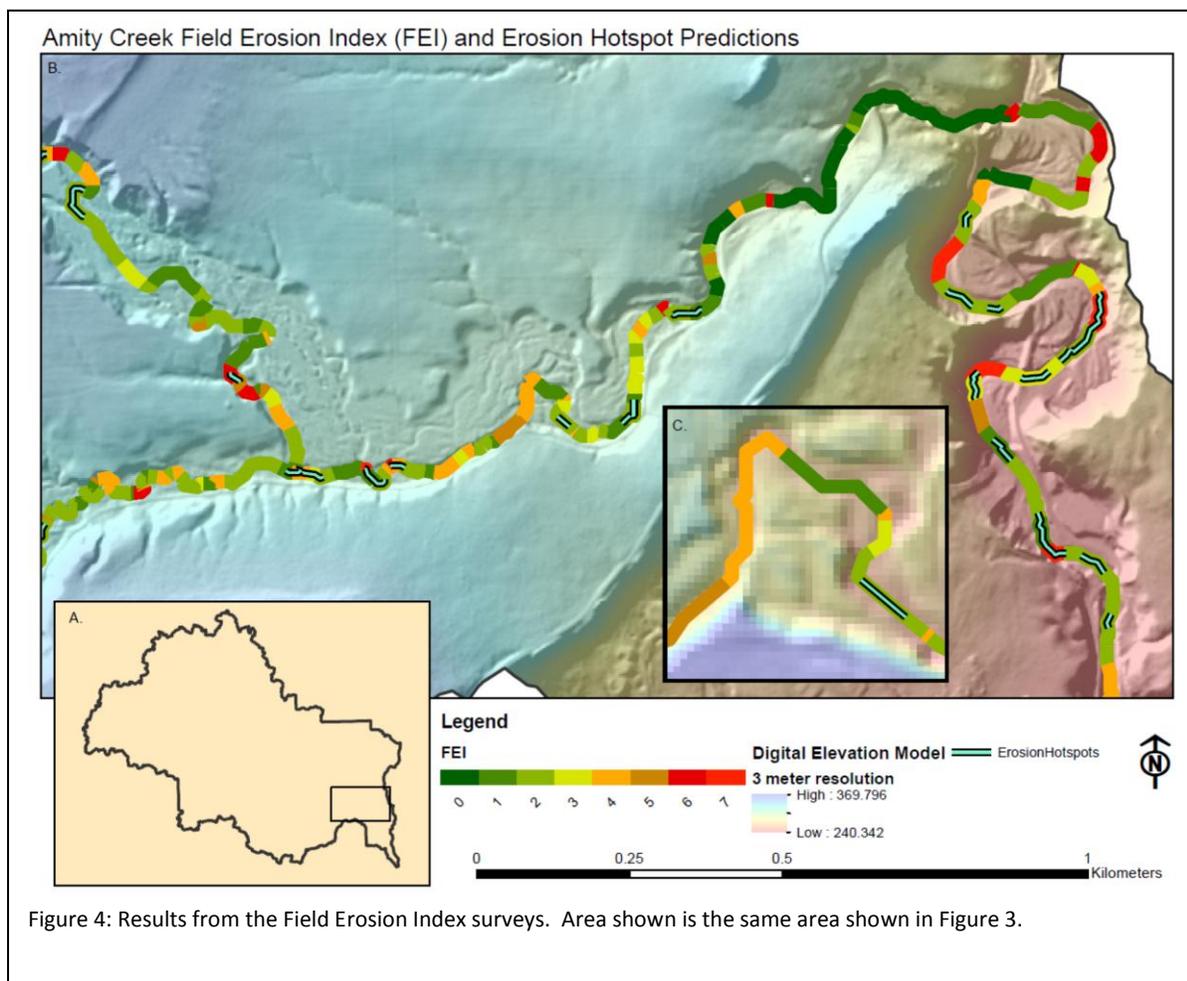


Figure 3 ctd: Results from erosion predictor analyses. The portion of the watershed shown in all tiles is outlined in the watershed map in tile A. Tile C shows angle of impingement. Tile D shows bedrock exposure, with the Feature Analyst map shown in green (mapped only in 300-meter corridor along channel), and the MGS bedrock exposure maps (for entire watershed) shown in purple.



### Preliminary Conclusions and Project Status

We have completed hydrologic conditioning and stream network delineations on the following streams: Amity, Talmadge, French, Lester, Sucker, Knife, Split Rock, Beaver, Baptism, Poplar, Cascade, Cross, Brule, and Flute Reed. These stream network delineations will be available to the public through the Lake Superior Streams website by early summer 2013.

We have determined three useful erosion potential predictors. We are in the process of combining them in order to identify erosion hotspots on Amity Creek using different models. Once we establish the most effective combination of predictors to identify erosion hotspots on Amity Creek, we will test our model on the Talmadge and French Rivers. After these steps are complete, we will have maps of erosion hotspots located along these three watersheds that will be made publicly available on the Lake Superior Streams website.

After the most successful model is established, we will finish completing the three main predictor analyses (stream power, bluff proximity, and angle of impingement) on the remaining watersheds along the North Shore. Because bedrock exposure maps are not available for the entire North Shore, and mapping them remotely is outside the scope of this project, we will be unable to produce final erosion hotspot predictions for all watersheds. The individual predictor layers will be made available on the Lake Superior Streams website, but not the final erosion hotspot maps because their utility is limited without prior knowledge of bedrock outcrop locations.

The lack of high-resolution bedrock exposure data is a major limitation of completing this analysis on other North Shore watersheds. We had hoped to use the SSURGO data to get information on erodibility at high-resolution. Unfortunately, SSURGO soil erodibility data are still not high enough resolution to help this project, and they lack data on bedrock exposure. For Amity Creek, we were able to use prior knowledge of the watershed along with air photos and lidar data to produce a bedrock exposure map, but this may be difficult in other North Shore watersheds due to a lack of data availability and computing power limitations.

Other limitations of this approach involve temporal and spatial scales of erosion. Erosion in a single event is dependent upon fine-scale features like vegetation, large woody debris, or even culverts or other infrastructure. Although in the long-term, erosion rates may be greater in areas with erodible substrates, tight bends, high cliffs, and high stream power, in a single event it is more difficult to predict the exact locations where erosion will occur. Thus, our comparisons between erosion in the June event and predicted erosional hotspots is challenged by our inability to use remote data to predict fine-scale variation of vegetation and large woody debris that may actually dictate erosion in a single event. These fine-scale variations may account for the poor regressions between our predictors and our field datasets. In addition, the 500-year event that our FEI dataset is based on may introduce additional uncertainty due to the magnitude of the event compared to a typical bankfull flood event. Erosion in a typical bankfull flood would be more limited spatially.

Even with these limitations, these analyses may be helpful as a screening tool to locate potential field sites or sites for management or protection. However, background knowledge of the watershed characteristics such as vegetation patterns, land use, and surficial geology will be very helpful in order to use this beneficially.

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#### 2) Publications:

Wick, M. J. and Gran, K.B., 2012. Identifying Riverine Erosional Hotspots Using Airborne Lidar. Abstract G23A-0897 presented at 2012 Fall Meeting, AGU, San Francisco, Calif., 3-7 Dec.

#### 3) Student Support:

This project provided summer RA support for one M.S. student, Molly Wick, during summer 2012. She will be defending her thesis in summer 2013.

This project also provided support for one undergraduate student, Ryan Peterson, during both the summer 2012 and fall semester 2012.

#### 4) Presentations:

We gave one presentation specifically on the erosion model and preliminary results:

Wick, M. J. and Gran, K.B. December 2012. Identifying Riverine Erosional Hotspots Using Airborne Lidar. Abstract G23A-0897, poster presented at 2012 Fall Meeting, AGU, San Francisco, Calif., 3-7 Dec.

Three additional presentations were given that focused more generally on erosion in Duluth-area streams:

Karen Gran and Molly Wick presented "Duluth Flood of June 2012: Stream Visual Assessments" at Living with Uncertainty: Duluth Streams in the Aftermath of the 2012 Floods, Minnesota Pollution Control Agency Offices, Duluth, MN. Wednesday, October 31, 2012.

Molly Wick presented "Unique Aspects of how North Shore Streams Flow and Respond to Storms." at Lake Superior Watershed Ditch and Culvert Design Workshop, EPA Mid Continent Ecology Division, Duluth, MN, March 6, 2013.

Faith Fitzpatrick gave a talk in which Molly Wick and Karen Gran assisted with slides and were co-authors: Fitzpatrick, F. A., Gran, K. B., Wick, M. J., and Czuba C. R., Influence of Drainage-Network Position and Geologic Setting on Channel Responses to Floods for Duluth-Area Streams. St. Louis River Estuary Summit, Superior, Wisconsin, February 2013.

*5) Awards:*

None

*6) Related Funding:*

K. Gran (PI) received an internal grant from the University of Minnesota's Center for Urban and Regional Affairs (CURA)'s Faculty Interactive Research Program for a project entitled "Identifying and mitigating impacts from expanding urbanization to Duluth-area streams" (\$37,220). This project also involves hydrologic conditioning and analyses of lidar data in Duluth-area streams, using many techniques developed as a result of WRRF funds. Project period: 3/13-6/14.

# Improving treatment: Understanding the effect of organic carbon on the biodegradation of two endocrine disrupting compounds

## Basic Information

<b>Title:</b>	Improving treatment: Understanding the effect of organic carbon on the biodegradation of two endocrine disrupting compounds
<b>Project Number:</b>	2012MN322B
<b>Start Date:</b>	3/1/2012
<b>End Date:</b>	2/28/2014
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	5th
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Wastewater, Treatment
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Paige J Novak

## Publications

1. Tan, D.T., W. A. Arnold, and P. J. Novak, 2012. Impact of Organic Carbon on the Biodegradation of Estrone in Mixed Culture Systems. Submitted to Water Research, under review.
2. Tan, D.T., W.A. Arnold, and P.J. Novak, 2012. The Impact of Organic Carbon on the Biodegradation of Steroid Estrogens: Competition and Community. Poster presented at the Minnesota Conference on the Environment.
3. Tan, D.T., W.A. Arnold, and P.J. Novak, 2012. The Impact of Organic Carbon on the Biodegradation of Steroid Estrogens: Competition and Community. Poster presented at the Gordon Research Seminar, Environmental Sciences: Water, June 24-29, 2012, Holderness, New Hampshire.

**Annual Program Report for “Improving treatment: Understanding the Effect of Organic Carbon of Two Endocrine Disrupting Compounds”**

1. Research Synopsis

Report attached below

2. Publications

“Impact of Organic Carbon on the Biodegradation of Estrone in Mixed Culture Systems,” David T. Tan, William A. Arnold, and Paige J. Novak. Submitted to *Water Research*, under review.

3. Student Support

None

4. Presentations

Tan, D.T., Arnold, W.A., Novak, P.J., 2012. “The impact of organic carbon on the biodegradation of steroid estrogens: competition and community.” Poster presented at the Minnesota Conference on the Environment.

Tan, D.T., Arnold, W.A., Novak, P.J., 2012. “The impact of organic carbon on the biodegradation of steroid estrogens: competition and community.” Poster presented at the Gordon Research Seminar, Environmental Sciences: Water.

5. Awards

None

6. Related Funding

None

## Research Synopsis

The impact of organic carbon concentrations and loads on the biodegradation of estrone (E1) by a mixed consortium of bacteria was investigated in laboratory scale batch and membrane-coupled bioreactor systems. E1 is an endocrine disruptor and has negative impacts on aquatic life when discharged to surface water via treated wastewater effluent. The overall goal of this work is to find ways in which existing biological treatment systems can be optimized and altered to enhance the removal of E1. The work has resulted in a manuscript submitted to *Water Research*, attached as Appendix A (paper) and Appendix B (supplemental information).

In summary, key findings from this project are: (1) substrate competition does not inhibit E1 biodegradation in mixed microbial systems containing a variety of organic carbon sources (i.e. wastewater-like systems), (2) starvation of microbial biomass in batch systems improves E1 biodegradation ability over short time periods, and (3) low organic loads to continuous-flow systems may inhibit E1 biodegradation. Results from this research are consistent with E1 degradation by multiple substrate-utilizing microorganisms rather than specialized E1 degraders. These results are presented and discussed in detail in the submitted manuscript, which is therefore attached as part of this report (Appendices A and B).

The absence of substrate competition during E1 biodegradation suggests that wastewater treatment plants do not need to achieve a high removal of effluent BOD to facilitate the removal of E1. The positive impact of starvation on E1 biodegradation indicates that opportunities exist, and should be explored, to enhance E1 degradation. For example, detaining solids rather than immediately recycling them to activated sludge systems may improve E1 degradation over time. Current research is being conducted to further explore the effect of solids detention on the

development and enrichment of E1 degradation capacity and to establish if longer-term starvation continues to have positive impacts on the enrichment of E1 degradation ability.

## **Appendix A: Impact of Organic Carbon on the Biodegradation of Estrone in Mixed Culture Systems, Submitted to Water Research**

David T. Tan, William A. Arnold, and Paige J. Novak.

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### **Abstract**

The effect of organic carbon concentrations on the degradation of estrone (E1) was examined under various conditions in batch and membrane-coupled bioreactors (MBRs). Organic compounds did not inhibit E1 degradation via substrate competition, but conditions during which there was prolonged (28 hr) incubation in the absence of organic carbon (“starvation”), resulted in larger increases in E1-degrading capability compared to conditions during which organic compounds were fed every 12 hrs (“feast-famine”). Low concentrations of organic compounds in the influent, however, appeared to inhibit E1 degradation in MBRs. Taken together, these results suggest the importance of multiple substrate utilizers in E1 degradation. They also suggest that while the initial growth of biomass depends on the presence of sufficient organic carbon, further enrichment of these organisms under starvation conditions may improve E1 degradation capability via the enrichment and/or stimulation of multiple substrate utilizers.

### **Keywords**

Estrone; Estrogen; Wastewater; Wastewater strength; Bulk organic carbon; Multiple substrate utilization; Biodegradation

### **1. Introduction**

The presence of endocrine disrupting compounds (EDCs) in municipal wastewater is detrimental to aquatic life downstream of discharge points (Tetreault et al., 2012; Jobling et al.,

2002). Wastewater-derived EDCs are present in surface waters throughout the US (Kolpin et al., 2002) and cause a wide range of effects, including inhibition of predator avoidance behavior (McGee et al., 2009), alteration of nest guarding behavior (Lavelle and Sorenson 2011), and production of vitellogenin and female gonadal tissue in male fish (Balch et al. 1998; Routeledge et al. 1998). These effects lead to reproductive disruption that can cause population collapses (Kidd et al., 2007). Of the EDCs discharged in municipal wastewater, estrone (E1) is of particular importance because it is thought to be the largest contributor of estrogenic activity in treated effluent (Salstee et al., 2007; Onda et al., 2003).

While activated sludge systems can effectively remove steroid estrogens, removal of E1 and 17 $\beta$ -estradiol (E2) from wastewater varies widely across treatment plants and within individual treatment plants over time (Baronti et al., 2000). A survey of the removal of general estrogenicity (E2-equivalent) also showed wide variability between treatment facilities (Holbrook et al., 2002). It is known that critical solid retention times (SRTs) must be met for the degradation of various EDCs in municipal wastewater treatment and that increased sludge age correlates with enhanced removal for EDCs (Clara et al., 2005). It is also hypothesized that slower growing organisms that scavenge a variety of carbon sources are one reason for the importance of SRT in steroid estrogen removal (Koh et al., 2009). Systems with longer hydraulic residence times (HRT) appear to have better removal of estrogenic activity as well (Svenson et al., 2003). Nonetheless, these parameters are insufficient to account for the variability in the removal of E1, E2, and estrogenicity observed in the field (Holbrook et al., 2002; Baronti et al., 2000). Though multiple attempts have been made to establish correlations between steroid estrogen removal and operating or effluent water quality parameters in full-scale treatment plants, they have met with little success (Fernandez et al., 2008; Onda et al., 2003).

Many operational parameters in wastewater treatment, including SRT, HRT, and food to microorganism ratio, are related to the concentration of organic compounds present, which may in turn affect E1 removal in a number of ways. Estrogen degradation was poor or non-existent in low strength greywater representative of space waste streams, in spite of long HRT, infinite SRT, and individual estrogen concentrations ranging from tens to over a hundred  $\mu\text{g/L}$  (Kavanli et al., 2008). It is possible that the low concentration of organic compounds present was inherently detrimental to E1 removal or that low biomass concentrations resulting from substrate scarcity negatively impacted removal via multiple substrate utilization and/or co-metabolism.

Conversely, high food to microorganism ratios can result in substrate competition. This was suggested as an explanation for discrepancies in E1 degradation rate coefficients between laboratory batch experiments and a full-scale treatment system (Joss et al., 2004). Later studies, however, failed to find evidence for substrate competition (Koh et al., 2009). Lastly, wastewater strength could affect the structure and performance of the microbial community present (Luo et al., 2008; Docherty et al., 2006; Eiler et al., 2003), leading to changes in micropollutant removal via changes in the individual populations present (i.e., Helbing et al., 2012).

This study was performed to determine the effects of wastewater strength on E1 removal. Understanding the effect of organic carbon on E1 biodegradation will enable better design and operation of wastewater treatment systems and thus allow treatment plants to achieve high and consistent removal of E1 from wastewater. This research will also lead to better understanding and evaluation of alternative wastewater collection and treatment design options (e.g., separate collection and treatment of urine and feces from greywater) and may have broader implications for our understanding of the roles organic carbon plays in EDC removal.

## 2. Methods

### 2.1 Chemicals And Synthetic Wastewater

E1 and deuterated and  $^{13}\text{C}$ -labeled E1 were obtained from Sigma and Cambridge Isotopes, respectively. The recipe for synthetic septage was adapted from Boeijs et al. (1999) and contained (per L): 75 mg urea, 11 mg ammonium chloride, 12 mg sodium uric acid, 25 mg magnesium phosphate dibasic trihydrate, and 20 mg potassium phosphate tribasic. The septage also contained a carbon source made up of the following (per L, for 100 mg COD/L nominal concentration): 6 mg bacteriological peptone, 51 mg sodium acetate, 6 mg dry meat extract, 17 mg glycerine, 21 mg potato starch, and 25 mg low fat milk powder. The carbon source was diluted or concentrated for carbon feeds of various strengths.

### 2.2 Experimental Set-Up

#### 2.2.1 Sludge Seed

Biomass used to start each experiment was taken from the Metropolitan Wastewater Treatment Plant in St. Paul, Minnesota. A single sample of activated sludge was triple-washed with phosphate-buffered saline, divided into 3.5 mL aliquots, and cryopreserved in 15% glycerol (v/v) at  $-80^{\circ}\text{C}$  until use. A single sludge aliquot was used to start each reactor.

#### 2.2.2 Competition Experiment

A substrate competition experiment was performed to test if spikes in wastewater strength have short-term inhibitory effects on E1 degradation. A 1-L batch reactor was seeded with cryopreserved activated sludge and synthetic septage with 100 mg/L COD and operated for 15 d, during which E1 degradation capability was observed. Reactor solids were filtered and

used to seed a 14-L batch reactor containing synthetic septage with 100 mg/L COD, and E1 at a concentration of 5 µg/L. After a 48-h period, this 14-L reactor was split into two sets (competition and control) of triplicate 2-L batch reactors. E1 was added to each reactor at a concentration of 5 µg/L. At 8 h, competition reactors received a synthetic septage spike, increasing the COD in the reactor by an additional 100 mg/L. Reactors were sampled every two h over a 16-h period to measure E1 and absorbance at 600 nm.

### *2.2.3 Starvation and Feast-Famine Conditions*

The impact of starvation and feast-famine reactors on E1 degradation was studied in two sets of duplicate batch reactors. A 1-L batch reactor was seeded with cryopreserved activated sludge and synthetic septage media at 100 mg/L COD. After 24 h, reactor solids were filtered using glass fiber filters and were used to seed two sets of duplicate 2-L batch reactors containing synthetic septage media at 100 mg/L COD and E1 at 2 µg/L.

During the first phase, reactors were run for a 72-h period. After this, a second phase was initiated when an additional 2 µg/L of E1 was added to each reactor. Feast-famine reactors also received a synthetic septage spike, increasing reactor COD by 100 mg/L. Reactors were sampled every 2 h over a 12-h period to monitor E1 degradation and absorbance at 600 nm. At the end of the second phase, feast-famine reactors were diluted by adding an equivalent volume of no-COD septage (no carbon source added) so that microbial density, as measured by OD<sub>600</sub>, was comparable to the starvation reactors. After a 4 h interval, an additional 2 µg/L of E1 was added to each reactor, and reactors were sampled during the third phase over a 12-h period as described above.

#### 2.2.4 Membrane Coupled Bioreactor (MBR) experiment

Three continuous flow membrane-coupled bioreactors were operated to test the impact of influent wastewater strength on E1 degradation. Reactors (150 mL) were operated with a HRT of 8 h and an SRT of 10 d. Influent wastewater strengths to the three reactors were 20, 75, and 350 mg/L COD, and all reactors were fed E1 at a concentration of 2 µg/L. All treatments were also set-up and analyzed in triplicate. An additional control reactor was fed distilled water containing sodium azide at 1% by weight to assess loss of E1 as a result of sorption to the reactor or membrane.

The MBRs were operated for a period of 36 d. Reactor effluent was sampled twice weekly for E1, pH, ammonia, and COD. The reactor solids stream was also sampled twice weekly to determine biomass concentration and perform microbial community analysis.

#### 2.3 DNA Collection, Processing, and Analysis

All samples for DNA analysis were collected and processed in triplicate. Reactor liquor (1.5 mL) was centrifuged and decanted, after which the pellet underwent three consecutive freeze-thaw cycles and an incubation of 90 min at 70 °C to lyse cells. DNA was extracted from lysed cells with the FastDNA spin kit (MP Biomedicals, Solon, OH) and stored at -20°C until further processing.

Automated Ribosomal Intergenic Spacer Analysis (ARISA) was conducted as described by Nelson et al. (2010). Briefly, the ribosomal intergenic spacer (ITS) regions of *Bacteria* were amplified using primers ITSF (50-GTC GTA ACA AGG TAG CCG TA-30) and ITSReub (50-GCC AAG GCA TCC ACC-30) (Cardinale et al., 2004). Fragment analysis was performed by denaturing capillary electrophoresis at the Biomedical Genomics Center at the University of

Minnesota using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA).

Fragment length was estimated using the MapMarker 1000 size standard.

## 2.4 Analytical Methods

### 2.4.1 *Sample Extraction and Cleanup*

Solid phase extraction (SPE) and silica gel clean-up procedures were adapted from Ternes et al. (1999). Briefly, samples of 100 mL were collected for E1 analysis, acidified to pH 3 with concentrated sulfuric acid, and amended with a labeled surrogate, (2,4,16,16-D<sub>4</sub>-estrone). Resprep Bonded Reversed Phase SPE cartridges (6 mL, Restek) were prepped with two column volumes each of acetone and Milli-Q water. Samples were then loaded onto the cartridges at a flowrate of ~3 mL/min. Samples were eluted from the column with two column volumes of acetone.

Eluted samples were blown down to dryness with nitrogen and resuspended in 2 mL of hexane for silica gel cleanup. Silica gel columns were prepared by packing 3 cm of silica gel into pasture pipettes and then washing with two column volumes of hexane. Samples were then loaded onto the column and eluted with three column volumes of a 65:35 mixture of acetone/hexane (v/v), blown down to dryness with nitrogen, and resuspended in a 60:40 mixture of methanol and water (v/v) containing an internal standard (13,14,15,16,17,18-<sup>13</sup>C<sub>6</sub>-estrone). The sample was then stored at 4°C until analysis via liquid chromatography-mass spectrometry (LC/MS). Average sample recovery was 66% with a standard deviation of 12%.

### 2.4.2 *LC-MS analysis*

E1 samples were quantified via LC/MS using an HP 1050-series LC coupled to an Agilent/HP 1100 Series G1946D mass spectrometer detector. E1 was separated on a Synergi 4u Polar-RP 80A 150 × 2.00 mm 4 μm particle size column (Phenomenex). A binary gradient consisting of a pH 4 ammonium acetate buffered solution (10 mM) in 90% water and 10% acetonitrile (A) and acetonitrile (B) at a flow rate of 0.2 mL/min was used. The gradient was as follows: 35% B for 17 minutes, followed by a linear increase to 100% B over 3 minutes, held at 100% B for 5 minutes, and stepped down to 35% B for equilibration over 5 minutes.

The mass spectrometer was operated in negative ion, selected ion monitoring mode at 269, 273, and 275 for the detection of estrone, the surrogate, and the internal standard respectively. Standard curves of at least seven points were used in sample quantification. Blanks of 40:60 methanol water, as well as method blanks were run at the beginning of each sample analysis, as well as intermittently between samples. Typical instrument quantification limits were 25 μg/L (sample quantification limits of 200 ng/L). In-vial concentrations of E1 and surrogate recovery were corrected for by the internal standard. Sample concentrations of E1 were further corrected for by surrogate recovery.

#### *2.4.3 Biomass, chemical oxygen demand, and ammonia, determination*

Biomass concentrations in reactors were measured via absorbance at 600 nm with a Beckman DU 530 UV/Vis spectrophotometer. For the membrane bioreactors, a standard curve comparing volatile suspended solids to OD<sub>600</sub> was created. The range of the curve was 15-1500 mg/L, with an R<sup>2</sup> value of 0.99. Chemical oxygen demand was measured using accu-Test Low Range and Mid Range Micro COD vials (Bioscience) and a DR/890 colorimeter (Hach). Triplicate readings had a standard deviation of 2 mg/L COD at readings below 20 mg/L COD,

and a standard deviation of 15% at higher COD concentrations. Ammonia measurements were taken using an Orion 9512HPBNWP ammonia probe (Thermo Scientific), and a 5-point standard curve ranging from 1.4 to 140 mg/L as ammonium (typical  $R^2$  values of 0.99) was used to quantify samples.

## 2.5 Data Analysis

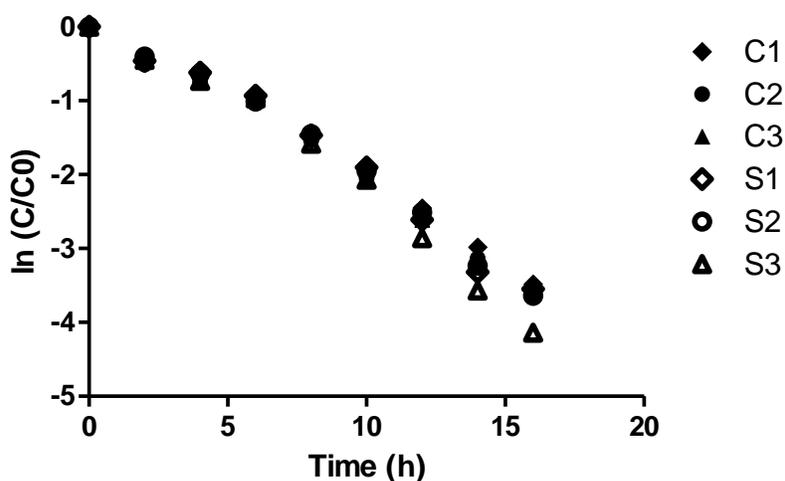
Nonmetric multidimensional scaling (nMDS) was used on triplicate ARISA profiles to compare microbial community profiles in samples as described in LaPara et al. (2011). Relative peak intensity was used in this analysis, excluding peaks falling below 0.5% of total peak intensity. nMDS was performed using the ade4 package in R, version 2.4.1.33. E1 degradation rates from linear regression and the Student t-test were performed in Microsoft Excel.

## 3 Results

On a short time scale (16 h), the addition of organic substrate (synthetic septage) to cultures actively degrading E1 did not affect E1 biodegradation rates (Figure 1). Neither percentage removal of E1 in the 2 h period immediately following addition of synthetic septage, nor the degradation rates over the entire 16 h period (Supplemental Figure 1) were statistically distinguishable between treatments ( $P = 0.19$  and  $P = 0.29$  respectively). This demonstrates that the organic carbon present in synthetic septage does not directly inhibit the biological degradation of E1 in mixed wastewater communities via substrate competition.

Average values of  $OD_{600}$  in the control and wastewater spike reactors prior to addition of synthetic septage were 0.067 and 0.071, respectively, and were not statistically distinguishable ( $P = 0.032$ ). Following the addition of synthetic septage, the  $OD_{600}$  in the amended reactors

increased to 0.140 by the end of the experiment, while the  $OD_{600}$  in the control reactors remained relatively constant at 0.072. This increase in biomass in the septage-amended reactors did not have an effect on E1 degradation rate or percentage removal, as the rates of degradation (non-biomass normalized) were equivalent. Nevertheless, the  $OD_{600}$  was low in all reactors and it is possible that the biomass concentration was not yet large enough to cause a statistically distinguishable increase in degradation rate upon biomass growth.

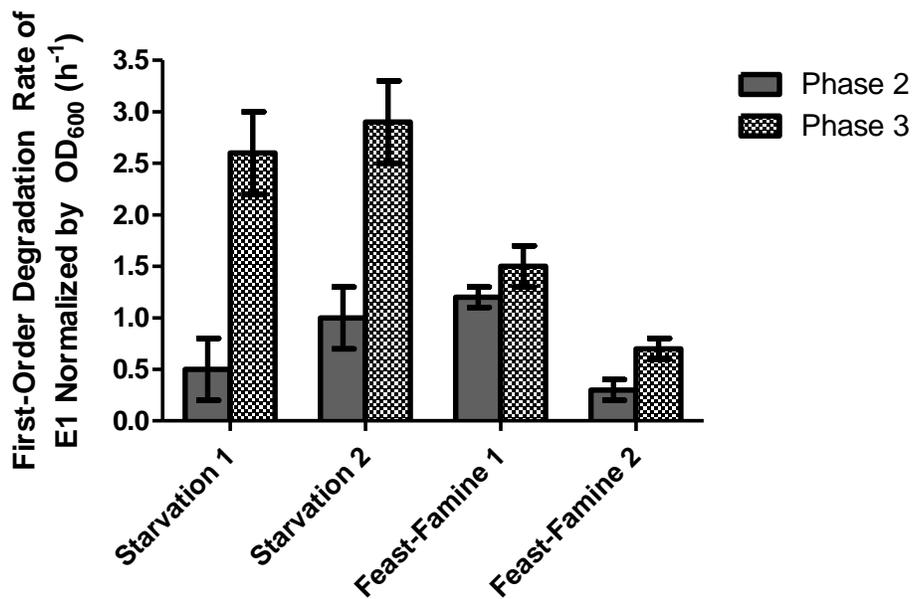


**Figure 1:** Semi-log plot of E1 in triplicate control reactors (C) and triplicate reactors amended with synthetic septage at 8 h (S). No change in E1 degradation performance was observed following the septage amendment.

In additional batch experiments comparing E1 degradation in reactors to which synthetic septage was or was not added, a similar, but wide range of biomass-normalized E1 degradation rates were observed in an initial 12-hour incubation period (phase 2, Figure 2). As was observed in the competition experiment, the addition of synthetic septage did not slow E1 degradation, again, pointing to a lack of substrate competition in these systems. Biomass did increase by 2-3 times upon septage addition, but this did not have a consistent and reproducible impact on non-

biomass normalized E1 degradation, likely because of the variability in the data and the separate incubation of reactors for 72 hours prior to the study.

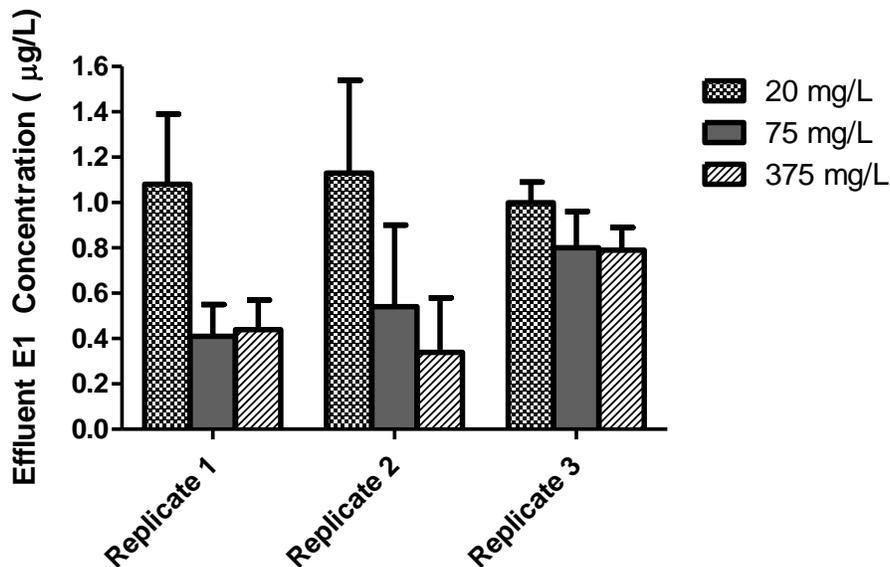
Further enrichment or stimulation of E1 degraders took place in these batch reactors over the 28-h time frame comprising phases 2 and 3 (Supplemental Figure 2). During this period of enrichment or stimulation, an increase in E1 biomass-normalized degradation rates was observed in both septage-fed and unfed treatments, particularly by phase 3 (Figure 2); nevertheless, the increase in rate was much larger in the reactors subjected to 28 hours of organic compound starvation compared to those re-fed synthetic septage every 12 hours (feast-famine conditions).



**Figure 2:** Biomass-normalized first-order degradation rates of E1 in reactors subject to starvation and feast-famine conditions during phase 2 and phase 3; similar biomass-normalized degradation rates are shown during phase 2 but greater increases in biomass-normalized E1 degradation rates were observed in phase 3 in the reactors subject to starvation. Error bars represent 95% confidence intervals derived from regressions (see supplemental Figure 2).

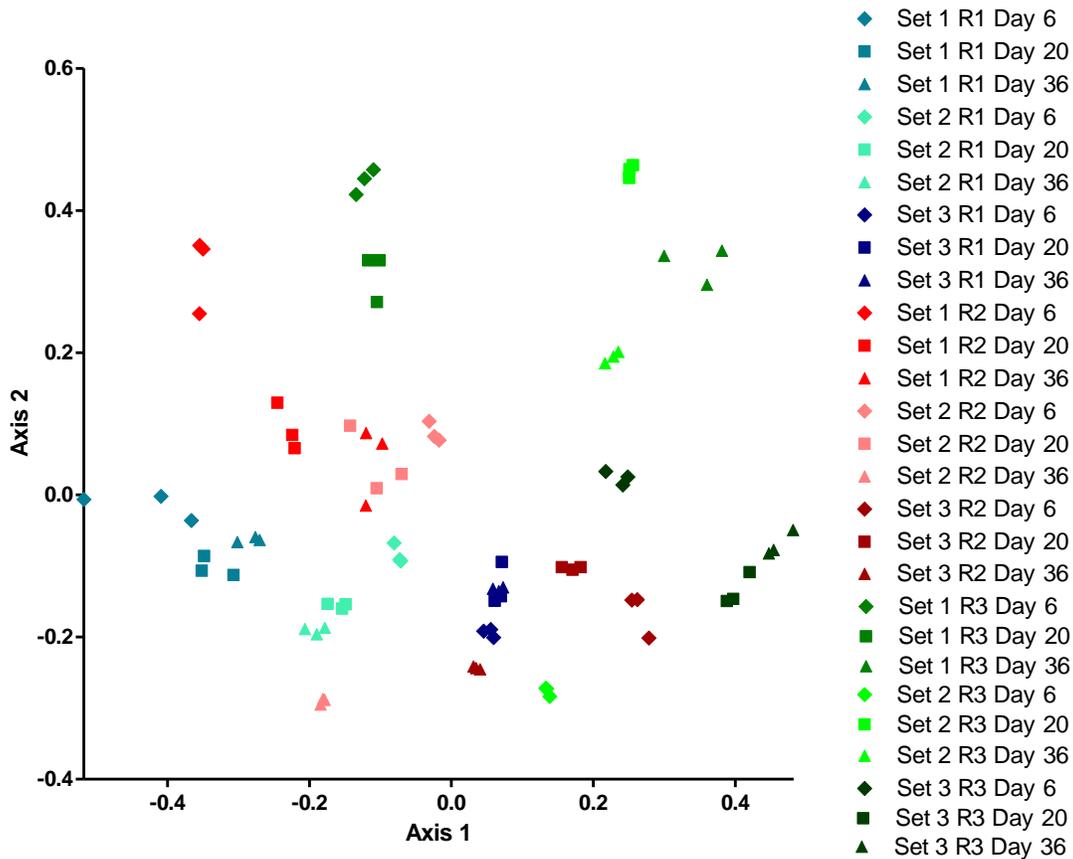
In completely mixed MBRs with similar operational and effluent conditions but different incoming synthetic septage strength, degradation of E1 was hindered by low influent septage strength ( $P = 0.018$ ). No difference in E1 removal was observed between reactors receiving

moderate and high strength septage ( $P = 0.76$ ). Biological removal of E1 was observed in all reactors and was generally greater than 50%, with effluent concentrations of E1 around or below  $1 \mu\text{g/L}$  (Figure 3). Loss of E1 to sorption was initially observed in killed-control reactors, but decreased over time, with effluent concentrations of E1 matching influent concentrations by Day 10 (Supplemental Figure 3). Similarly, E1 effluent concentrations in biologically active reactors initially increased over time, but leveled out by Day 16 (Supplemental Figure 3). As such, only E1 data on and after Day 16 were considered in this analysis. All effluent and operating conditions except for influent wastewater strength and biomass concentration ( $650 \text{ mg/L VSS}$ ,  $140 \text{ mg/L VSS}$ , and  $80 \text{ mg/L VSS}$  in the reactors receiving  $375$ ,  $75$ , and  $20 \text{ mg/L}$  influent COD, respectively) were similar across reactors. Food to microorganism ratios (F/M) appeared to be lower in MBRs receiving low strength wastewater, but this difference was not statistically significant ( $P = 0.27$ ).



**Figure 3:** Average E1 effluent concentrations between Days 17 and 31 from MBRs fed with synthetic septage containing 20, 75, and 375 mg/L COD. MBRs fed 20 mg/L COD had poorer E1 removal compared to the MBRs fed 75 and 375 mg/L COD. Error bars represent the standard deviation of the effluent [E1] from 5 different sampling periods in a single reactor. Three replicate reactors for each COD influent level were tested and results from each are shown.

Analysis of ARISA profiles of microbial communities in the MBRs via nMDS showed that communities in MBRs receiving synthetic septage with COD values of 20 and 75 mg/L tended to converge over time, but MBRs receiving synthetic septage with a COD value of 375 mg/L developed distinct communities (Figure 4). The two similar communities in reactors receiving the low and moderate strength synthetic septage stands in contrast to the statistically different E1 removals observed (Figure 3), while statistically similar E1 removals were observed in reactors containing the divergent communities receiving moderate and high strength synthetic septage.



**Figure 4:** nMDS analysis of ARISA of microbial communities in reactor sets 1, 2, and 3 on days 6, 20, and 36 shows microbial communities in R1 and R2 converge and are distinct from communities in R3 by the end of the experiment. R1, R2, and R3 are reactors with influent COD loads of 20, 75, and 375 mg/L respectively.

## 4 Discussion

Conventional wisdom has held that low substrate conditions are beneficial for the removal of micropollutants. This work shows a more complicated relationship because these conditions are (1) neutral, as shown by the absence of substrate competition; (2) beneficial, as seen in the increase in E1 degradation rate under prolonged starvation conditions; or (3) problematic, as observed in MBR experiments.

The absence of substrate competition and the improved degradation of E1 under starvation conditions are consistent with E1 removal by microorganisms that are multiple substrate utilizers. This has also been suggested by others (Koh et al., 2009; Gaulke et al., 2008) and is consistent with previous work that shows that prior exposure to E1, E2, and E3 is not necessary for good removal of these estrogens (Bagnall et al., 2012). Multiple substrate utilization relies on broad expression of catabolic enzymes instead of specialization and strict metabolic control via mechanisms such as catabolite repression (Egli 2010). Multiple substrate utilizers may also be able to degrade a myriad of compounds at low concentrations, enhancing their ability under starvation conditions to compete with typical heterotrophs that are less-adept at degrading trace organics like E1. Indeed, our results support this, for E1 degradation rates increased substantially during enrichment under “starvation” conditions. Because there is unlikely to be a catabolite repression-like mechanism occurring with respect to E1 degradation in such a system, once multiple substrate utilizers are enriched, potentially competing substrates do not inhibit E1 degradation and may actually have some positive impact (Bagnall et al., 2012; Muller et al., 2010). This was also observed in the competition experiments in which there was no change in E1 degradation upon addition of synthetic septage.

Interestingly, the poor removal of E1 in the MBR receiving low strength septage suggests that there may be a COD threshold below which E1 degradation suffers. Though somewhat surprising, this finding is in agreement with work by others that showed very poor to no estrogen degradation in low strength greywater systems (Kavanli et al., 2008) and shorter lag times to E1 degradation in the presence of another carbon source (Muller et al., 2010). Previous work has also shown that higher F/M ratios lead to faster biodegradation of several micropollutants, including estrogens (Lim et al., 2008). Taken together, these results suggest that organic compounds are required for the initial generation of biomass, while enrichment or stimulation of E1 degradation activity occurs most readily under starvation conditions.

If multiple substrate utilizers are responsible for E1 removal, biomass concentration alone cannot be indicative of E1 degradation rates. This has been noted in studies of wastewater treatment systems (Koh et al., 2009) and was observed in each experiment carried out in the current study. In the competition experiment, the COD spike resulted in a doubling of biomass by the end of the experiment but did not alter degradation rates, albeit biomass concentrations were quite low. When reactors underwent prolonged starvation conditions, biomass concentrations remained steady, while rates increased substantially. Finally, similar E1 effluent concentrations were observed in the MBRs receiving 75 mg/L and 375 mg/L COD influent, despite a fivefold difference in biomass concentrations. While the possibility that these reactors were threshold limited cannot be ruled out, threshold limitation seems unlikely, given that much lower E1 concentrations are seen in wastewater treatment and were observed in the batch experiments described herein.

Based on the results from this study and others, a clearer picture of E1 degradation is emerging, from which creative design of reactors and treatment trains may improve E1 removal.

Some minimum COD threshold is needed to generate adequate biomass levels to ensure good E1 degradation. In fact, the poorer removal of estrogens in low strength wastewater suggests that chemical treatment of certain types of wastewater (i.e., source-separated urine) may be an excellent option for removal of E1 and other micropollutants if additional COD is not added. Once a reasonable quantity of biomass is present, further enrichment of the multiple substrate utilizers that seem to be particularly active in E1 degradation is clearly enhanced by organic carbon-starved conditions. Some mechanism for biomass retention, a biofilm system or an MBR, would probably facilitate such enrichment in full-scale systems. In addition, if adequate land is available, a separate holding tank or pond for recycle solids, in which biomass may be allowed to undergo enrichment under starvation conditions prior to reintroduction, could also be used to achieve such enrichment.

## **5 Conclusions**

The impact of general organic carbon on the degradation of E1 was investigated in this study. Key findings are as follows:

- Substrate competition via general organic carbon does not affect the biodegradation of E1
- Starvation conditions results in greater improvement in E1 degradation rates over time compared to feast-famine conditions
- Low-strength influent wastewater may negatively impact E1 removal in continuous flow systems
- Multiple substrate utilizers appear to be critical for E1 removal and it may be possible to design wastewater treatment systems to enrich for these organisms

## **Acknowledgments**

This work was supported by the U of MN Graduate School via a fellowship to D.T.T., the U.S. EPA Great Lakes National Priorities Office (project #4641), and the Minnesota Water Resources Center (with #30612). This work has not been subject to EPA review.

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Appendix B: Supplemental Data in Impact of Organic Carbon on the Biodegradation of Estrone in Mixed Culture Systems, Submitted to Water Research

1. FIRST-ORDER KINETICS IN SUBSTRATE COMPETITION EXPERIMENTS

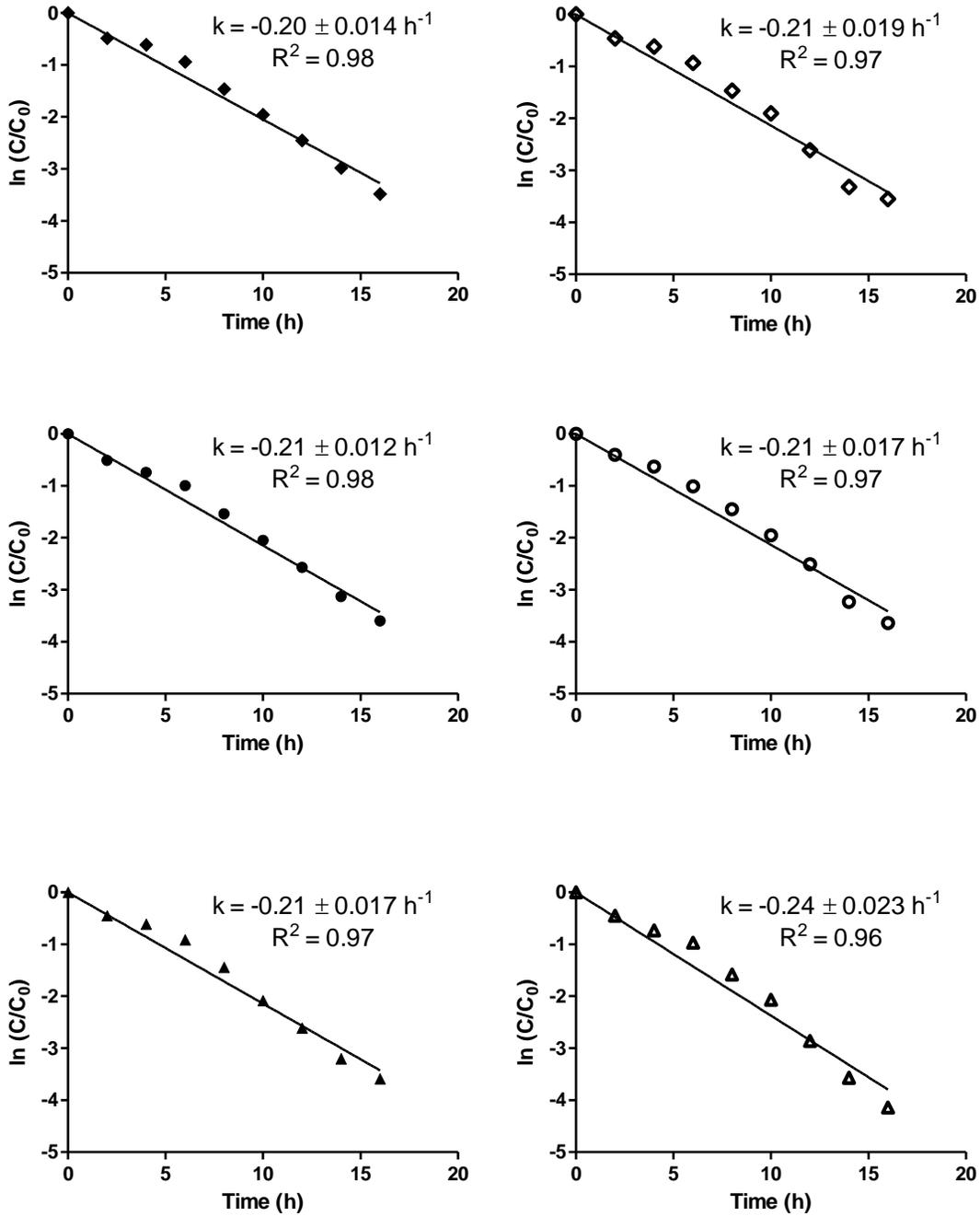
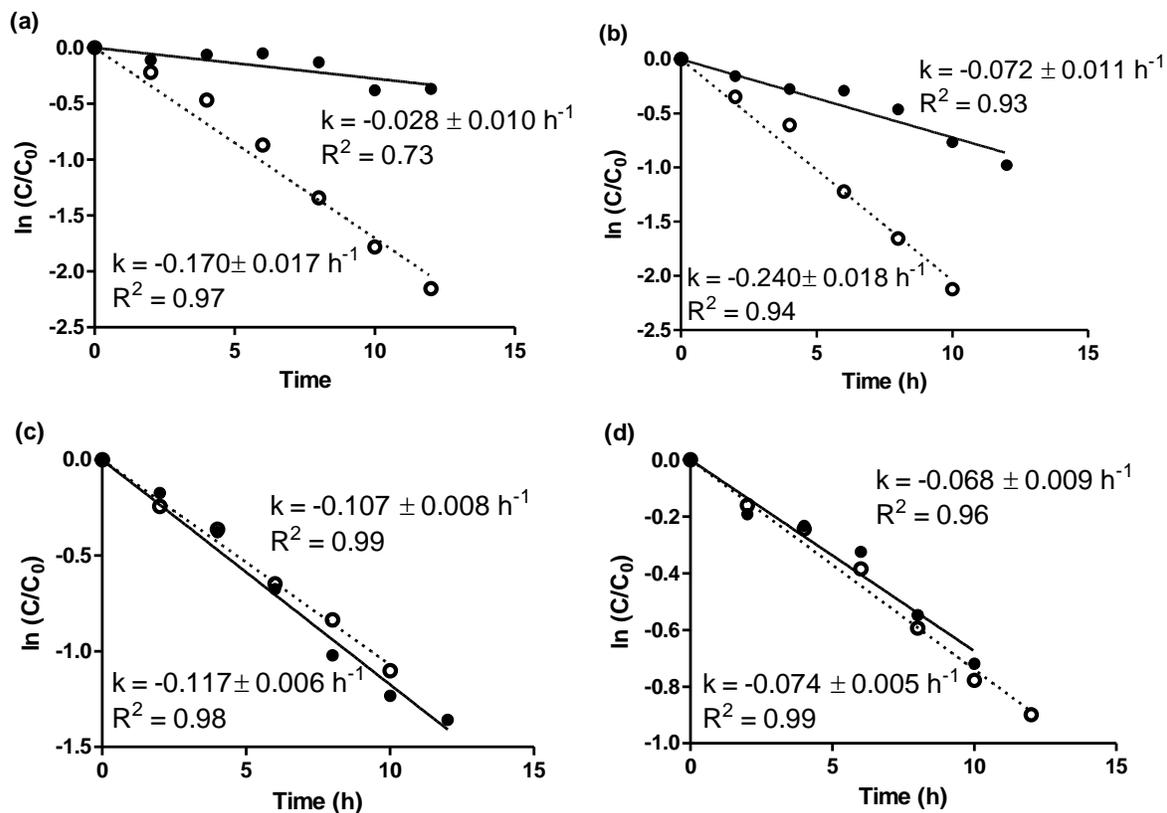


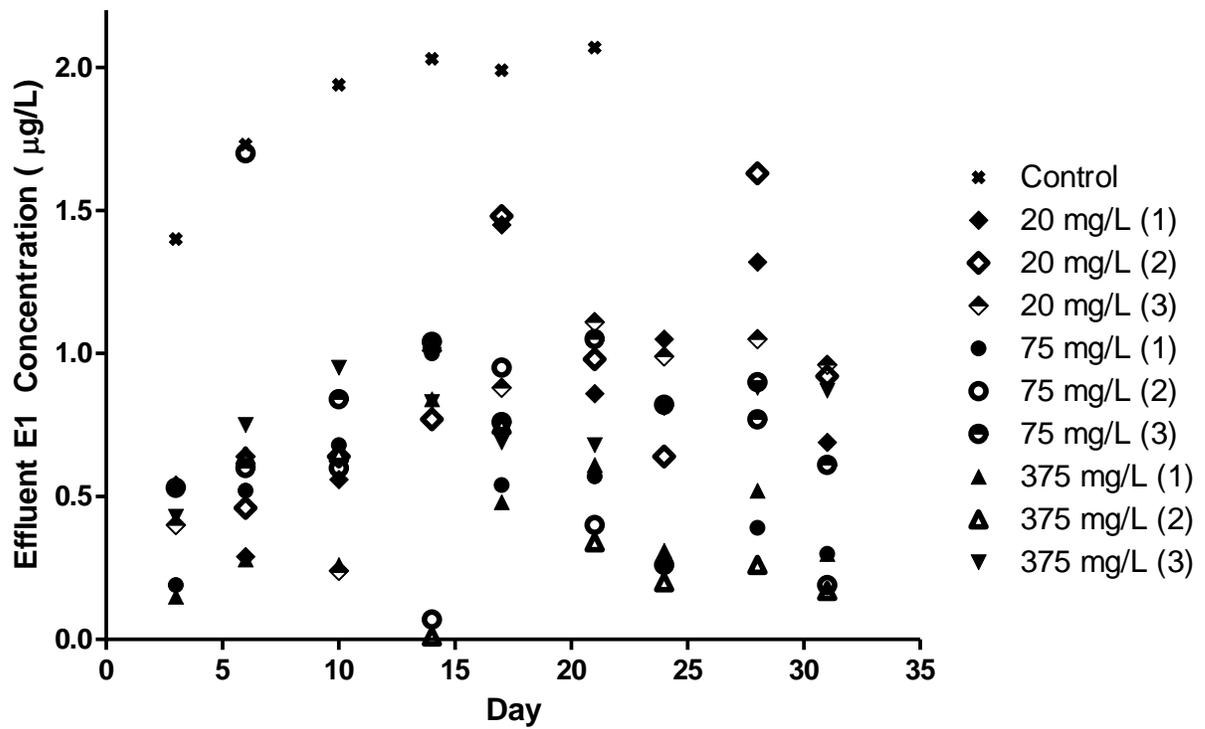
Figure S.1: First-order degradation rates of E1 in control reactors (closed symbols) and reactors receiving a wastewater spike at 8 h (open symbols). Error terms represent 95% confidence intervals from regression.

## 2. FIRST-ORDER KINETICS IN STARVATION VS. FEAST FAMINE EXPERIMENTS

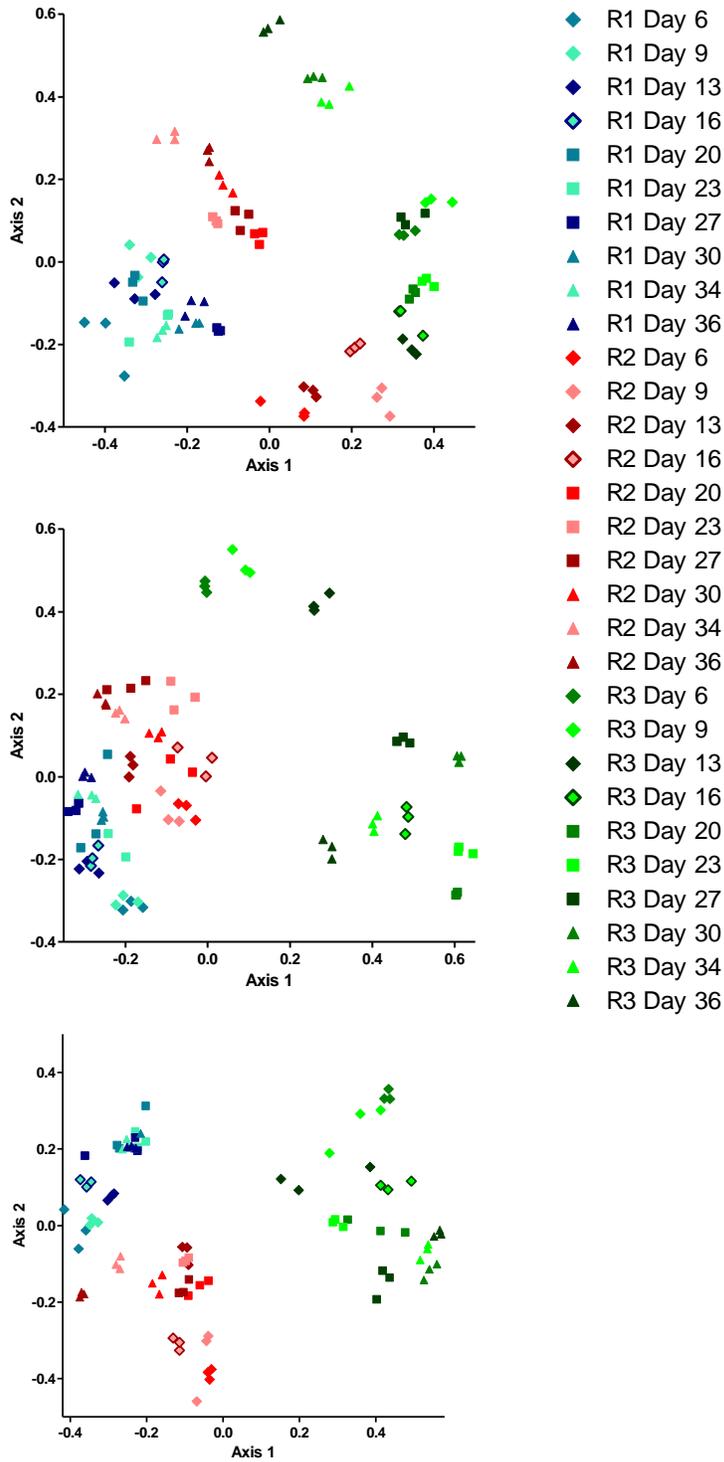


**Figure S.2:** First-order degradation rates of E1 in starvation reactors (a) and (b) and feast-famine reactors (c) and (d) during phase 2 (closed circles) and phase 3 (open circles) show greater increase in E1 degrading capacity under starvation conditions. Feast-famine reactors were diluted by thirty percent between phase 2 and phase 3.

### 3. MBR EXPERIMENTS



**Figure S.3:** E1 effluent concentrations from MBRs fed with synthetic septage containing 20, 75, and 375 mg/L COD. Reactor E1 removal performance stabilized by day 17.



**Figure S.4:** nMDS analysis of ARISA of microbial communities in reactor sets 1, 2, and 3 in (a), (b), and (c) show formation of distinct communities at varying influent COD concentrations. R1, R2, and R3 are reactors with influent COD loads of 20, 75, and 375 mg/L respectively.

# Understanding Pesticide Photolysis in Prairie Potholes for Water Management Strategies

## Basic Information

<b>Title:</b>	Understanding Pesticide Photolysis in Prairie Potholes for Water Management Strategies
<b>Project Number:</b>	2012MN344G
<b>Start Date:</b>	9/1/2012
<b>End Date:</b>	8/31/2014
<b>Funding Source:</b>	104G
<b>Congressional District:</b>	MN05
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Surface Water, Non Point Pollution, Hydrology
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	William Alan Arnold

## Publications

There are no publications.

# Understanding Pesticide Photolysis in Prairie Potholes for Water Management Strategies Project Number 2012MN344G

## Principal Investigator

William Arnold, Department of Civil Engineering, University of Minnesota

September 1, 2012-August 31, 2014

1) Research: The primary goal of this research is to quantify the importance of pesticide photolysis processes in prairie pothole lakes/wetlands (PPLs) such that appropriate, adaptive water management strategies can be developed to handle agricultural runoff and drainage. This includes both the design of constructed wetlands and the optimization of transient drainage features. PPLs have unique water chemistry (e.g., high levels of dissolved sulfate and natural organic matter (NOM)) and shallow depths, suggesting direct and indirect photolysis processes may be active in degrading pesticides in PPLs. The central hypothesis is that the high levels of photosensitizers present in such systems will increase the importance of indirect photolysis as a pesticide loss process in PPLs. Using probe and quencher experiments, we will determine the steady state concentrations of a suite of photochemically produced reactive intermediates (PPRIs; triplet organic matter, <sup>3</sup>OM, singlet oxygen <sup>1</sup>O<sub>2</sub>, and hydroxyl radical ·OH) in PPL waters. Photolysis experiments with target pesticides (atrazine, *s*-metolachlor, mesotrione, bentazon, and diuron) will be used to determine the relative importance of different photolysis processes. By comparing permanent, drained, and reconstructed PPLs in North Dakota and Minnesota/Iowa, we will be able to compare varying drainage strategies and water chemistries and how they affect the fate of pesticides and potential impacts on the wetlands, surface waters, and groundwater that interact with PPLs.

Over the past six months, we have located all necessary sampling sites, obtained permission to collect samples, and have begun collecting surface water samples from each site. These sites include the Cottonwood Lakes Study Area near Jamestown, ND, Glacial Ridge National Wildlife Refuge near Crookston, MN, and a private farm in Tracy, MN. The sampling locations include one native/temporary wetland, two native/permanent wetlands, and one reconstructed wetland that is not directly affected by cropland runoff. The impacted sampling sites include a native, permanent wetland, a drained wetland, and a reconstructed wetland that each receives direct runoff from cropland. Surface and porewater samples from the PPLs will be collected seasonally (spring, summer, and fall until summer 2014). At the time of collection, temperature, pH, and dissolved oxygen are recorded for each wetland. Nitrate, dissolved organic matter, and sulfate/sulfide concentrations are measured in the laboratory.

The proposed sampling regimen will allow study of both categorical and seasonal variations in pesticide degradation among PPLs. Understanding these variations will be integral for future reconstruction of drained and agriculturally affected PPLs. It is expected that the characteristics of DOM will change as land use surrounding PPLs changes (i.e. from active crop land to reconstructed wetland).

Preliminary tests measuring the steady state concentrations of photochemically produced reactive intermediates have been performed. In the coming months, filter-sterilized surface waters will be modified with environmentally appropriate concentrations of pesticides (atrazine, *s*-metolachlor, mesotrione, bentazon, diuron) and the time required to achieve acceptable

pesticide concentrations will be recorded. Reactions will be conducted both outdoors and indoors. Parent pesticide compounds and degradation products will be quantified by gas chromatography/mass spectrometry (GC/MS). Reactive intermediate quenchers will be used to quantify the contributions of direct and indirect photolysis: isopropanol and methanol for  $\cdot\text{OH}$ , sodium azide for  $^1\text{O}_2$ , and isoprene for  $^3\text{DOM}^*$ . Because dissolved oxygen acts as a  $^3\text{DOM}^*$  quencher, samples will be sparged with nitrogen gas to examine the effect of deoxygenation on pesticide degradation. Dark controls will be incorporated to confirm that sunlight is required for significant pesticide degradation. Blank controls will be used to ensure no cross-contamination between samples.

2) Publications: None to date.

3) Student Support: One MS/Ph.D. student, Mr. Andrew McCabe, has been supported by the project.

4) Presentations: Invited Seminar, William A. Arnold, *Abiotic Transformations of Pesticides in Prairie Potholes*, University of Colorado-Boulder, November 30, 2012.

5) Awards: None to date.

6) Related Funding: None to date.

# **Information Transfer Program Introduction**

We have not funded Information Transfer projects.

# USGS Summer Intern Program

## Basic Information

<b>Start Date:</b>	3/1/2012
<b>End Date:</b>	2/28/2013
<b>Sponsor:</b>	USDI USGS
<b>Mentors:</b>	Paul D. Capel
<b>Students:</b>	Kshiteesh Hegde

## Internship Evaluation

Question	Score
Utilization of your knowledge and experience	Good
Technical interaction with USGS scientists	Very Good
Treatment by USGS as member of a team	Very Good
Exposure and access to scientific equipment	Good
Learning Experience	Very Good
Travel	Too Little
Field Experience Provided	Too Little
Overall Rating	A

## Additional Remarks

<b>Student Support</b>					
<b>Category</b>	<b>Section 104 Base Grant</b>	<b>Section 104 NCGP Award</b>	<b>NIWR-USGS Internship</b>	<b>Supplemental Awards</b>	<b>Total</b>
<b>Undergraduate</b>	3	0	0	0	3
<b>Masters</b>	2	0	1	0	3
<b>Ph.D.</b>	2	1	1	0	4
<b>Post-Doc.</b>	0	0	0	0	0
<b>Total</b>	7	1	2	0	10

## Notable Awards and Achievements

Arnold, William, staff, Malcolm Pirnie/AEESP Frontier in Research Award 2012

Arnold, William, staff, Outstanding Mentor Award, President's Distinguished Faculty Mentor Program, Multicultural Center for Academic Excellence, Office of Equity and Diversity 2012

Arnold, William, staff, Frontiers of Engineering Education Symposium Participant, National Academy of Engineering, 2011

Arnold, William, staff, Resident Fellow, Institute on the Environment, 2011-2014

Arnold, William, staff, Triclosan report highlighted in the State of the River report ([www.stateoftheriver.com](http://www.stateoftheriver.com))

Easter, William, staff, retired from the University in 2012 after 42 years of teaching and research

Gulliver, John, staff, Resident Fellow, Institute on the Environment, University of Minnesota, 2012 – present

Gulliver, John, staff, Resident Fellow Minnesota Federation of Engineering and Science Technologist Societies Distinguished Engineer of the Year, 2012

Gulliver, John, staff, Resident Fellow Center for Transportation Studies Partnership Award, 2011, for "Assessment and Recommendation for Operation of Standard Pumps as Best Management Practices for Stormwater Treatment." The award recognizes research projects that have resulted in significant impacts on transportation

Mulla, David, staff, Pierre C. Robert Precision Agriculture Research Award, 2012 from the International Soc. Prec. Ag

Mulla, David, staff, National Academy of Sciences Member Comm. Water Quality Standards for Nutrients in Lakes and Flowing Waters of Florida. 2011

Allen, Joshua, M. S. student, GK 12 Fellowship

Chraibi, Victoria, M. S. student, "Fulbright Canada-RBC Eco-Leadership grant 2010-2011, \$4000 Project: Science Institute for Educators: teacher training in current water issues. Partner: Great Lakes Aquarium

Chraibi, Victoria, M. S. student, NSF-IGERT fellowship from the University of Nebraska-Lincoln to study resilience and adaptive management

Chraibi, Victoria, M. S. student, received a nomination for the 2012 Jim LaBounty Best Paper Award

Shaw Chraibi VL, Bennett EM, Gregory-Eaves I. 2011. Conservation of a transboundary lake: historical watershed and paleolimnological analyses can inform management strategies. *Lake and Reservoir Management*, 27:4, 355-564

Dietz, Robert, Ph. D. student, received the Moos Graduate Research Fellowship in Aquatic Biology, 2012

Dietz, Robert, Ph. D. student, Graduate and Professional Student Assembly Scholarly Travel Grant, 2012

Dietz, Robert, Ph. D. student, Herbert E. Wright, Jr. Quaternary Paleoecology Fellowship, 2011

Donovan, Kyle, M. S. student, Excel Energy Grant

Fairbairn, David Ph. D. student, Smith Partners Sustainability Fellowship, funded by Smith Partners PLLP, \$2500

Grundtner, Ashley, M. S. student, WRS Travel Grant May 2012

Kelly, Megan, Ph. D. student, American Chemical Society - Graduate Student Paper Award, highest award given to students by the Division of Environmental Chemistry

Krogman, Ann, M. S. student, College of Biological Sciences Outstanding Teaching Assistant Award, \$250.00

Kronholm, Scott, Ph. D. student, accepted to Phi Kappa Phi honor society

Kruger, Brittany Ph. D. student, Butler and Jessen Fellowship

Macdonald, Meg M.S. student, WRS Travel Grant \$500

Macuiane, Messias, Ph. D. student, Office of International Programs (OIP), student award

Mazack, Jane, M. S. student, WRS Travel Grant \$500

Meester, Jennifer, M. S. student, Water Resources Science Fellowship, \$25,000

TenEyck, Matthew, Ph. D. student, Characterizing the Risk-Release of Aquatic Invasive Species in the Great Lakes. Funded by The Great Lakes Protection Fund, \$996,000

Thompson, Seth, M. S. student, Moos Graduate Fellowship in Aquatic Science, funded by the Freshwater Society

Thompson, Seth, M. S. student, Itasca Director's Graduate Fellowship, funded by the College of Biological Sciences Scholarships

Titze, Daniel, M. S. student, First year fellowship, WRS

Wasik, Jill, Ph. D. student, \$1300.00 Honorarium/scholarship to participate in School for Environmental Science and Synchrotron Radiation at Argonne National Laboratory's Advance Photon Source

Wick, Molly, M. S. student, Geology Department Travel Award

Wisker, James, M. S. student, BWSR Watershed Employee of the Year for Outstanding Contribution to Water Resources

Wisker, James, M. S. student, Certificate of Appreciation for contribution as elected official on Hennepin SWCD

## Publications from Prior Years

1. 2011MN288B ("Stratigraphic Distribution and Mineralogical Sources of Arsenic to Minnesota Glacial Aquifers") - Articles in Refereed Scientific Journals - Toner, B. M., S. L. Nicholas, L. J. Briscoe, A. R. Knaeble, J. A. Berg, and M. L. Erickson. 2011. Natural Sources of Arsenic to Minnesota Groundwater. CURA Reporter 41, 3-10.
2. 2011MN288B ("Stratigraphic Distribution and Mineralogical Sources of Arsenic to Minnesota Glacial Aquifers") - Articles in Refereed Scientific Journals - Nicholas, S. L., B. M. Toner, M. L. Erickson, L. G. Woodruff, A. R. Knaeble, and G. N. Meyer. 2011. Mineral Sources of Arsenic from Glacial Aquifer Sediments to Well Water in Minnesota, USA. Goldschmidt, Prague, Czech Republic. Mineralogical Magazine 75.
3. 2011MN288B ("Stratigraphic Distribution and Mineralogical Sources of Arsenic to Minnesota Glacial Aquifers") - Articles in Refereed Scientific Journals - Nicholas, S. L., B. M. Toner, M. L. Erickson, A. R. Knaeble, L. G. Woodruff, and G. N. Meyer. 2011. Speciation and Mineralogy of Arsenic in Glacial Sediments and their Effect on Arsenic Concentrations in Groundwater, Minnesota, USA. Geological Society of America, Minneapolis, MN, 283-5.
4. 2011MN288B ("Stratigraphic Distribution and Mineralogical Sources of Arsenic to Minnesota Glacial Aquifers") - Other Publications - Nicholas, S.L. 2011. Identifying Mineral Sources of Arsenic Affecting West-Central Minnesota Well-Water. Invited talk. Annual meeting of the Minnesota chapter of the American Waterworks Association, Duluth, MN.  
<http://www.mnawwa.org/news/breeze/Fall2011Breeze.pdf>
5. 2011MN291B ("Persistence of the Fecal Indicator Bacteroides in Sand and Sediment") - Other Publications - Eichmiller, J.J., R. E. Hicks and M. J. Sadowsky. 2012. Influence of Moisture and Temperature on the Persistence of Molecular Marker Genes to Detect Fecal Pollution in Sand and Sediment. American Society for Microbiology Annual Meeting, San Francisco, California.
6. 2010MN269B ("The role of sulfate reduction in sediment of the St. Louis River estuary") - Other Publications - Beck, B. F. and N. W. Johnson. 2011. Sulfur and Carbon Controls on Methylmercury in St. Louis River Estuary Sediment. SETAC North America, Boston, MA. Beck, B. F. and N. W. Johnson. 2011. Characterizing the Relationship Between Sulfate Reduction and Mercury Methylation in St. Louis River Sediment. Minnesota Water Resources Conference, St. Paul, MN.
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