Nebraska Water Resources Center
Annual Technical Report
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Introduction

Dr. Suat Irmak, professor in the Biological Systems Engineering Department at the University of Nebraska-Lincoln, took over as the interim director of the Water Center as of January 1, 2012. Lorrie Benson, J.D. serves as the assistant director. Steve Ress and Tricia Liedle serve as the communications specialist and program specialist, respectively. The Water Center staff also includes Rachael Herpel as the water outreach specialist. The Water Center also underwent a name change in February 2012 becoming the Nebraska Water Center, a part of the Daugherty Water for Food Institute at the University of Nebraska.

The Nebraska Water Center is currently physically housed in the School of Natural Resources, located in Hardin Hall (3310 Holdrege, Lincoln, NE 68583-0979). However, the Center is now part of the Robert B. Daugherty Water for Food Institute, which is located in Whittier Building in the Robert B. Daugherty Water for Food Institute, University of Nebraska, 234 Whittier Research Center, 2200 Vine Street, Lincoln, NE 68583-0857 U.S.A. The Nebraska Water Center remains in the physical location of Hardin Hall on East Campus.

The Nebraska Water Center was the lead organizer for two major events fall 2012. First was a one-day science and policy symposium showcasing water-related research and programming in Nebraska, with a focus on Water: Science, Practice and Policy. The event, which was co-sponsored by the USGS Nebraska Water Science Center, featured speaker Matthew Larsen, associate director. The second event was a one-day water law conference, designed for practicing attorneys, but attended by many water policy makers and managers. It was co-sponsored by the University of Nebraska College of Law. The Nebraska Water Center also assisted the UNL Office of Research with the fourth annual Water for Food conference an event drawing international speakers and attendees and sponsored by the University of Nebraska's Robert B. Daugherty Water for Food Institute.

The Nebraska Water Center has continued to assist with development of the UNL water portal (water.unl.edu) and maintains the NIWR website (snr.unl.edu/niwr) through the Nebraska Water Center. Along with these websites, we continue to focus on the Nebraska Water Center's home website (http://watercenter.unl.edu).

Research Program Introduction

The Nebraska Water Center continued its traditional outreach programs and activities as well as serving as a liason for connecting researchers and stakeholders for addressing water related issues throughout Nebraska as well as neighboring states. In addition to these activities, the center continued to administer the USGS 104(b) grant program. For the 2012 fiscal year, three research seed grants received funding through the USGS 104(b) program. Areas chosen for funding were: (1) analysis of potential groundwater trading programs for Nebraska; (2) developing a two-tier screen to evaluate the health of Nebraska's wetlands; and (3) direct monitoring of knickpoint progression; An additional two seed research grants were selected for possible funding during the 2013 fiscal year. Areas chosen for 2013 were: (1) development of an affinity-based concentrator-detection kit for monitoring emerging contaminants in recycled water; and (2) an innovative graphene oxide filter for drinking water contaminants removal.

The Water Sciences Laboratory (WSL) core facility, a part of the Nebraska Water Center, is a state-of-the-art laboratory designed to provide technical services and expertise in analytical and isotopic methods. The facility provides specialized instrumentation and methods for organic, emerging contaminants, heavy metals, and for stable isotope mass spectrometry. Faculty, staff, and students have analyzed thousands of samples at the facility since it was established in 1990. The facility recently obtained external funding for additional ground water age-dating instrumentation and plans to begin offering a totally new isotope analysis capability during the second half of 2013.

A Cost Effective Fixed Film Atrazine Treatment Utilizing Nitrate as a Nutrient

Basic Information

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There are no publications.

A COST EFFECTIVE FIXED-FILM ATRAZINE TREATMENT UTILIZING NITRATE AS A NUTRIENT

Mohamed F. Dahab, Ahmed Hosni, and Allison Cole

Department of Civil Engineering

University of Nebraska-Lincoln

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Abstract

The popular broadleaf herbicide atrazine is often found in contaminated groundwater along with other agricultural chemicals, such as nitrate. Mulch biowalls, a passive treatment placed *in situ*, can inexpensively remediate groundwater by intercepting and treating a contaminant plume. Three types of organic mulch: cedar, cypress, and hardwood were evaluated for their ability to act as supporting materials for a biowall to simultaneously remove atrazine and nitrate from groundwater. Physical and chemical properties of the mulch were characterized. Cedar mulch had the highest organic carbon content, 996 mg/g. The adsorptive capacity of the mulch for atrazine and nitrate, in mono and binary adsorbate systems were evaluated in a series of isotherm experiments. There was no statistical difference in the ratio of q_e/C_e (equilibrium concentration on the mulch/equilibrium concentration in solution) for atrazine or nitrate among the three types of mulch, except for atrazine in the pairs of cedar-hardwood and cypress-hardwood in the binary adsorbate system. Atrazine adsorption appeared to exhibit a C-type isotherm, due to the range of concentrations examined; A wider range of atrazine concentrations may show a more distinct L-type isotherm. Atrazine adsorption was not affected by the presence of nitrate. Nitrate adsorption did not clearly exhibit a specific isotherm type and was affected by surface properties of the mulch as well as the presence of atrazine. The adsorption behaviors of atrazine and nitrate were quantified from Langmuir and Freundlich isotherms. Atrazine adsorption was best modeled by the Freundlich isotherm, while nitrate adsorption was best modeled by the Langmuir. Qualitatively, cypress mulch exhibited the greatest sorption capacity for atrazine and nitrate and was selected to examine the feasibility of a mulch biowall using a laboratory-scale biotic column. The cypress column was not able to remove nitrate because the concentration of dissolved oxygen was too high, even after the addition of an external carbon source. The column was not able to remove atrazine because the concentration of nitrate was too high for bacterial degradation of the herbicide to occur.

INTRODUCTION

Background

Nebraska is a land of agriculture. Ninety three percent of Nebraska is farmland, and Nebraska ranks in the top ten states for crop production (Nebraska Agricultural Fact Card 2011). This substantial agricultural activity is made possible by the extensive use of fertilizers and pesticides to enhance production.

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] is a popular broadleaf herbicide, typically used on corn. It is normally applied at 2.2 kg/hectare or less (Solomon et al. 1996). Between 30,000 and 34,000 tons of atrazine are used annually in the United States (Solomon et al. 1996; Hayes et al. 2002). However, about 10% of the atrazine applied washes off fields, moving away from target sites toward areas devoid of oxygen, like groundwater (Ma and Selim 1996; Gu et al. 2003). Forty five percent of groundwater contamination cases are attributed to point source contamination of atrazine (Silva et al. 2004).

More than 50% of United States population derives its primary drinking water from groundwater (Kross et al. 1992). Atrazine is the second most frequently detected pesticide in drinking water wells (U.S. EPA Office of Pesticide Programs 1993). In 2010, the Nebraska Department of Environmental Quality found that 5% of groundwater samples exceeded the reporting limit for atrazine (Nebraska Department of Environmental Quality, 2010). The maximum contaminant level for atrazine in drinking water is 3 μg/L, as set by the Environmental Protection Agency (EPA). Exposure to atrazine causes endocrine disruption in frogs, rats, and humans (U.S. EPA Office of Pesticide Programs 1993; U.S. EPA Office of Pesticide Programs 2002; Villanueva et al. 2005).

Atrazine and nitrate are often found together in the groundwater of agricultural states (Ritter 1990). In 2010, the Nebraska Department of Environmental Quality found that 94% of groundwater samples exceeded the reporting limit for nitrate (Nebraska Department of Environmental Quality, 2010). The maximum contaminant level for nitrate in drinking water is 10 mg NO_3-N/L as nitrogen, as set by the EPA (Nebraska Department of Environmental Quality, 2010). Nitrate can cause methemoglobinemia, or “blue baby syndrome,” because it interferes with the body’s ability to carry oxygen in its red blood cells (Skipton and Hay 1998).

Literature has shown that researchers have tested several processes in the treatment of pesticide contamination in both soil and water including: chemical and biological treatment processes. Waria et al. (2009) used zero valent iron and ferrous sulfate to degrade atrazine chemically in soil. Soybean oil was also added to provide a carbon source for biological activity. Atrazine, initially at a concentration of 500 mg/kg soil, was reduced by 79% in 342 days. Tafoya-Garnica et al. (2009) used a fluidized bed reactor containing biological granular activated carbon to achieve high degradation rates. Modin et al. (2008) used a methane fed bioreactor intended to remove both atrazine and nitrate. However, atrazine removal was not successful (Modin et al. 2008). Bianchi et al. (2006) successfully used photolysis, photocatalysis (with TiO_2), and ozonation for atrazine degradation.

Processes such as these require the presence of a nutrient source, such as methane or soybean oil, and specialized treatment, such as ultraviolet radiation or biological activated carbon. These additions can greatly increase the cost of treatment, especially when the price of highly trained operators is factored in.

Passive treatment, such as a biowall, is inexpensive when compared to methods discussed above because it is placed *in situ*. Biowalls are bacteria supported on a natural substrate that is placed to intercept contaminated groundwater flow. Removal is accomplished through adsorption or biological degradation, as the contaminated plume passes through a permeable remediation well or trench placed perpendicular to groundwater flow. Biowalls are low maintenance and can endure changes in operating conditions (Kao et al. 2001, Schipper et al. 2004; Seo et al. 2007). Biowalls supported on a natural substrate, such as mulch or peat moss, have been studied for naphthalene (Seo et al. 2007), tetrachloroethylene (Kao et al. 2001), and denitrification (Schipper et al. 2004; Ilhan et al. 2011), but rarely for atrazine removal. Ilhan et al. (2011) examined the removal of atrazine and nitrates in a woodchip bioreactor. The bulk of the atrazine removal appeared to be due to physical, rather than biological methods.

Low concentrations of nitrate, ~1mM, do not interfere with atrazine degradation (Crawford et al. 1998, 2000). Some atrazine-degrading bacteria, such as *Pseudomonas* sp. ADP, can use nitrate as an electron acceptor under anoxic conditions (Shapir et al. 1998). However, when nitrate is present in excess, some atrazine-degrading bacteria may prefer to use nitrate as a source of nitrogen instead of atrazine (Hunter and Shaner 2010). This relationship may be dependent on the bacteria species present as well as the background concentration of nitrate.

OBJECTIVES

The objective of this research is to examine a cost-effective and reliable biological treatment method for the co-removal of atrazine and nitrate from groundwater. Two approaches were established to achieve the objective. First, the adsorption capacities of three types of common gardening mulch for both atrazine and nitrate were examined. Second, the type of mulch exhibiting the largest adsorption capacity was used for further experimentation in a laboratory-scale biotic column experiment to examine the feasibility of implementation of this type of biowall in contaminated groundwater.

REVIW OF PHYSICAL AND BIOLOGICAL REMEDIATION OF ATRAZINE

Background

Atrazine is one of the most widely used herbicides for the control of broad-leafed weeds. It was developed in Switzerland in 1958 by the Geigy Chemical Company, and became registered for use in the United States in 1959 (Solomon et al. 1996). Between 30,000 and 34,000 tons of atrazine are used annually in the United States, normally applied at 2.2-4.5 kg/ha (1.1-2.2 μg /g soil) (Yeomans and Bremner 1987; Solomon et al. 1996; Hayes et al. 2002).

However, about 10% of atrazine applied washes off fields, moving away from target sites toward areas devoid of oxygen, like groundwater (Ma and Selim 1996; Gu et al. 2003). The rest is retained in the soil; atrazine’s vapor pressure is so low that volatilization is negligible (2.89E-7 mm of Hg at 25°C) and it is not photodegradable at wavelengths >300nm (Solomon et al. 1996). Forty five percent of groundwater contamination cases come from point source contamination of atrazine (Silva et al. 2004).

More than 50% of the United States population derives its primary drinking water from groundwater (Kross et al. 1992). The Environmental Protection Agency (EPA) has set the maximum contaminant level in drinking water for atrazine at 3 μg/L, whereas the European Union has set the level at 0.1 μg/L (Wilber et al. 1995; Faur et al. 2005; Zadaka et al. 2009). Atrazine is the second most frequently detected pesticide in drinking water wells (U.S. EPA Office of Pesticide Programs 1993). It has been found at levels exceeding the maximum contaminant level because of its popularity and low biodegradability (Somasundaram and Coats 1990; Sene et al. 2010).

The degradation of atrazine occurs through one of two pathways; it can be dehalogenated to form hydroxyatrazine (HYA) or dealkylated to form deisopropylatrazine (DIA) or deethylatrazine (DEA). Without dehalogenation, the dealkylated metabolites still retain the phytotoxic properties and possibly the endocrine-disrupting potency of atrazine, making further degradation or removal of metabolites desirable (Boundy-Mills et al. 1997; Silva et al. 2004).

Atrazine is also frequently found in conjunction with its metabolites. In a national study of groundwater quality in the United States, 49.4% of sites where pesticides had been detected contained both atrazine and DEA. All but two of the sites conformed to drinking water criteria; yet, current drinking water criteria only enforce one compound at a time (Kolpin et al. 2000). Another study of vernal pools in protected areas in the United States found atrazine was the most frequently detected pesticide (53%), followed closely by DEA (47%), HYA (44%), and DIA (29%) (Battaglin et al. 2008). Synergistic effects from multiple compounds are unknown and cannot be predicted based on the toxicity of a single component (Marinovich et al. 1996).

However, regulations are changing to include metabolites. The European Union has set a limit of 0.5 μg/L for the combination of atrazine and its degradation products, known as Total Chloro-s-Triazine (TCT). The EPA is considering similar strict regulations for TCT (Faur et al. 2005; Jiang and Adams 2006). As these regulations reach the United States, further research should focus on elucidating the toxicity of the metabolites, which is still largely speculative. Generally speaking, atrazine, DIA, DEA, and didealkylatrazine share a common mechanism of toxicity with respect to endocrine disruption (U.S. EPA Office of Pesticide Programs 1993; Jiang and Adams 2006). However, relative toxicity studies with bioluminescent bacteria have shown that DEA and DIA are less toxic than atrazine (Kross et al. 1992).

The toxicity of atrazine has been researched in a variety of animals. Studies on atrazine levels in fish species revealed that atrazine does not tend to bioconcentrate, like the infamous pesticide DDT (dichlorodiphenyltrichloroethane). Male frogs in water contaminated with greater than 0.1 μg atrazine/L show hermaphroditism and retarded gonadal development. In rodents, atrazine is embryotoxic and embryolethal, but not teratogenic (Villanueva et al. 2005). In adult rats, atrazine causes mammary gland tumors. Though this cancer mechanism is different in humans, it doesn't rule out the possibility of reproductive developmental effects by another mechanism (U.S. EPA Office of Pesticide Programs 2002). Health effects in humans from acute exposure to atrazine levels above the maximum contaminant level include "congestion of heart, lungs and kidneys; hypotension; antidiuresis; muscle spasms; weight loss; adrenal degeneration" (U.S. EPA Office of Pesticide Programs 1993).

Atrazine is not the only contaminant in natural waters. A study on leopard frogs by Hayes et al. (2006) used a low concentration (0.1 ppb) of a nine-pesticide mixture, including atrazine, to simulate a low runoff concentration. Tadpoles exposed to the mixture took a longer time to metamorphose, were smaller, and had weakened immune systems, making them vulnerable to predation and bacterial infections. Though the toxicity of atrazine is relatively well characterized, the way it interacts in the environment with other pesticides or its own metabolites is still greatly unknown.

Removal via Adsorption

Background

Adsorption occurs at a surface of a solid adsorbent, which forms chemical or physical bonds to remove a component, such as atrazine, from the fluid phase (Foo and Hameed 2010). The atrazine reaches the adsorbent after undergoing three types of diffusion. First, film diffusion moves the atrazine from the bulk phase to the adsorbent surface. Second, particle diffusion moves the atrazine to the interior of the adsorbent. Third, the atrazine is adsorbed onto the surface of the adsorbent (Chingombe et al. 2006). Compared to chemical or biological removal methods, adsorption has a low initial cost, offers flexibility and simplicity of operation, and doesn't form harmful intermediates (Ahmad et al. 2010).

Soil Adsorption

Atrazine enters soil environments through land application. Atrazine adsorption on soils is influenced by many factors, including: organic matter, pH, conductivity, alkalinity, suspended solids, dissolved salts, and water content (Seol and Lee 2000). This section will discuss the influence of pH and organic materials on soil adsorption and show how the structure of atrazine influences its affinity to soil. Lastly, soil remediation methods with activated carbon, hydrolysis, and wastewater application will be discussed.

A study by Clay and Koskinen (1990) showed that atrazine and hydroxyatrazine are more strongly adsorbed to soils at lower pH, 4, compared to a more neutral pH, 6, because atrazine and hydroxyatrazine are weak bases and have greater protonation at lower pH values. Atrazine desorption was hysteretic, which could have occurred for many reasons, including: equilibrium was not attained, precipitates, changes in desorption solution composition, degradation, or irreversible binding to soil.

Atrazine adsorbs rapidly to organic components of soils, especially polysaccharides, lignin, and humic substances (Ma and Selim 1996; Masaphy and Mandelbaum 1997). However, the origin of the organic matter influences the adsorption. In a study by Laird et al. (1994), atrazine chemisorbed to the organic matter in coarse silicate clays, whereas atrazine only physisorbed to the organic matter in fine silicate clays, because the organic material in the finer clays had fewer organic functional groups.

Though Granular Activated Carbon (GAC) is more conventionally used for wastewater treatment, it has been used for soil remediation in the past. Gunther and Gunther (1970) suggested a rule of thumb for application rates of 200 lb/acre of activated carbon for every 1 lb/acre of atrazine. This is slightly higher than the 120 lb/acre suggested by Harvey (1973). Both application rates can be reduced with band applications or root dips (Gunther and Gunther 1970). However, the high rate of application required makes GAC feasible only for high value land or crops (Harvey 1973). Harvey (1973) also noted that freeze-thaw cycles were detrimental to the effectiveness of the activated carbon, which may limit its usefulness.

Dehalogenation of Atrazine

As stated in Section 2.1, dehalogentation of atrazine is highly desired due to the possible phytotoxicity and endocrine-disrupting potency of the halogenated metabolites (Boundy-Mills et al. 1997; Silva et al. 2004). Dehalogenation can occur through both chemical and biological processes.

Xu et al. (2001) tested the ability of freeze dried samples of sodium-saturated ferruginous smectite to adsorb atrazine. Reduced clay adsorbed 31% of the atrazine from solution, but further High-Performance Liquid Chromatography (HPLC) analysis revealed a high concentration of hydroxyatrazine. Xu et al. believes that the atrazine was hydrolyzed via a nucleophilic displacement of chlorine by hydroxide. Chemical hydrolysis was favored in the reduced clay environment because a greater electron density in the alkaline environment (Xu et al. 2001). Armstrong et al. (1967) also noted the benefits of an alkaline environment as well as an acidic one. Alkaline hydrolysis occurred through a direct nucleophilic displacement. Acid hydrolysis occurred through protonation of a side chain or ring nitrogen atom and than nucleophilic displacement by water (Armstrong et al. 1967).

Hydrolysis can also occur biologically, with the enzyme AtzA. This enzyme was discovered by Mandelbaum et al. (1995) and characterized in the mid-nineties by deSousa et al. (1996). Both chemical and biological processes may be at work; Houot et al. (1998) hypothesized that increased formation of hydroxyatrazine was due to a combination of chemical and biological hydrolysis caused by a lower pH in soils from the addition of composted straw.

Stimulating Soil Microbial Activity with Wastewater

Addition of organic matter from wastewater treatment plant effluent causes an increase in the organic content of soil, which affects atrazine sorption (Barriuso et al. 1997; Masaphy and Mandelbaum 1997; Celis et al. 1998). For example, remediation with *Pseudomonas* sp. ADP was only 20% effective on soils that had been sprayed with treated wastewater, compared to 60-80% effective in soils without wastewater (Masaphy and Mandelbaum 1997). Conversely, addition of high concentrations (1058 mg of organic carbon/L) of dissolved organic matter from sewage sludge has the reverse effect: increasing desorption of atrazine, due to site competition or surface modification (Celis et al. 1998). However, in the presence of small concentrations of dissolved organic matter, up to 150 mg of organic carbon/L, atrazine adsorption is not suppressed (Seol and Lee 2000). The effects of wastewater application have important ramifications for the irrigation of farmland with effluent, and warrant further study.

This section has shown that the adsorption of atrazine on soils is strongly influenced by the presence of organic carbon as well as pH. Soil remediation with activated carbon is expensive. Recycling of wastewater treatment plant effluent onto soil may either decrease atrazine adsorption or increase it, depending on the concentration and properties of the organic material. Further research should examine the influences of soil properties on atrazine adsorption and the effects of irrigation with wastewater treatment plant effluent.

Activated Carbon Adsorption

Atrazine may enter drinking water through groundwater or runoff into surface water. Drinking water treatment plants typically use activated carbon treatments to remove atrazine or other residual compounds. This section will discuss the two most common types of activated carbon, Granular Activated Carbon (GAC) and Powdered Activated Carbon (PAC), as well as two variations: Biological Granular Activated Carbon (BGAC) and Activated Carbon Fibers (ACF).

Drinking water treatment plants use activated carbon to remove organics, residual inorganics, and taste/odor-causing compounds (Tchobanoglous et al. 2003). Activated carbon has been designated the best available technology for the removal of herbicides from drinking water by the EPA (Adams and Watson 1996). Activated carbon has a large porous surface area, controllable pore structure, low acid/base reactivity, and thermal stability. It has a low initial cost and high adsorption and regeneration capacities. It is easy to control the dosage of activated carbon and it does not form any oxidation byproducts as in ozoneation (Adams and Watson 1996; Foo and Hameed 2010).

There are two types of activated carbon: Granular Activated Carbon (GAC) and Powdered Activated Carbon (PAC). GAC has larger particles with a diameter greater than 0.1 mm. In a water treatment process, it is contained in a pressurized contact basin. Conversely, PAC particles are smaller with a diameter less than 0.074 mm, and can be added at any point during the process (Tchobanoglous et al. 2003). Typically, PAC is added at the raw water intake, the rapid mix tank, or in a slurry contactor (Crittenden et al. 2005). Later in the process PAC must be settled out in a contacting basin or removed with filtration (Tchobanoglous et al. 2003). GAC requires less activated carbon, has easier handling, and can be regenerated, but has higher operation, maintenance, and capital costs. PAC has a low capital cost and offers flexibility of operation; however, it is hard to fully utilize its entire adsorption capacity and it requires an additional filtration procedure (Kyriakopoulos and Doulia 2006). Both types of activated carbon can be designed to contain varying pore sizes.

Atrazine is 9.6 x 8.4 x 3 Å and prefers primary or secondary micropores (Li et al. 2004a). However, in a drinking water treatment plant, atrazine is not the only target compound for removal, and must compete for adsorption sites with other compounds, including Natural Organic Matter (NOM). NOM is typically found at 3.7 ppm in natural waters, making it 1000 times greater than a typical concentration of atrazine (Kyriakopoulos and Doulia 2006; Zadaka et al. 2009). NOM affects atrazine adsorption in two ways: direct site competition and pore blockage (Zadaka et al. 2009).

NOM is larger than atrazine. If preloaded, it will block openings to smaller pores, reducing the surface area available for atrazine adsorption. Atrazine will have to move around the blockage or displace the NOM to adsorb. However, if NOM is in direct competition with atrazine, as in a batch reactor, pore blockage is not an issue, because atrazine can quickly adsorb to micropores before they are blocked (Li et al. 2003, 2004a).

Knappe et al. (1997) used RSSCT (Rapid Small Scale Column Tests) to examine the effect of NOM preloading on atrazine adsorption by GAC. Both virgin GAC and GAC that had been preloaded for 5 months effectively removed atrazine. However, a longer preloading time, 20 months, was not successful due to enhanced adsorption and polymerization of NOM in the presence of oxygen. In a later study, Knappe et al. (1999) discovered that after preloading, there was no competition between NOM and atrazine for sites. Also, adsorption capacity could be increased by grinding preloaded GAC into PAC. The grinding increased the surface area by opening up pore space.

The PAC dose in a treatment plant is typically 1-2 mg/L for odor and taste control (Jiang and Adams 2006). If NOM is present, 10-16 times more PAC is required to achieve 90-99% removals of atrazine, because NOM has slower adsorption kinetics; NOM moves down the column quickly, preloading the bottom of the column before atrazine can get there (Li et al. 2003).

Ding et al. (2008) found that there was less site blockage in PAC with pores 15-50 Å. NOM favors this pore size, leaving smaller micropores unblocked for atrazine adsorption. Similarly, Li et al. (2003) found that PAC with a greater percentage of mesopores had better atrazine adsorption. Due to site competition, atrazine adsorption is not related to the total surface area, but rather to the number of available micropores (Ding et al. 2008). Thus, adsorption kinetics is more important than adsorption capacity.

There are many options to decrease the effect of NOM, including: pulse input of PAC, aeration, and optimizing the membrane cleaning interval. A pulse input of PAC results in a greater amount of contact time with a greater amount of PAC, lessening the effect of pore blocking materials (Li et al. 2004b). The adsorption capacities and lifespan of the PAC can be increased with intermittent high intensity aeration (2.7 L/min with a 2 second pause). These bubbles generate microscale high intensity eddies that shrink the resistance of the boundary layer (Jia et al. 2006). Lastly, the performance of a small reactor can be optimized to avoid influence from pore blocking materials with a short membrane cleaning interval (MCI) and a low PAC dose (Li et al. 2004b).

Zhang and Emary (1999) used jar tests to simulate a drinking water treatment plant environment. PAC alone exhibited a 40-50% removal of atrazine. Typical drinking water treatment plant additions, such as alum coagulant or lime, had negligible effect on the atrazine removal. However, the combination of a lowered pH with sulfuric acid (5.8), alum coagulation, and PAC increased atrazine removal to over 60%. The lower pH increased the hydrophilic properties of atrazine and lowered the charge density on NOM, making atrazine more susceptible to removal by coagulation or adsorption.

GAC and PAC are the two most common types of activated carbon and are typically used in drinking water treatment plants. Two variations, more common in a laboratory setting, are Biological Granular Activated Carbon (BGAC) and Activated Carbon Fibers (ACF).

Herzberg et al. (2004) compared anaerobic atrazine degradation by *Pseudomonas* sp. ADP on an adsorbent medium (GAC) and non-adsorbent medium. The BGAC (Biological Granular Activated Carbon) column degraded more atrazine by two orders of magnitude than the column with non-adsorbent media, due to a “double flux” of atrazine through the biofilm and the adsorbent media. In a similar study, Feakin et al. (1995a) successfully used *Rodococcus rhodochrous* in a BGAC column for atrazine degradation. The authors hypothesized that atrazine adsorbed on the GAC is not bioavailable to the bacteria; atrazine must desorb into the liquid phase to become bioavailable (Feakin et al. 1995a). BGAC has limited application to drinking water treatment plants due to environmental regulations. In the United Kingdom, influent bacteria counts must be approximately equal to effluent bacteria counts. If bacterial counts are greater than 10^3, chlorination is advised (Feakin et al. 1995b).

Scientists that are specifically interested in examining pore blockage effects in activated carbon often turn to ACF (Activated Carbon Fibers). ACF are synthetic materials from polymeric substances. They are specifically engineered to have a uniform and continuous pore structure. GAC is exactly the opposite; It is made from impure, non-uniform feedstocks, it is not homogenous, and doesn’t have continuous micropores (Pelekani and Snoeyink 2000). ACF have faster initial adsorption rates as well as a greater adsorption capacity than GAC (Faur et al. 2005). Pelekani and Snoeyink (2000) studied the competitive adsorption between atrazine and methylene blue (a compound of similar size to atrazine) on ACF. Similar to previous studies with NOM, the impact of preloading with the competing substance decreased as pore size increased. Also, increasing the volume of secondary micropores relative to primary micropores increased the adsorption of atrazine. This allowed atrazine to directly compete for sites, instead of finding primary micropores blocked by the competing substance.

In conclusion, activated carbon can be an effective tool for atrazine removal, provided enough micropores are not blocked by competing substances, such as NOM. BGAC have promise for use in the drinking water industry, but more experimentation is required to select robust strains of bacteria with higher survival rates. ACF are useful on a laboratory scale to examine pore blockage effects, but is too expensive for most real world applications. Activated carbon, though capable of removing 98% of atrazine, is costly, leading researchers to examine cheaper alternatives: recycled activated carbon, modified soils, oil seed press cakes, switchgrass, and recycled materials.

Natural Materials Adsorption

PAC is typically used for six months to a year, and then it is discarded. Ghosh and Phillip (2005) found a way to reuse it as Powdered Waste Activated Carbon (PWAC). The PWAC removed 17.19 mg atrazine/g carbon, and when washed for reuse, removed 13.24 mg atrazine/g carbon. The PWAC also supported the growth of atrazine-degrading bacteria that grew on the surface of the activated carbon without causing a biofilm or a pressure drop (Ghosh and Phillip 2005).

Modified soils have been examined by Bottero et al. (1993) and Zadaka et al. (2008) for atrazine removal. Zeolites were not able to outperform activated carbon (Bottero et al. 1993). However, Zadaka et al. (2008) was able to achieve 93-96% removal rates of atrazine, outperforming activated carbon by 10%, with montmorillonite soils pre-adsorbed with 10% poly(4-vinylpyridine-*co*-styrene), or PVPco-S90%-mont. Also, the PVPco-S90%-montmorillonite was not as affected by addition of dissolved organic matter, was more structurally compatible with atrazine, and had a higher charge density than other modified soil.

Boucher et al. (2007) examined the adsorption of atrazine by oil seed press cakes. The press cakes adsorbed 58% of the atrazine, out-performing the seeds alone or ground seeds. This was due, in part, to a mass transfer effect; the oil particles were smaller in the press cakes and less blocked by other structures.

Atrazine adsorption on or near a field is highly desired to reduce runoff and groundwater contamination. The adsorption capacities of thatch and fresh switchgrass were evaluated in a laboratory setting to approximate the behavior of a vegetative filter. Both were able to effectively adsorb atrazine. The adsorption coefficients after 24 hours were 81.1 and 21.4 Lkg^{-1} for switchgrass and thatch, respectively. However, cut ends of switchgrass, not generally present in the field, may have skewed the adsorption data (Mersie et al. 2006). An earlier study by Mersie et al. (1999) used planted boxes of switchgrass instead of switchgrass cuttings. Bacterial degradation was faster in beds planted with switchgrass, and switchgrass plots successfully adsorbed atrazine. Similarly, Selim and Zhu (2005) found that sugarcane mulch residue left in the field after harvest exhibits strong atrazine retention, with a partitioning coefficient of 16.4 Lkg^{-1}.

A wide variety of recycled materials have been investigated as cheaper alternatives to activated carbon: wood charcoal, rubber granules, bottom ash, coconut fiber, and sawdust. Wood charcoal is the best alternative for adsorption, though it is not as efficient as activated carbon, with removal rates of 95-97%. However, rubber granules also have a high removal rate (82%) (Alam et al. 2000; Sharma et al. 2008). Alam et al. (2000) recommends rubber granules over wood charcoal because the disposal of the charcoal causes air pollution, whereas rubber granules can be recycled into rubberized asphalt.

Alternatives for atrazine adsorption have varying degrees of effectiveness. However, none is equal to the power of activated carbon. Future research should focus on finding a cheap, fast, effective alternative for atrazine adsorption and should continue to elucidate the kinetics of atrazine adsorption.

Metabolite Adsorption

Atrazine is often found in conjunction with its metabolites in natural waters. Atrazine and its metabolites have different solubilities in water, as seen in Table 2.3. This affects their adsorption capacities, according to Lundelius rule: The extent of adsorption of a solute is inversely proportional to its solubility in the solvent (Adams and Watson 1996).

Table 1: Solubility of atrazine and its metabolites (Steinheimer 1993; Faur et al. 2005)

Compound	**Solubility in Water** *(mg/L)*
Atrazine	34.7
Deethylatrazine (DEA)	3200
Deisopropylatrazine (DIA)	670
Hydroxyatrazine (HYA)	7

DEA and HYA are the two most prevalent metabolites found in soils (Liu et al. 1996; Mudhoo and Garg 2011). As shown in Table 1, HYA is strongly adsorbed to soils. HYA has stronger adsorption because it has a higher protonation than atrazine at the same pH. However, HYA adsorption does not interfere with atrazine adsorption, because the two compounds prefer different types of sites (Ma and Selim 1996).

Faur et al. (2005) studied atrazine, DEA, and DIA adsorption on activated carbon fibers. They found that atrazine adsorbed the strongest, followed by DIA and DEA, confirming Lundelius rule. In binary systems, such as atrazine-DEA and atrazine-DIA, the adsorbate of lower solubility, atrazine, was favored for adsorption, while the other, DIA or DEA, did not adsorb and had no influence on atrazine adsorption.

Standard drinking water treatment plant processes such as coagulation, flocculation, sedimentation, free chlorine, lime, or soda ash do not reduce the concentration of atrazine, DEA, or DIA. Ozone at a dose of 3-5 mg/L reduced TCT (Total Chloro s-Triazine) concentration in river water by 32% and in DI water by 70%, because of the persistence of the metabolites. Higher removals were seen with powdered activated carbon (0.55 m^3/g). In river water, 90% of TCT was removed with PAC concentrations of 20-50 mg/L and 80% was removed with PAC of 5 mg/L. However, using PAC concentrations more typical to treatment plants, 1-2 mg/L, only 40% removal could be achieved, due to the high solubilities of the metabolites and fowling by natural organic matter (Jiang and Adams 2006). A similar study showed using PAC to treat DEA and DIA requires 3.1-4.5 times more activated carbon than it would to treat atrazine alone, because of the higher solubilities of these metabolites (Adams and Watson 1996).

The varying solubilities of atrazine metabolites pose a special challenge for removal. As regulations change in the coming years to include metabolites, further research should focus on adsorption kinetics and examine cheaper adsorption materials.

Bacterial Removal

Background

Bacteria provide an alternative method for atrazine removal, either by degradation or mineralization. Atrazine degradation is the disappearance of the parent compound, atrazine, into intermediate compounds, or metabolites; atrazine mineralization is the complete transformation of atrazine and its metabolites into carbon dioxide (Ellis and Wackett 2011a, 2011b, 2011c).

In soils, the half-life of atrazine is 35-50 days with little mineralization of the s-triazine ring by indigenous bacteria (Topp 2001). The time required for indigenous bacteria to mineralize the s-triazine ring, thereby degrading atrazine into less toxic metabolites, has been estimated to be 60-360+days. Complete mineralization is estimated to occur only to less than 40% of applied atrazine. However, more rapid mineralization of atrazine has been reported in agricultural soils that frequently come in contact with atrazine. Repeated dosing of atrazine naturally selects bacteria with an enhanced ability to degrade atrazine (Alvey and Crowly 1996). Isolation of some of these indigenous atrazine-degrading bacteria began in the nineties and continues to the present day.

Uncharacterized Consortia

Indigenous soil bacteria can be acclimated to degrade atrazine, if given time for the proliferation of a degrading population that selects and expresses the right genes for degradation (Silva et al. 2004). In top soil, atrazine degradation occurs in 60 days, whereas, degradation in subsurface soils or in groundwater, takes significantly longer (U.S. Environmental Protection Agency 1988). Under aerobic conditions in soil, the half-life for atrazine is 3.6 ± 0.4 years; under denitrifying conditions, the half-life is over 500 years, suggesting that atrazine degradation is dependent on soil depth (Nair and Schnoor 1992; Kruger et al. 1993). However, a study by Wilber and Parkin (1995) found that atrazine degradation by a natural soil consortia was not different between aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions. However, degradation by the consortia was rapidly decreased under aerobic and nitrate-reducing conditions once the primary substrate, acetate, was depleted.

Isolates and Isolated Consortia

Isolated bacterial strains for atrazine mineralization have existed since the 1990's (Wackett et al. 2002). *Pseudomonas* sp. ADP was one of the first strains to be isolated. It was isolated and characterized by Mandelbaum et al. (1995) and continues to be studied due to its high removal efficiency. *Pseudomonas* sp. ADP can utilize atrazine as a nitrogen source, but not as a carbon source. If a carbon source is added, such as citrate, removal efficiencies of 80% (Shapir et al. 1998) up to 95% (Katz et al. 2001) have been seen. The ratio for complete denitrification was calculated to be 5.11 g citrate g^{-1} NO_3-N (Katz et al. 2000).

However, the requirement of a carbon source puts *Pseudomonas* sp. ADP at a disadvantage compared to other isolates, such as *Pseudominobacter* sp. and *Nocardioides* sp. These isolates, especially *Pseudominobacter* sp., outperformed *Pseudomonas* sp. ADP because they can utilize atrazine both as a carbon and a nitrogen source (Topp 2001). Compounds with this capability have greater potential uses as bioremediation agents, because they do not require additional chemical stimulation.

Consortia are more common in nature than single strains and they can be more effective at atrazine removal. A consortia was isolated from atrazine degrading soil by Kolic et al (2007): *Arthrobacter* sp. AG1, *Arthrobacter keyseri* 12B, *Ochrobactrum* sp., and *Pseudomonas* sp. It was able to achieve 78% mineralization, because it shared carbon and nitrogen sources, and cross-fed metabolites.

A larger consortia was characterized by Smith et al (2005): *Agrobacterium tumefaciens*, *Caulobacter crescentus*, *Pseudomonas putida*, *Sphingomonas yaniokuyae*, *Nocardia* sp., *Rhizobium* sp., *Flavobacterium oryzihabitans*, and *Variovorax paradoxus*. The pivotal members of this consortia were *Nocardia* sp. and *Rhizobium* sp. *Nocardia* sp. was the only member that could use the enzyme TrzB to transform atrazine into hydroxyatrazine. Next, *Rhizobium* sp. used AtzB to transform hydroxyatrazine to the next product, *N*-ethylammelide, which all members could further degrade.

Characterization of natural consortia illuminated the metabolic pathway of atrazine, providing new bacteria that work either individually or together to perform fast and effective degradation. However, the impact of the natural bacteria that cannot be cultured must also be considered for their role in atrazine degradation (vanVeen 1999; Smith et al. 2005).

Co-removal of Atrazine and Nitrate

Atrazine and nitrate are often found together in groundwater in agricultural areas, so their simultaneous removal is often desired (Ritter 1990). Katz et al. (2001) used *Pseudomonas* sp. ADP in anoxic non-sterile reactors that had a high removal efficiency of atrazine, >95%, for the first month, and then lost effectiveness, 10-25%, due to competitive nitrifying bacteria that could not degrade atrazine. Herzberg et al. (2004) also saw a similar decrease in atrazine removal efficiency in a reactor filled with non-adsorptive media. However, this effect was not present in similar reactor with adsorptive media due to a “double flux” of atrazine through the biofilm and the adsorbent media.

Clausen et al. (2002) suggested that a high concentration of nitrate interferes with the degradation capabilities of *Pseudomonas* sp. However, this conclusion was based on a 14-day reaction time, which may not be long enough to see the full effect of an excess of nitrate.

Early reports by Cervelli and Rolston (1983) claimed that atrazine applied at 3 μg/g soil inhibited denitrification, specifically the reduction of N_2O to N_2. However, this was later disproved by Yeomans and Bremner (1987), who found no inhibition of denitrification when atrazine was applied at 5, 10, 25, or 100 μg/g soil.

Isolate M91-3 has been studied extensively for use in denitrification coupled with atrazine degradation in glass media columns by Crawford et al (2000). Atrazine degradation was achieved in both aerobic and anaerobic zones in the column. Low concentrations of nitrate, ~1 mM, did not interfere with atrazine degradation (Crawford et al. 1998, 2000). Also, the addition of glucose accelerated the anaerobic degradation of atrazine in the presence of nitrate (Crawford et al. 2000).

Hunter and Shaner (2010) used a double column containing a vegetable oil based denitrifying biobarrier followed by an aerobic reactor with an atrazine-degrading consortia to simulate nitrate and atrazine removal from groundwater. The denitrifying section removed 98% of the supplied nitrate and 30% of the atrazine, while the aerobic reactor removed the remaining 70% of the atrazine. Atrazine removal varied considerably in the denitrifying biobarrier, because of the interference of the nitrate, an easier source of nitrogen for column bacteria than atrazine.

As the above results show, co-removal of atrazine and nitrate is possible, though sometimes difficult, depending on the electron acceptor conditions, the presence of a carbon source, and the presence of other competing bacteria.

Biostimulation and Bioaugmentation

Laboratory studies using natural soils revealed the effects of bioaugmentation and biostimulation on indigenous bacteria. Bioaugmentation is the addition of non-indigenous microbial strains (i.e. *Pseudomonas* sp. ADP) to the environment for purposes of remediation. Biostimulation is the addition of chemicals (i.e. citrate) to the environment to stimulate naturally occurring bacteria for purposes of remediation. Biostimulation is approved faster by government agencies, whereas, bioremediation, especially with genetically modified organisms, causes closer scrutiny (Wackett et al. 2002). Bioremediation is often preferred over physical or chemical remediation because it can be done *in situ* with lower costs and environmental impacts (Sturman et al. 1995; Newcombe and Crowley 1999).

Biostimulation has been researched with a number of materials: municipal solid waste compost, straw compost, rice hulls, sodium citrate, urea, Sudan hay, glucose, mannitol, acetic acid, and starch (Houot et al. 1998; Assaf and Harris 1994; Alvey and Crowley 1995; Chung et al. 1996; Getenga 2003). Rice hulls had the highest mineralization of atrazine, 88%, according to Alvey and Crowley (Alvey and Crowley 1995; Houot et al. 1998).

In a study by Houot et al. (1998), municipal solid waste compost adsorbed 75% of applied atrazine, 1 kg/ha, making it unavailable for biodegradation. Conversely, in a study by Getenga (2003), municipal solid waste compost applied at 5000 ppm resulted in 55% mineralization of atrazine. It is unknown whether this effect is due to additional bacteria present in the compost or if the compost provided carbon and nitrogen for the soil bacteria to utilize.

Assaf and Harris (1994) found that their soil bacteria benefited from addition of mannitol, which produced a 17% increase in CO_2 evolution, suggesting a rate-limiting step in the metabolization of atrazine. Acetic acid additions to soil reduced the half-life of atrazine from 224 to 164 days. However, because only the level of atrazine was measured, these values are not indicative of complete mineralization (Chung et al. 1996). The varied results from these studies show that further research in this area would benefit from a better understanding of bacterial community population dynamics to select the correct compound for biostimulation.

Silva et al. (2004) showed that biostimulation with citrate alone inhibited atrazine mineralization by indigenous bacteria, because their mineralization pathway may be different from that of *Pseudomonas* sp. ADP. However, bioaugmentation and biostimulation of *Pseudomonas* sp. ADP and citrate together reduced atrazine concentrations by 80% in 2 days. Rousseaux et al. (2003) found biostimulation and bioaugmentation with *Chelatobacter heintzii* Cit1 and sodium citrate was not effective in soils that already had an indigenous population of atrazine degrading bacteria. However, in soils without an indigenous population, biostimulation and bioaugmentation resulted in a 3-fold increase in mineralization capacity of atrazine. As the above discussion show, the addition of natural materials can either help or hinder atrazine removal. The natural materials add new bacteria or fungi that may either compete with or assist local populations of atrazine-degrading microbes. The natural materials also may act as a source of nutrients for bacterial populations. However, atrazine may adsorb into pore space in the natural material, making it less bioavailable for degradation. The fastest mineralization rate was 80% in ten days with the addition of wastewater by Masaphy and Mandelbaum (1996). Rice hulls were the next most effective, with 88% mineralization in 77 days (Alvey and Crowley 1995).

From the Laboratory to the Field

Laboratory conditions are easier to control and are often less harsh to atrazine degrading bacteria than field conditions. Though some laboratories attempt to replicate conditions in the field, field conditions are hard to control such as: a non-uniform distribution of atrazine, the presence of other contaminants or metabolites, ambient temperature, and mass transport limitations for the contaminant, bacteria, and nutrients (Sturman et al. 1995; Strong et al. 2000; Silva et al. 2004).

One laboratory-scale study strived to replicate field conditions, using simulated rainwater, commercial herbicides, and earthworms to examine the survival rates and effectiveness of *Pseudomonas* sp. ADP. Chelinho et al. (2010) set up soil microcosms in the laboratory with earthworms (*Eisenia andrei*) and springtails (*Folsomia candida*) that were dosed with *Pseudomonas* sp. ADP and Atrazerba FL, a commercial herbicide containing atrazine. Both the herbicide and the bacteria were dispersed throughout the soil with simulated rainwater, to imitate field conditions. Mineralization of atrazine after 42 days of exposure was 99%, which was slower than similar studies without invertebrates, suggesting that the invertebrates may have contributed to a decline in the numbers of *Pseudomonas* sp. ADP.

Unfortunately, in field studies, bioaugmented bacteria often exhibit a low survival rate or lose their degradation ability over time. This could be due to a lack of available nutrients, competition with indigenous populations, or other conditions not favorable to bacterial growth (Sturman et al. 1995; Newcombe and Crowly 1999; Silva et al. 2004). Before introducing bacteria, vanVeen et al. (1999) recommends the use of microbiosensors to assess the soil environment for availability, distribution, and movement of soil nutrients. Thus, biostimulation, in addition to bioaugmentation, is recommended for field studies.

The natural fluctuations of soil conditions may stress bioaugmented bacteria. Inoculated bacteria survival is based on their ability to colonize soil particles. Stress on bioaugmented bacteria can be cushioned with a carrier, such as peat moss, to provided protected pore space and nutrients. Carriers must be nontoxic, biodegradable, and of consistent quality (vanVeen et al. 1999).

To overcome the loss of degradation ability, Newcombe and Crowly (1999) used a batch fermenter to deliver a bacterial consortia containing *Pseudomonas* sp. strain CN1 and *Clavibacter michiganese* ATZ1 to soils contaminated with 100 μg atrazine/g soil at different frequencies. In laboratory tests, soils that were inoculated once had a mineralization rate of 17%, but soils that were inoculated every three days had mineralization rates of 64%. In field tests, no significant mineralization occurred in soils that were only inoculated once. However, 72% mineralization was seen in soils that had eight inoculations over 12 weeks. Lima et al. (2009) saw similar results with *Pseudomonas* sp. ADP and citrate. At low concentrations (6 μg/g soil) the citrate addition was unnecessary. However, at high concentrations (62 μg/g soil) a single inoculation mineralized 87% whereas the same single inoculation spread over three days mineralized 99%. This demonstrates the vast difference between laboratory and field conditions, and that repeated applications are one strategy to improve microorganism survival rates in the field.

Another strategy, suggested by Alvey and Crowly (1996), is to plant corn. Corn did not affect the mineralization rate of the bacterial consortia (*Pseudomonas* sp. strain CN1 and *Clavibacter michiganese* ATZ1), but it did increase the survival rate of the bacterial consortia. Survival of the bacterial consortia was 30 times higher in the planted soil compared to non-planted soil for low atrazine concentrations.

Once the enzymes responsible for atrazine degradation with *Pseudomonas* sp. ADP were illuminated, biochemists began creating their own bioaugmentation sources using chemically killed, recumbent organisms engineered to overproduce enzymes of interest. The first field-scale study of this in the United States was performed by Strong, et al. (2000) using *Escherichia coli* that had been engineered to produce AtzA, *atrazine chlorohydrolase*, the enzyme responsible for the dehalogenation of atrazine into hydroxyatrazine. In the laboratory studies, 84% of initial atrazine concentration was degraded, whereas field scale studies only achieved 77% degradation. This difference in performance is attributed to field conditions, which are harder to control, including distribution of atrazine and temperature.

The public may have a negative perception of bioaugmentation with exotic genetically-modified strains of bacteria (Shapir et al. 1998). However, this may be avoided by using a method proposed by Perumbakkam et al (2006). AtzA was delivered to two biofilm populations on a laboratory scale using plasmids: a mixed culture of indigenous bacteria and *Acinetobacter* sp. BD413. The gene augmentation was successful on both accounts, resulting in 80-85% degradation of 20 mg atrazine/L. Zhao et al. (2003) also used the AtzA gene to augment naturally occurring soil bacteria. The effectiveness of the AtzA gene was compared to *Pseudomonas* sp. ADP in both aged and un-aged soils. The AtzA gene resulted in faster degradation than by *Pseudomonas*; however, the degradation of both was slowed by aging.

The possibilities of inoculation of indigenous bacteria with necessary genes for degradation of contaminants is promising, especially if complete mineralization could be achieved. However, the cost of widespread use of engineered microorganisms is yet to be shown. Also, the economics and practicality of frequent re-inoculation must also be considered when designing large-scale projects (Topp 2001).

Aging

Remediation of older sites poses special challenges: the older the site, the greater the opportunity for atrazine or its metabolites to adsorb into inaccessible pore spaces, limiting the interaction with plants, animals, bacteria, and transport. Bound residues of atrazine are assumed to be unavailable, but they may not be truly unavailable to bacteria or other organisms (Barriuso et al. 2004). Therefore, bioavailability is best described for a specific organism and a specific mode of transport (Alexander 2000).

As a contaminant is sequestered in soil, its toxicity generally decreases with time, because the contaminant moves into the pore space or within the organic matter matrix. Desorption out of these two areas is very slow. However, risk of exposure is not completely eliminated because pockets of unadsorbed contaminant may still remain. Pockets like these are aged, but not sequestered (Alexander 2000).

By convention, pesticide concentrations in soil are measured as total concentration after extraction with harsh solvents. These solvents may be releasing more pesticide than is actually bioavailable, and may be causing unnecessary remediation in places where much of the contaminant is sequestered (Alexander 2000). Barriuso et al. (2004) proposed a milder extraction technique: a mix of calcium chloride and methanol. The introduction of *Pseudomonas* sp. ADP after the extraction didn't result in further degradation of atrazine, demonstrating that most of the bioavailable atrazine had been extracted. Milder extraction methods better simulate natural conditions and this approach could impact environmental regulations.

Bioavailability is an important consideration for remediation. It is influenced by the age of a site and whether a compound is truly sequestered, and will remain sequestered. Different soils may influence bioavailability differently. It is unknown which soil properties are most important to simulate in a laboratory setting. Differences in different types of soil, such as bulk soil and rhizosphere soil should be considered. Lastly, much research has been done with atrazine, but there are far fewer papers that examine the toxicity and bioavailability of its metabolites (Sturman et al. 1995; Chung and Alexander 1998; Alexander 1999; vanVeen et al. 1999; Alexander 2000).

EVALUATION OF MULCH PROPERTIES FOE ATRAZINE CONTAMINATED GROUNDWATER REMEDIATION

Background

Atrazine is a popular broadleaf herbicide, typically used on corn (Solomon et al. 1996). Atrazine has a moderate solubility in water (34.7 mg atrazine/L at 25˚C), and a slow biodegradation rate (Faur et al. 2005). After application, atrazine can slowly infiltrate through soil to groundwater (Ma and Selim 1996; Gu et al. 2003). In 2010, the Nebraska Department of Environmental Quality found that 5% of groundwater samples exceeded the reporting limit for atrazine (Nebraska Department of Environmental Quality, 2010).

The maximum contaminant level (MCL) for atrazine in drinking water is 3 μg/L, as set by the EPA (Wilber et al. 1995). The European Union has not only set the MCL at 0.1 μg/L, but has also banned the use of atrazine due to its persistence in the environment (Wilber et al. 1995; Faur et al. 2005; Sass and Colangelo 2006; Zadaka et al. 2009). Atrazine was the most frequently detected pesticide (53%) in of water samples from vernal pools in protected areas in the United States (Battaglin et al. 2008). Exposure to atrazine causes endocrine disruption in frogs, rats, and humans (U.S. EPA Office of Pesticide Programs 1993, 2002; Villanueva et al. 2005).

Indigenous soil bacteria, when exposed to atrazine over long periods of time, may gradually increase their capacity to degrade it. Specific strains have been isolated and characterized from indigenous consortia and reapplied for remediation via bioaugmentation (Mandelbaum et al. 1995; Alvey and Crowly 1996; Newcombe and Crowley 1999; Silva et al. 2004; Smith et al. 2005; Kolic et al. 2007; Lima et al. 2009). Unfortunately, survival rates of isolated strains of bacteria in the field are low, making multiple reapplications necessary (Silva et al. 2004; Newcombe and Crowly 1999; Sturman et al. 1995).

Bacteria supported on a substrate, or a biowall, avoids the hassle of re-application rates and is better able to endure changes in operating conditions (vanVeen et al. 1997). *In situ* treatments like these can be placed to intercept the contaminant plume to prevent the spread of further contamination. Biowalls consist of bacteria supported on a natural substrate, placed to intercept contaminated groundwater flow. Removal is accomplished through adsorption or biological degradation, as the contaminated plume passes through a permeable remediation well or trench placed perpendicular to groundwater flow. This treatment is inexpensive when compared to pump and treat methods, especially when the supporting substrate is cheap and abundant, like mulch (Kao et al. 2001; Schipper et al. 2004; Seo et al. 2007). Substrates made of natural materials have been used as a supporting material for biowalls to remove naphthalene (Seo et al. 2007) and tetrachloroethylene (Kao et al. 2001), but rarely for atrazine. Ilhan et al. examined the removal of atrazine and nitrates in a woodchip bioreactor. The bulk of the atrazine removal appeared to be due to physical, rather than biological methods (Ilhan et al. 2011).

Atrazine and nitrate often coexist in groundwater (Ritter 1990). In 2010, the Nebraska Department of Environmental Quality found that 94% of groundwater samples exceeded the reporting limit for nitrate (Nebraska Department of Environmental Quality, 2010). The MCL for nitrate is 10 mg NO_3-N/L ("Basic Information" 2012). Co-removal of atrazine and nitrate, often desired in agricultural states like Nebraska, is possible with biological treatment if a carbon source is provided. This carbon source can be added separately, or could be part of the biowall itself (Schipper et al. 2004).

Materials and Methods

Organic Mulch

Three types of common gardening mulch were selected as possible substrates for biofilm support: cedar, cypress, and hardwood. The mulch was purchased from a local gardening supplies store in Lincoln, NE., USA.

Mulch samples were prepared using the method reported by Seo et al. (Seo et al. 2007), with modifications. Mulch samples were dried in a fume hood overnight, ground for 30 seconds using a Black and Decker food processor for 30 seconds, and sieved to a #10 mesh size (2 mm). To remove the influence of natural bacteria and fungi, the mulch samples were autoclaved twice before they were finally dried in a 105°C oven.

Physical and Chemical Properties of the Mulch

The pH, conductivity, water content, organic content, and cation exchange capacity (CEC) of each mulch was measured to determine the best kind of mulch to use as a supporting material for a biowall. The pH, conductivity, and organic content (Loss-On-Ignition method) were determined using techniques from the Methods of Soil Analysis (Klute et al. 1994; Weaver et al. 1994). The pH and conductivity measurements were taken after 10 grams of mulch sample mixed with 100 mL of nanopure water for 10 minutes. The organic content (Loss-On-Ignition method) measurements were performed using a weight difference in mulch samples before and after ignition in a muffle furnace (550°C).

The water content was determined by taking the difference in weights between unprepared mulch and after drying it in a 105°C oven overnight.

The values for organic content (High Range COD and Low Range COD) were found using the reactor digestion method with high and low range COD digestion vials, respectively, available from Hach Chemicals (Loveland, CO, USA).

The Cation Exchange Capacity (CEC) was determined using a modification of the barium chloride method (Ross 1995; Ciesielski and Sterckeman 1997), wherein the concentrations of barium and magnesium ions were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Absorption Spectroscopy (AAS), respectively, rather than by accurate weighing. In this method, the adsorbed barium exchanges with magnesium and precipitates as $BaSO_4$. Briefly, 2.5 g of mulch sample was shaken with 30 mL of a 0.1 M $BaCl_2$ solution for one hour. The supernatant was collected and analyzed by ICP-MS. The mulch samples were equilibrated with 30 mL of 2 mM $BaCl_2$. Lastly, the mulch samples were shaken with 0.02M $MgSO_4$ for two hours.

Each datum point represents an average of three samples. The mulch properties were compared with SigmaPlot 12 (Systat Software, San Jose, CA, USA) using a one-way analysis of variance (ANOVA) with a significance level of α=0.05.

Isotherm Experiments

The adsorptive capacity of mulch for removal of atrazine, nitrate, and both atrazine and nitrate was quantified by conducting isotherm experiments. Sodium nitrate and atrazine were purchased from chemical suppliers. Amber glass bottles with Teflon caps were used to minimize the effect of light on the samples. The bottles were filled with varying weights of mulch and filled to the rim with selected concentrations of solution to avoid any headspace (Environmental Protection Agency 1992). The bottles were then sealed with Parafilm, capped with a Teflon cap, and again sealed with Parafilm. Control tests were conducted to ensure that the mulch samples did not contain atrazine or nitrate and atrazine or nitrate were not adhering either to the glass container or to the filter paper.

Weights and concentrations were selected so that the final concentration of a chemical in solution would be either less than 90% or greater than 10% of the initial solution concentration. The mono adsorbate data were obtained for concentration ranges of 0.5-20 mg NO_3-N/L and 5-10 mg atrazine/L. The nitrate adsorption data were obtained using two concentration ranges, 0.5-3.4 mg NO_3-N/L and 0.5-20 mg NO_3-N/L. The lower range reflects the median background concentration of nitrate in Nebraska Groundwater in 2009, 4.7 mg NO_3-N/L ("Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater" 2011).

Binary adsorbate data were obtained for two concentrations: 7 and 3.5 mg NO_3-N/L, paired with 5 and 2.5 mg atrazine/L, respectively. The nitrate concentration was selected based on the average background concentration of nitrate in Nebraska groundwater in 2009, 7.8 mg NO_3-N/L ("Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater" 2011).

The initial atrazine concentration in solution for both the mono and binary adsorbate isotherms is more than a thousand times higher than typical background concentrations in groundwater, which are typically less than 1.5 µg atrazine/L (Nebraska Department of Environmental Quality, 2010). Higher concentrations were used to increase the concentration gradient and ensure more reliable adsorption data.

The bottles were tumbled at 18 rpm for 5 days. The equilibrium time was selected based on a literature review (Seo et al. 2007). After 5 days, the solutions were filtered using a filtration apparatus with glass fiber filter paper to remove particulate matter, and concentrations of the chemicals were determined, as described in Section 3.2.4. The adsorption capacity of nitrate and atrazine were calculated from the Langmuir and Freundlich data.

The equation for the Langmuir isotherm is given as

$$\frac{1}{q_e} = \frac{1+K_L C_e}{q_{max}\, K_L C_e} \tag{1}$$

or, in a linearized form,

$$\frac{C_e}{q_e} = \frac{1}{q_{max} K_L} + \frac{C_e}{q_{max}} \tag{2}$$

where q_e is the amount of adsorbed compound on the mulch at equilibrium (mg/kg), q_{max} is the maximum adsorption capacity (mg/kg), K_L is the Langmuir constant (L/mg), and C_e is the concentration of compound in solution at equilibrium (mg/L) (Tchobanoglous, et al. 2003).

The equation for the Freundlich isotherm is given as

$$q_e = K_F C_e^{1/n} \tag{3}$$

or, in a linearized form,

$$\log(q_e) = \log(K_F) + \frac{1}{n}\log(C_e) \tag{4}$$

where K_F ((mg/kg)(mg/L)$^{-n}$) and n are constants representing sorption capacity and intensity, respectively (Tchobanoglous, et al. 2003).

Data were discarded if the equilibrium concentration in solution was greater than 90% or less than 10% of the initial solution concentration to ensure that the adsorption quantity was accurate. A 95% confidence interval was used to test for possible outliers before fitting to adsorption models. The ratio of q_e/C_e and the average value of isotherm coefficients were compared between data sets using a one way analysis of variance (ANOVA) with a significance level of 0.05.

Instrumental Analysis

Nitrate was analyzed using a Dionex Ion Chromatograph (ICS-90) (Sunnyvale, CA, USA) with a Dionex AS40 Autosampler. Sample size was 5 mL. The column was a 4x250mm IonPac AS14. An isocratic mobile phase consisted of 3.5 mM Na_2CO_3 and 1 mM $NaHCO_3$. The data were analyzed with Chromeleon v. 6.7, Build 1820.

Atrazine was analyzed using a Waters Alliance 2695 High-Performance Liquid Chromatograph (HPLC) (Milford, MA, USA) connected to a Waters 2996 Photodiode Array (PDA) detector. The sample size was 25 μL and was injected at 1 mL/min. The mobile phase was a gradient of water and methanol, as shown in Figure 1. The column type was Kromasil 100-5C8 and was 4.6 m in length and 250 mm in diameter. The column temperature was 50°C. The detector wavelength was set at 222 nm. The data were analyzed with Waters Empower Software Build #1154.

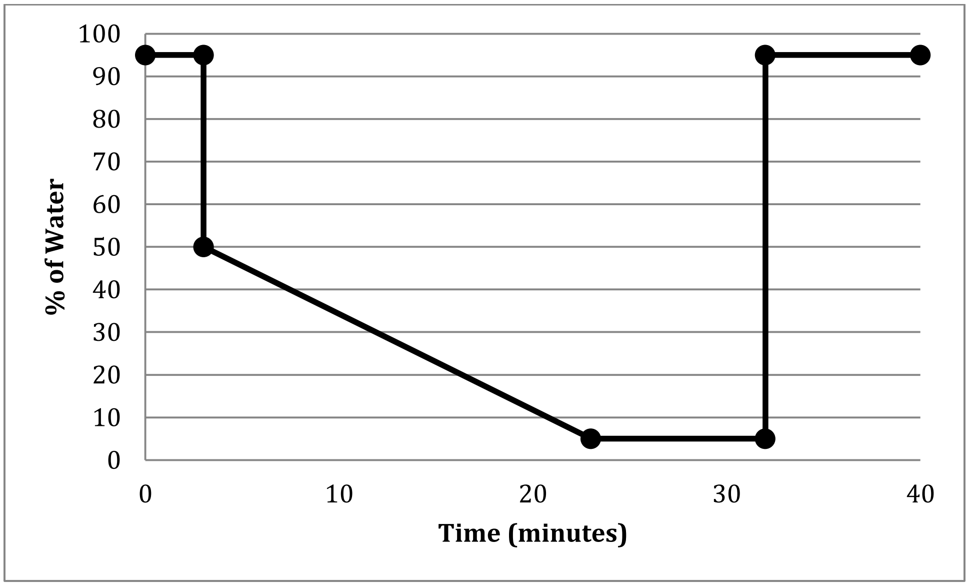

Figure 1: High-performance liquid chromatography gradient for atrazine analysis

RESULTS

Physical and Chemical Properties of the Mulch

The pH, conductivity, water content, organic content, and cation exchange capacity (CEC) were measured to determine the best kind of mulch to use as a supporting material for a biowall (Table 2). Each data point represents an average of three samples.

Table 2: Physical and chemical properties of cedar, cypress, and hardwood mulch. Superscripts indicate pairings of statistical significance.

	Cedar	**Cypress**	**Hardwood**
Ph	6.73 ± 0.73^{a}	5.32 ± 0.02^{a}	5.98 ± 0.51
Conductivity (µS/cm)	129.1 ± 25.3	99.2 ± 6.5	105.8 ± 17.1
Water content (%)	44.89 ± 5.14	53.82 ± 3.98	40.42 ± 7.68
Organic content (mg/g)			
Loss-On-Ignition	976.95 ± 2.52^{b}	996.35 ± 2.16^{b}	984.17 ± 0.9^{b}
Low Range COD	1186.67 ± 323.93^{c}	$2000 \pm 144.22^{c,d}$	1253.33 ± 106.92^{d}
High Range COD	1246.73 ± 118.34	1301.33 ± 370.91	1005.08 ± 2.8
Cation Exchange Capacity (meq/100g)	7.97 ± 1.45	6.73 ± 0.68	7.23 ± 1.25

Loss-on-Ignition was the only property measured that showed a statistically significant difference between all three types of mulch. The pH difference between cedar and cypress mulch is statistically significant and the Low Range COD difference between the pairs of cedar-cypress and cypress-hardwood is statistically significant. More accurate measuring equipment or methods could be used to verify these statistics in the future.

Qualitatively, among the three types of mulch, cedar mulch has the highest pH, conductivity, and CEC, whereas cypress mulch has the lowest values. Seo et al. (2009) demonstrated a similar pattern: mulches with a high conductivity also exhibited a high CEC. This association can be expected, because CEC is a measure of the ability of the material to exchange cations and electrical conductivity is a measure of the ion content of a solution (Henry 1997).

Henry (1997) reported that CEC was inversely proportional to water content. This is shown in Table 2; qualitatively, cypress has the highest water content and the lowest CEC. Research by the Virginia Extension Board concluded the inverse: High CEC was linked to high organic and water content in clay soils (Grisso et al. 2009). This is most likely because mulch has different properties than clay soil. Further investigation is needed to verify this.

Surface area measurements were beyond the scope of this limited project. However, the surface areas of the cypress and hardwood mulch were assumed to be similar to results from Seo et al. (2009). The authors performed a Brunauer, Emmett, and Teller (BET) isotherm revealing that cypress and hardwood mulch had a surface area of 11-18 m^2/g and 25-32 m^2/g), respectively.

Isotherm Experiments: Mono Systems

Isotherm experiments were performed to quantify the adsorptive capacity of cedar, cypress, and hardwood mulch for the mono systems of atrazine and nitrate, as well as the binary system of atrazine-nitrate. The raw data for the mono-system isotherms for atrazine and nitrate are given in Appendices A and B, respectively.

The raw isotherm data were graphed (Cole, 2012) for atrazine and nitrate to determine which of the Giles isotherm types would best describe the data (Giles et al. 1974a). The atrazine data were linear, thus appearing to show that atrazine is exhibiting C-type, or constant partitioning, adsorption. However, Calvet (1989) suggests that an L-type, or Langmuir adsorption, better describes atrazine adsorption. The atrazine isotherm appears to be a C-type, likely due to the range of concentrations examined; a wider range of atrazine concentrations may show a more distinct L-type isotherm.

The raw isotherm data for nitrate did not show a distinct isotherm type (Cole, 2012). Nitrate should be exhibiting a H-type, or high affinity, adsorption (Giles et al. 1974b). Nitrate is a negatively charged ion that has a high affinity for positively charged sites on the surface of the mulch. However, organic materials generally don't have a high number of positive sites. The number of positive sites can be increased with surface treatments, such as acidification (Cays-Vesterby 2009).

Table 3: Langmuir and Freundlich constants for mono atrazine isotherm

	Langmuir Isotherm				Freundlich Isotherm			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	-1.79	-0.03	0.30	11	0.79	0.85	0.91	12
Cypress	1.16	0.06	0.16	12	0.05	1.04	0.94	12
Hardwood	-0.65	-0.06	0.73	9	0.07	0.84	0.94	11

Table 4: Langmuir and Freundlich constants for mono nitrate isotherm

	Langmuir Isotherm				Freundlich Isotherm			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.12	-4.53	0.92	12	-0.02	2.45	0.87	8
Cypress	0.18	2.11	0.90	22	-0.001	1.47	0.02	8
Hardwood	0.13	-1.94	0.98	17	-0.01	1.93	0.62	11

Table 5: Langmuir and Freundlich constants for mono nitrate isotherm for C_o<3.4 mg NO_3-N/L

	Langmuir Isotherm				**Freundlich Isotherm**			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.34	-5.53	0.86	9	-0.10	3.73	0.14	7
Cypress	0.06	-3.55	0.81	17	-0.03	1.54	0.09	8
Hardwood	0.17	-5.86	0.79	12	-0.04	1.99	0.12	9

Atrazine adsorption from the aqueous phase was correlated using the Langmuir isotherm, as shown in Equation 2, and the Freundlich isotherm, as shown in Equation 4. The mono atrazine isotherm results are shown in Table 3. Nitrate adsorption from the aqueous phase was also correlated using the Langmuir and Freundlich isotherms. The isotherm results for nitrate are shown in Table 4 for all concentrations, and in Table 5 for C_o<3.4 mg NO_3-N/L. The corresponding figures for nitrate for all concentrations and for C_o<3.4 mg NO_3-N/L can be found in Cole (2012). Based on the R^2 values, Tables 3, 4, and 5 show that the Freundlich isotherm best describes atrazine adsorption and the Langmuir isotherm best describes nitrate adsorption.

A statistical analysis of the ratio of the equilibrium concentration of a compound on the mulch and the equilibrium concentration of a compound in solution (q_e/C_e) was performed for each system using a one way ANOVA. The analysis revealed that there was no significant difference between the three types of mulch for either atrazine or nitrate adsorption. There was not a significant difference between the q_e/C_e ratio for the entire nitrate range and the small (C_o<3.4 mg NO_3-N/L) nitrate range. A statistical analysis comparing the average values of coefficients for all nitrate concentrations and for C_o<3.4 mg NO_3-N/L found the difference was not significant, for either the Freundlich or Langmuir isotherms.

Qualitatively, Tables 3 and 3.3 show that cypress mulch has the highest adsorption capacity for nitrate, 0.18 mg/g (q_{max}), and atrazine, 1.04 (mg/g)(mg/L)$^{-n}$ (K_F), while cedar and hardwood have lesser, similar values. At low concentrations of nitrate, as in Table 5, cedar mulch has the highest adsorption capacity for nitrate, 0.06 mg/g (q_{max}). The sorption capacity of atrazine on cedar and hardwood mulch, 0.8 (mg/g)(mg/L)$^{-n}$, was the same as the value found by Alam et al. (2000) for adsorption of atrazine on wood charcoal.

Isotherm Experiments: Binary Systems

The binary system of atrazine-nitrate was modeled similarly to the mono systems, using both Freundlich and Langmuir isotherms. The raw isotherm data were graphed (Cole, 2012) for atrazine and nitrate to determine which of the Giles isotherm types would best describe the data (Giles et al. 1974a). The data indicated that atrazine was still exhibiting linear adsorption, approximating the L-type, even in the presence of nitrate. Nitrate data showed that nitrate adsorption is highly dependent on surface property variations of the mulch.

The binary atrazine results are shown in Table 6. Binary nitrate isotherm results with initial concentrations of 7 and 3.5 mg NO_3-N/L can be seen in Tables 7 and 8, respectively. Based on the R^2 values, Tables 6, 7, and 8 show that the Freundlich isotherm continues to best describe atrazine adsorption, whereas neither the Langmuir nor the Freundlich isotherm best describes nitrate adsorption for all much types.

Table 6: Langmuir and Freundlich constants for atrazine in the binary isotherm

	Langmuir Isotherm				Freundlich Isotherm			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.45	0.24	0.58	7	0.06	1.08	0.92	10
Cypress	0.32	0.40	0.65	11	0.05	1.09	0.83	11
Hardwood	1.38	0.05	0.16	10	0.07	1.00	0.94	10

Table 7: Langmuir and Freundlich constants for nitrate in the binary isotherm with an initial concentration of 7 mg NO_3-N/L

	Langmuir Isotherm				Freundlich Isotherm			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.03	-0.32	0.02	5	0.07	0.56	0.04	5
Cypress	0.01	-0.23	0.89	5	-0.29	34.5	0.82	5
Hardwood	0.006	-0.21	0.85	6	-0.23	24.45	0.79	5

Table 8: Langmuir and Freundlich constants for nitrate in the binary isotherm with an initial concentration of 3.5 mg NO_3-N/L

	Langmuir Isotherm				**Freundlich Isotherm**			
	q_{max}	K_L	R^2	Number of Points	1/n	K_F	R^2	Number of Points
	mg/g	*L/mg*				*((mg/g)(mg/L)$^{-n}$)*		
Cedar	0.01	-0.47	0.74	7	-0.30	7.04	0.96	6
Cypress	0.03	-0.87	0.36	6	-0.02	1.28	0.33	6
Hardwood	0.004	-0.41	0.94	5	-0.82	203.66	0.99	6

A statistical analysis of the ratio of equilibrium concentration of a compound on the mulch and the equilibrium concentration of a compound in solution (q_e/C_e) was performed for each system using a one way ANOVA. The analysis revealed that the only significant difference between mulch types was during binary atrazine adsorption for the pairs of cypress-hardwood and cedar-hardwood. There was not a significant difference between the three types of mulch in the binary nitrate systems.

The differences in the q_e/C_e ratio between the mono and binary atrazine isotherms were significant for all systems. Also, the q_e/C_e ratio between the small concentration (C_o<3.4 mg NO_3-N/L) mono and the entire nitrate isotherm were significantly different from both binary nitrate systems, 3.5 and 7 mg NO_3-N/L.

In a binary system, the compound with lower solubility, atrazine, is favored for adsorption and that the co-adsorbate, nitrate, in this case, has no influence on adsorption (Faur et al. 2005). This relationship can be seen graphically in Figure 2. A similar comparison was done by Faur et al. (2005) with atrazine and deethylatrazine or deisopropylatrazine. When binary and mono systems for atrazine adsorption on cypress mulch are graphed together, the slope is nearly the same, as seen in Equations 5 and 6.

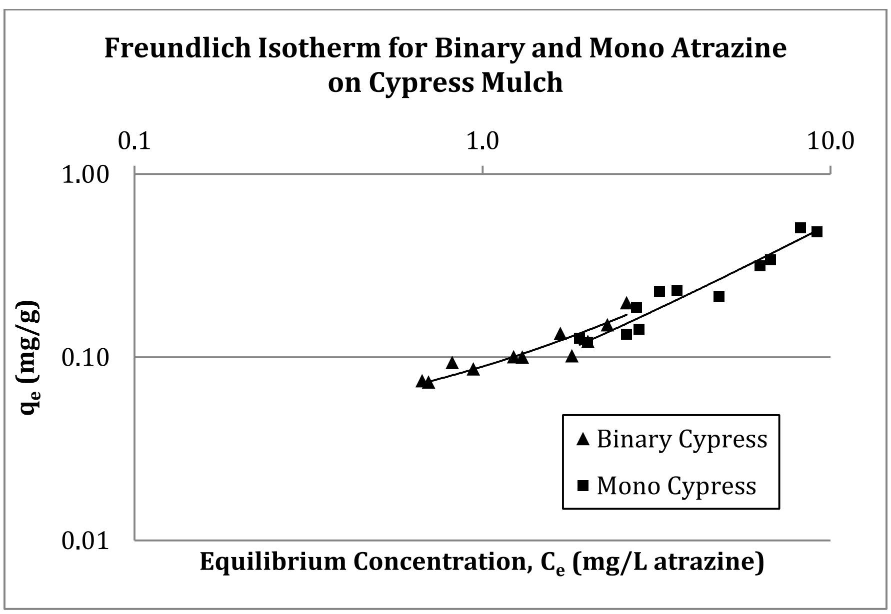

Figure 2: Freundlich adsorption isotherm for atrazine on cypress mulch in the presence and absence of nitrate

The equations for the best fit lines in Figure 2 are as follows:

In the mono cypress (absence of nitrate), the equation is:

$$\log(q_e) = 0.0517\log(C_e) + 0.0184 \quad (5)$$

where q_e is the amount of atrazine adsorbed on the mulch at equilibrium (mg/g) and C_e is the concentration of atrazine in solution at equilibrium (mg/L). This equation has an R^2 of 0.94.

In the binary cypress (presence of nitrate), the equation is:

$$\log(q_e) = 0.0511\log(C_e) + 0.0378 \quad (6)$$

This equation has an R^2 of 0.83.

A statistical analysis comparing the average values of the Langmuir and Freundlich coefficients for atrazine in the mono and binary systems (Tables 3 and 6) indicated that they are not significantly different.

Qualitatively, as in the mono system for atrazine, cypress mulch has the highest adsorption capacity, 1.09 (mg/g)(mg/L)$^{-n}$ (K_F). Also, cypress mulch has the lowest value of sorption intensity (1/n) for atrazine in both the mono and binary systems. For low values of 1/n, less energy is required for the adsorbate to adsorb on the surface; this low energy barrier results in faster adsorption (American Water Works Association 1999; Hristovski et al. 2009).

The binary nitrate isotherms were obtained for two initial concentrations: 7 and 3.5 mg NO_3-N/L, paired with concentrations of 5 and 2.5 mg atrazine/L, respectively. The Langmuir and Freundlich isotherm data can be seen in Tables 7 and 8 for 7 and 3.5 mg NO_3-N/L, respectively. A statistical analysis comparing the ratio of q_e/C_e between the 7 mg NO_3-N/L system and the 3.5 mg NO_3-N/L system revealed that the apparent differences were not statistically significant. Additionally, a statistical analysis comparing the average values of the coefficients for the two concentrations revealed that they were not significant for the Langmuir or the Freundlich isotherm.

Comparison of the average values of the Langmuir coefficients for nitrate in the mono and binary systems (Tables 4, 3.4, 7, and 8) revealed a significant difference in maximum adsorption capacity (q_{max}), but not the Langmuir constant (K_L), when the entire mono nitrate system is compared to both binary systems. The reverse is true when the small (C_o<3.4 mg NO_3-N/L) mono system is compared to both binary systems: the maximum adsorption capacity (q_{max}) is not significantly different, but the Langmuir constant (K_L) is different. The Langmuir constant is related to the energy of adsorption and is proportional to the adsorption bond (American Water Works Association 1999). Therefore, the presence of atrazine affects the capacity for nitrate adsorption at high concentrations of nitrate, but at low concentrations of nitrate, the energy of nitrate adsorption is affected. A statistical analysis comparing the average values of the Freundlich coefficients for nitrate in the mono and binary systems (Tables 4, 4, 7, and 8) revealed that there was no significant difference between coefficients.

Graphically, both the Langmuir and Freundlich isotherms for binary nitrate were nearly vertical, regardless of starting concentration. In contrast, the mono nitrate behavior exhibited smaller slopes at low concentrations for the Freundlich isotherm, and linear behavior for the Langmuir isotherms. This change of adsorption behavior in the presence of atrazine implies that atrazine is affecting nitrate adsorption. The differences between the binary and mono systems are most apparent in the Freundlich isotherm when binary and mono data are graphed together (Cole, 2012). This sudden increase in slope may have been caused by cation effects (Fawcett and Sellan 1977), variations in surface organic functional groups (Laird et al. 1994), other surface composition differences between samples or, most likely, the blocking of desirable nitrate sites with atrazine.

Qualitatively, the Langmuir isotherms for nitrate in the binary system reveal that, at the lower concentration (3.5 mg NO_3-N/L), the cypress mulch had the highest adsorption capacity (q_{max}), 0.03 mg/g. However, at the higher concentration (7 mg NO_3-N/L) the cedar mulch had the highest adsorption capacity (q_{max}), 0.03 mg/g. In both cases, the hardwood mulch had the lowest adsorption capacity (q_{max}), 0.004 mg/g for 3.5 mg NO_3-N/L and 0.006 mg/g for 7 mg NO_3-N /L. Qualitatively, the Freundlich isotherms for nitrate in the binary system suggest that cypress has the highest adsorption capacity (K_F) for nitrate at 7 mg NO_3-N/L, 34.5 (mg/g)(mg/L)$^{-n}$. However, hardwood has the highest adsorption capacity (K_F) for nitrate at 3.5 mg NO_3-N/L, 203.66 (mg/g)(mg/L)$^{-n}$.

ATRAZINE CONTAMINATED GROUNDWATER REMEDIATION USING MULCH BIOWALLS

Background

The extensive use of the broadleaf herbicide atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and its persistence in soil and groundwater is a worldwide concern. Atrazine and nitrate are often found together in groundwater of agricultural states (Ritter 1990).

Ongoing research on remediation techniques for pesticide contamination includes chemical and biological treatment processes. Waria et al. (2009) used zero valent iron and ferrous sulfate to degrade atrazine chemically in soil. Soybean oil was also added to provide a carbon source for biological activity. Atrazine, initially at a concentration of 500 mg/kg soil, was reduced by 79% in 342 days. Tafoya-Garnica et al. (2009) used a fluidized bed reactor containing biological granular activated carbon to achieve high degradation rates. Modin et al. (2008) used a methane-fed bioreactor intended to remove both atrazine and nitrate. However, atrazine removal was not successful. Bianchi et al. (2006) successfully used photolysis, photocatalysis (with TiO_2), and ozonation for atrazine degradation. Processes such as these require the presence of a nutrient source, such as methane or soybean oil, and specialized treatment, such as ultraviolet radiation or biological activated carbon. These additions may be worthwhile for short time periods, but would be costly over time, due to materials and operations costs.

Passive treatments are much more cost effective and require little specialized equipment. A permeable reactive barrier, or biowall, can be placed to intercept a contaminated groundwater plume, forming a bioreactive zone. Biowalls consist of bacteria supported on a natural substrate, placed to intercept contaminated groundwater flow. Removal is accomplished through adsorption or biological degradation, as the contaminated plume passes through a permeable remediation well or trench placed perpendicular to groundwater flow. Biowalls are typically made of cheap, abundant materials that perform remediation using a combination of bacterial growth and adsorption. *In situ* treatments such as these are low maintenance and can endure changes in operating conditions (Kao et al. 2001; Kalin 2004; Seo et al. 2007).

Biowalls have been tested extensively for denitrification. They can be placed either directly in aquifers or in vadose zones above aquifers (Kao et al. 2001; Kalin 2004). They can also be used to treat water from subsurface tile drainage (Ilhan et al. 2011). Denitrification requires a carbon source, which can be obtained from the organic content of the barrier itself, or added separately, as in Hunter (Schipper et al. 2004; Hunter 2009).

Biowalls supported on a natural substrate, such as mulch or peat moss, have been studied for naphthalene (Seo et al. 2007) and tetrachloroethylene (Kao et al. 2001) removal, but rarely for atrazine. Ilhan et al. examined the removal of atrazine and nitrates in a woodchip bioreactor. The bulk of the atrazine removal appeared to be due to physical, rather than biological methods (Ilhan et al. 2011).

Materials and Methods

Organic Mulch

Cypress mulch was selected as the supporting substrate for the biowall, based on physical and chemical analysis in comparison with cedar and hardwood mulch, as well as isotherm experiments, as described in Section 3. Physical and chemical properties of cypress mulch include a pH of 5.32$\pm$0.02, an electrical conductivity of 99.2$\pm$6.5μS/cm, a water content of 53.82$\pm$3.98%, and a cation exchange capacity of 6.73$\pm$0.68 meq/100g.

Cypress mulch was prepared using a modification of the method of Seo et al. (2007), as described in Section 3.3.1.

Isotherm Experiments

The adsorptive capacity of the mulch for removal of atrazine and nitrate was quantified with isotherm experiments, as described in Section 3. Various weights of mulch were paired with various concentrations of atrazine and nitrate, placed in brown glass bottles, and tumbled at 18 rpm for 5 days. Adsorption data was fitted to Freundlich and Langmuir isotherms. Qualitatively, for atrazine adsorption in the binary system, cypress mulch had the lowest 1/n value, 0.05, and the highest K_F value, 1.09 (mg/g)(mg/L)-n, meaning that it rapidly adsorbs atrazine and has a relatively high capacity for the adsorption of atrazine, respectively. Qualitatively, for nitrate adsorption in the binary system, cypress mulch had the highest q_{max} value, 0.03 mg/g, meaning that it has a relatively high capacity for nitrate adsorption.

Column Set Up

A laboratory-scale biotic column was used to simulate implementation of a biowall. The column and clamps were purchased from a glassware manufacturer and was the same model used by Seo et al. (2007). Figure 3 shows a schematic of the column set up. The column is 30 cm in length and has a 3.8 cm inner diameter. The body of the column has five sample ports and there are two additional ports on each of the end caps, for a total of seven sample ports.

Plastic tubing was used for connections. The tubing was attached to the pump using plastic hose barbs. Each of the seven sample ports were plugged using plastic septa. Both the column and the connecting tubing were wrapped in aluminum foil to keep out light. The feed solution was wrapped in towels to keep out light. A piece of vinyl tubing connected the effluent port to a hose barb, and then to the tubing, which ran to the waste container.

A glass groundwater feed container (22 L in volume) was capped with a green neoprene stopper to limit evaporation. A hose barb imbedded in the neoprene stopper allowed the tubing to enter the groundwater feed container. The waste container was a 113 L drum also affixed with a neoprene stopper with an imbedded hose barb to allow the passage of fluids.

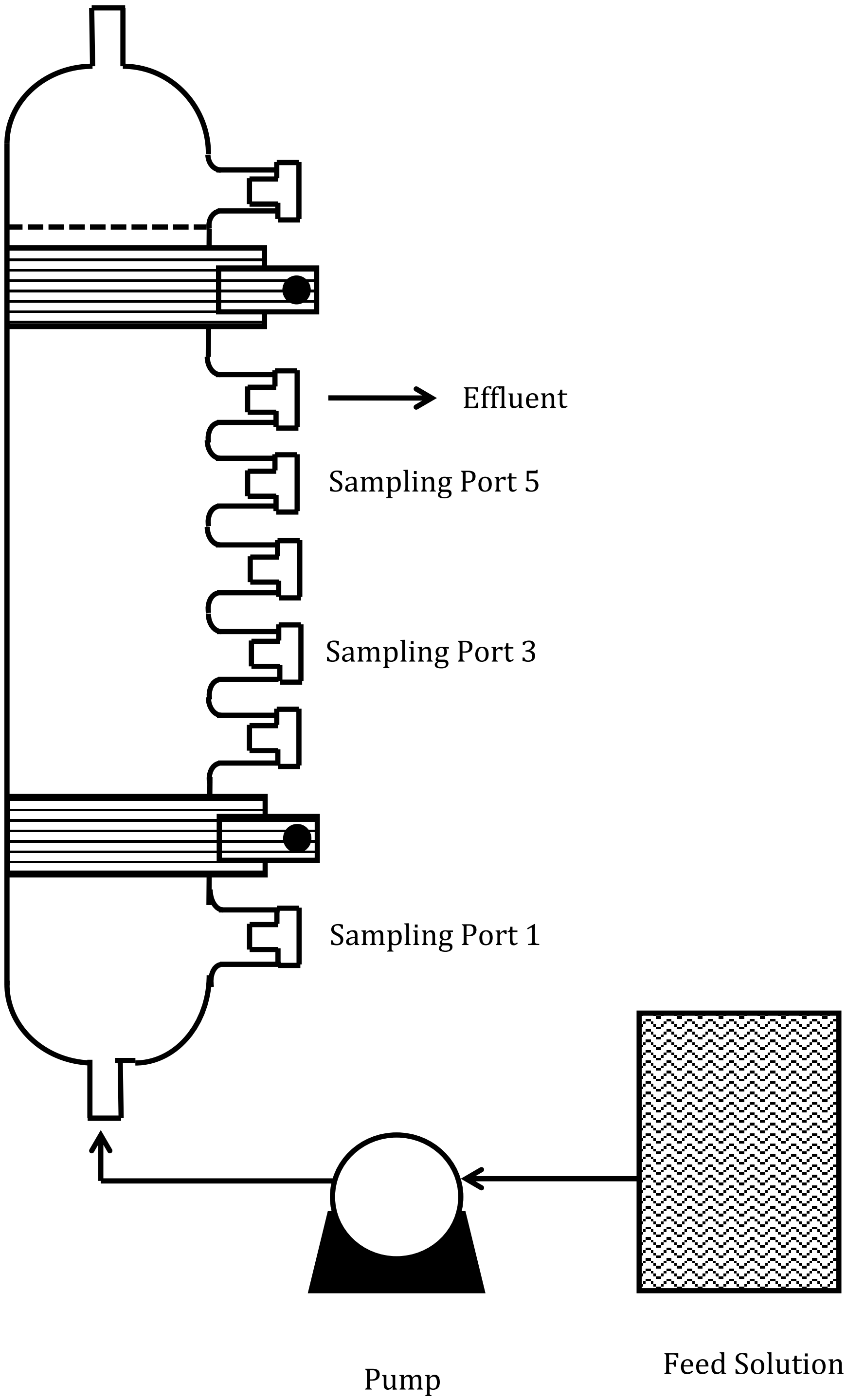

Figure 3: A schematic of the column set up

Forty-five grams of cypress mulch was added to the column. The mulch was held in the column at the influent and effluent ports with a fiberglass mesh. The column was seeded with 4 L of primary effluent from the Teresa Street wastewater treatment plant (Lincoln, NE, USA) pumped at 2.5 mL/min. This flow rate was chosen based on a literature review (Seo et al. 2007). Next, the column was fed with a simulated groundwater solution, whose composition can be seen in Table 9. The nitrate concentration was chosen based on the average background concentration of nitrate in Nebraska Groundwater in 2009, 7.8 mg NO_3-N/L (“Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater “ 2011). The composition of the remainder of the groundwater was selected based on a literature review (Dahab and Sirigina 1994; Nebraska Department of Environmental Quality, 2010).

The atrazine concentration in the column is a thousand times higher than the typical background concentration in groundwater, which is typically less than 1.5 µg atrazine/L (Nebraska Department of Environmental Quality, 2010). Atrazine is an unfavorable nitrogen source for bacteria (Clausen et al. 2002; Hunter and Shaner 2010). Using an initial concentration of atrazine that is the same order of magnitude as the initial concentration of nitrate was intended to encourage utilization of atrazine by bacteria.

Table 9: Chemical composition of synthetic groundwater solution (after Dahab and Sirigina 1994)

Compound	**Amount** (*mg/L*)
KH_2PO_4	150
K_2HPO_4	32.5
$FeSO_4 \cdot 7H_2O$	0.816
Na_2MoO_4	0.2365
$MnSO_4 \cdot 7H_2O$	0.1565
$CaCl_2 \cdot 6H_2O$	0.526
Na_2SO_3	250
$CoCl_2 \cdot 6H_2O$	1.052
$NaNO_3$	42.5 (7 mg NO_3-N/L)
Atrazine	1

The potassium phosphate buffers stabilized the pH. The ferrous sulfate, sodium molybdate, magnesium sulfide, and calcium chloride provided minerals necessary for bacterial growth. The sodium sulfite and cobalt chloride were added at twice their stoichiometric concentration in an effort to keep the dissolved oxygen level below 1 mg O_2/L to promote denitrification.

The synthetic groundwater solution was mixed in a 22 L glass container and fed to the column from the bottom. Dissolved oxygen and pH readings were taken biweekly from the influent and effluent ports. Samples were also taken biweekly using a syringe from ports 1 (influent), 3, 5, and 6 (effluent), as shown in Figure 3. The sample size was 12 mL. The samples were filtered to remove particulate matter. Concentrations of nitrate and atrazine were determined, as described in Section 4.3.4.

The average concentration of both atrazine and nitrate taken from each sample port were compared in SigmaPlot 12 (Systat Software, San Jose, CA, USA) using a one way analysis of variance (ANOVA) with a significance level of 0.05.

Results and Discussion

The column ran for three months with an average influent pH of 6.64 and average dissolved oxygen of 2.23 mg O_2/L. The raw column data can be found in Cole (2012). The oxygen scavengers, sodium sulfite and cobalt chloride, added at twice their stoichiometric concentrations, were not sufficient to overcome the daily diffusion of oxygen from the atmosphere into the feed solution. Oxygen rich conditions are not conducive to denitrification, because oxygen is desired over nitrate as an electron acceptor. Anoxic conditions are required for denitrification to occur, meaning a dissolved oxygen level below 0.5 mg O_2/L (van Haandel and van der Lubbe 2007).

During the course of the experiment, the cypress mulch did not degrade sufficiently to serve as an electron donor for denitrification. The rate of decomposition of mulch is inversely proportional to the ratio of lignin to nitrogen. The ratio of lignin to nitrogen is 125 in cypress mulch, making it very resistant to decomposition (Duryea et al. 1999). Atrazine makes a poor electron donor for nitrate reduction because only the carbon atoms in the side chains are a readily available energy source (Katz et al. 2000, 2001). Cypress mulch, as found in the mulch characterization experiments in Section 3.4.1, was the most acidic of the three types of mulch examined, with a pH of 5.32. Acidic conditions are not desirable for biological activity (Seo et al. 2007) and may have limited biofilm development.

Acetic acid was added on day 61 to serve as a carbon source to encourage denitrification. Using the suggested ratio of carbon to nitrogen of 1:1.45 for denitrification from Dahab and Lee (1988), 24.3 mL/L of glacial acetic acid was added. The acid was neutralized with 17,535 mg/L sodium hydroxide before being added to the groundwater solution. Both acetic acid and sodium hydroxide were purchased from EM Science (Gibbstown, NJ, USA).

The addition of acetic acid brought the influent dissolved oxygen down to 1.6 mg O_2/L, which is still too aerobic for denitrification. Additionally, the amount of acid added may not have been sufficient to satisfy the energy requirements of both aerobic and anaerobic bacteria.

Measured concentrations of atrazine and nitrate in the influent and effluent are shown in Figures 4 and 5, respectively. The influent concentrations of atrazine and nitrate were 1 mg atrazine/L and 7 mg NO_3-N/L, respectively. Note that on day 61, acetic acid was added as a carbon source.

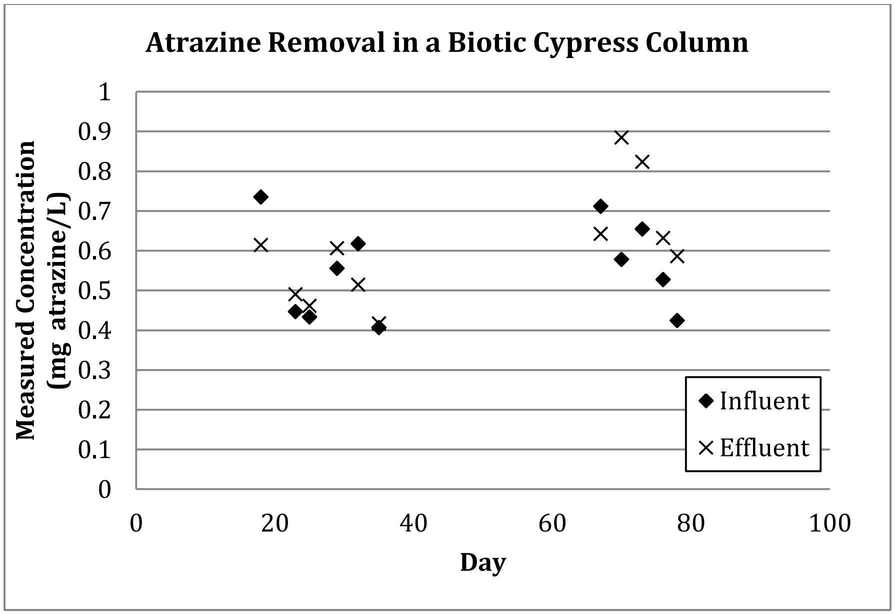

Figure 4: Measured atrazine concentrations in the influent and effluent ports of a biotic cypress column

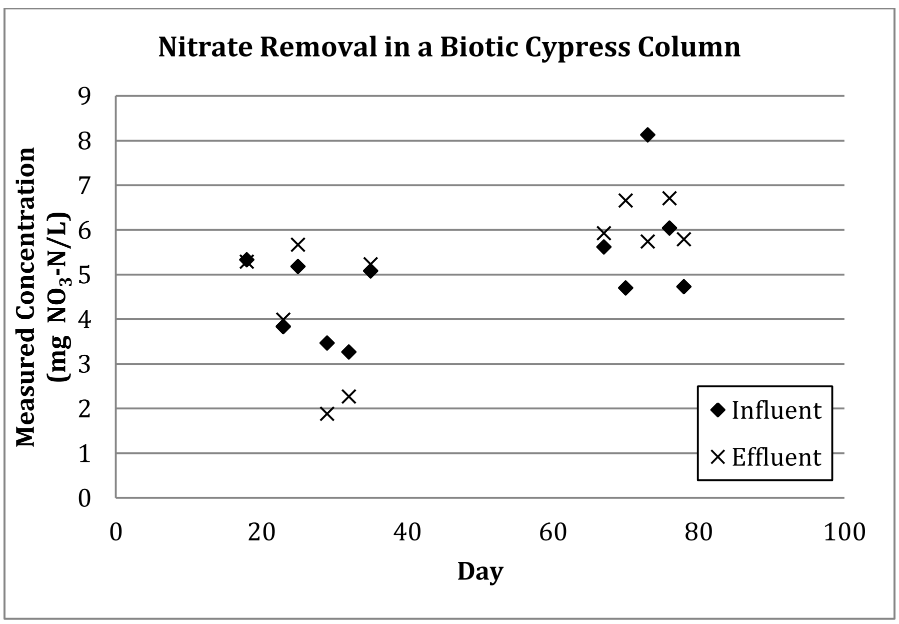

Figure 4.3: Measured nitrate concentrations in the influent and effluent ports of a biotic cypress column

Figures 4 and 5 show fluctuations in both influent and effluent concentrations, including times when the effluent concentration exceeds the influent concentration. A statistical analysis comparing average concentrations measured from different column ports found that these fluctuations are not significant. Graphs comparing the data from sample ports 3 and 5 can be found in Cole (2012).

Denitrification is not affected by the presence of atrazine (Yeomans and Bremner 1987; Ilhan et al. 2011). However, atrazine degradation can be affected by the presence of nitrate. When present in excess, nitrate provides a more readily accessible source of nitrogen for atrazine-degrading bacteria, thus inhibiting the degradation of atrazine (Clausen et al. 2002; Hunter and Shaner 2010). Physical removal of atrazine, as in Ilhan et al. (2011), was not occurring, because the adsorption capacity of the mulch was exhausted early in the experiment.

CONCLUSIONS

This study investigated the ability of a mulch biowall to remove atrazine and nitrate from contaminated groundwater. First, the physical and chemical properties of three types of mulch were characterized. Next, the adsorption capacity of the mulch for atrazine and nitrate was analyzed in a series of isotherm experiments. Finally, the feasibility of implementing a cypress mulch biowall with a laboratory-scale biotic column designed to remove atrazine and nitrate was evaluated. From this research, the following conclusions were made:

Mulch Characterization

- Cypress mulch had a significantly higher organic carbon content than cedar or hardwood mulch.
- Qualitatively, cypress mulch had the lowest pH, electrical conductivity, and cation exchange capacity.

Isotherm Experiment

- Based on the ratio of q_e/C_e (equilibrium concentration on the mulch over equilibrium concentration in solution), there was no statistical difference between the three types of mulch except for the pairs of cedar-hardwood and cypress-hardwood in the system of binary atrazine adsorption.
- The ratio of q_e/C_e was statistically different between mono and binary systems.
- . Atrazine adsorption appeared to exhibit a C-type isotherm, due to the range of concentrations examined; A wider range of atrazine concentrations may show a more distinct L-type isotherm.
- Nitrate does not significantly influence the adsorption of atrazine.
- Nitrate adsorption was highly dependent on mulch surface properties and did not exhibit a specific type of isotherm.
- Atrazine influenced nitrate adsorption.
- In the binary system, average values of Langmuir coefficients for nitrate adsorption were significantly different, indicating that atrazine is affecting the capacity of nitrate adsorption (q_{max}) at high concentration of nitrate, but at low concentrations of nitrate (C_e<5), the energy of nitrate adsorption (K_L) is affected.
- Qualitatively, cypress mulch exhibited the highest capacity for adsorption of atrazine and nitrate.
- Cypress was selected as the best substrate to support bacterial growth in a biotic column.

Column Experiment

- Nitrate removal was not effective because daily diffusion of oxygen into the feed container resulted in conditions unsuitable for denitrification.
- Atrazine removal was not effective because the nitrate concentration was too high.

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High-resolution imaging of the Platte River streambed using combined electromagnetic induction and hydraulic parameter estimation techniques

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Principal Investigators:	Jesse Thomas Korus, Xun-Hong Chen

Publications

There are no publications.

High-resolution imaging of the Platte River streambed using combined electromagnetic induction and hydraulic parameter estimation techniques

Jesse T. Korus and X. Hong Chen, School of Natural Resources, UNL

Statement of Problem

The exchange of water between aquifers and streams is a fundamental process linked to physical, chemical, and biologic conditions of riparian systems. This flow creates a shallow, narrow zone below the streambed with special significance for geochemical processes, water quality, aquatic habitat and biologic diversity, as well as water supply for human uses (Winter et. al, 1998). Many current water issues in Nebraska focus on processes in this zone. The vertical hydraulic conductivity (K_v) of streambed sediments is a key parameter controlling flows between aquifers and streams. Uncertainty and error associated with this parameter may result in poor management plans and stream/wetland restoration designs which could significantly impact environmental, agricultural, and socio-economic systems. Characterizing the spatial variability of streambed conductivity is therefore a basic research need in Nebraska.

Chen (2005, 2010) and Chen et al. (2008) clearly demonstrate cross-channel, downstream, and depth-dependent variation of K_v. This heterogeneity largely reflects lateral and vertical variations in the thickness of silt and clay layers and porosity variation with depth as opposed to salinity or temperature contrasts (Chen, 2010). Chen (2010) demonstrated an inverse correlation between electrical conductivity (EC) and K_v. This relationship suggests that EC contrasts obtained from geophysical methods can be exploited to infer gross 3-dimensionality of streambed K_v and perhaps to relate hydrologic and geophysical parameters.

This proposal focuses on using ground-based electromagnetic induction (EMI) because it measures subsurface electrical conductivity (EC) as a function of water content, porosity, salinity, temperature, soil texture, and mineralogy. These methods can be applied on the ground and through the air, allowing seamless 3-D integration of multi-scale datasets to create a high-resolution picture of the subsurface. EMI methods have furthermore proven useful for shallow subsurface characterization of stream channels (Crook et al., 2008; Sheets and Dumouchelle, 2009; Teeple et al., 2009).

Research Objectives

The goal of this project is to characterize the spatial variability of geophysical properties in relation to hydraulic properties in stream sediments. Our primary objectives are to 1) assemble existing and collect new geophysical and hydraulic data from multiple, co-located profiles at a stream site, and 2) assimilate data and generate multiple visualizations of apparent EC and streambed K_v. The work completed thus far has focused on assessing the effects of traverse direction during data collection on the EM results as well as finding the optimal EM signal frequency for mapping streambed K_v.

Methods

We used the EMP-400 field portable, multi-frequency EMI tool manufactured by Geophysical Survey Systems, Inc. of Salem, NH. This tool offers portability, a built-in GPS, multi-frequency operation between 1 and 16 kHz, and up to 3 frequencies recordable at one time, thus giving three effective depths of penetration. The maximum depth of penetration is about 6 m. The primary user attended a training course on the use of this tool at the manufacturer's headquarters in Salem, NH. Data was collected on Clear Creek, a small tributary of the Platte River near Columbus, Nebraska. A grid survey was conducted over 100 x 60 ft. grid on a point bar for which K_v estimates have been derived. Data was collected at 15, 8, and 3kHz in both X-

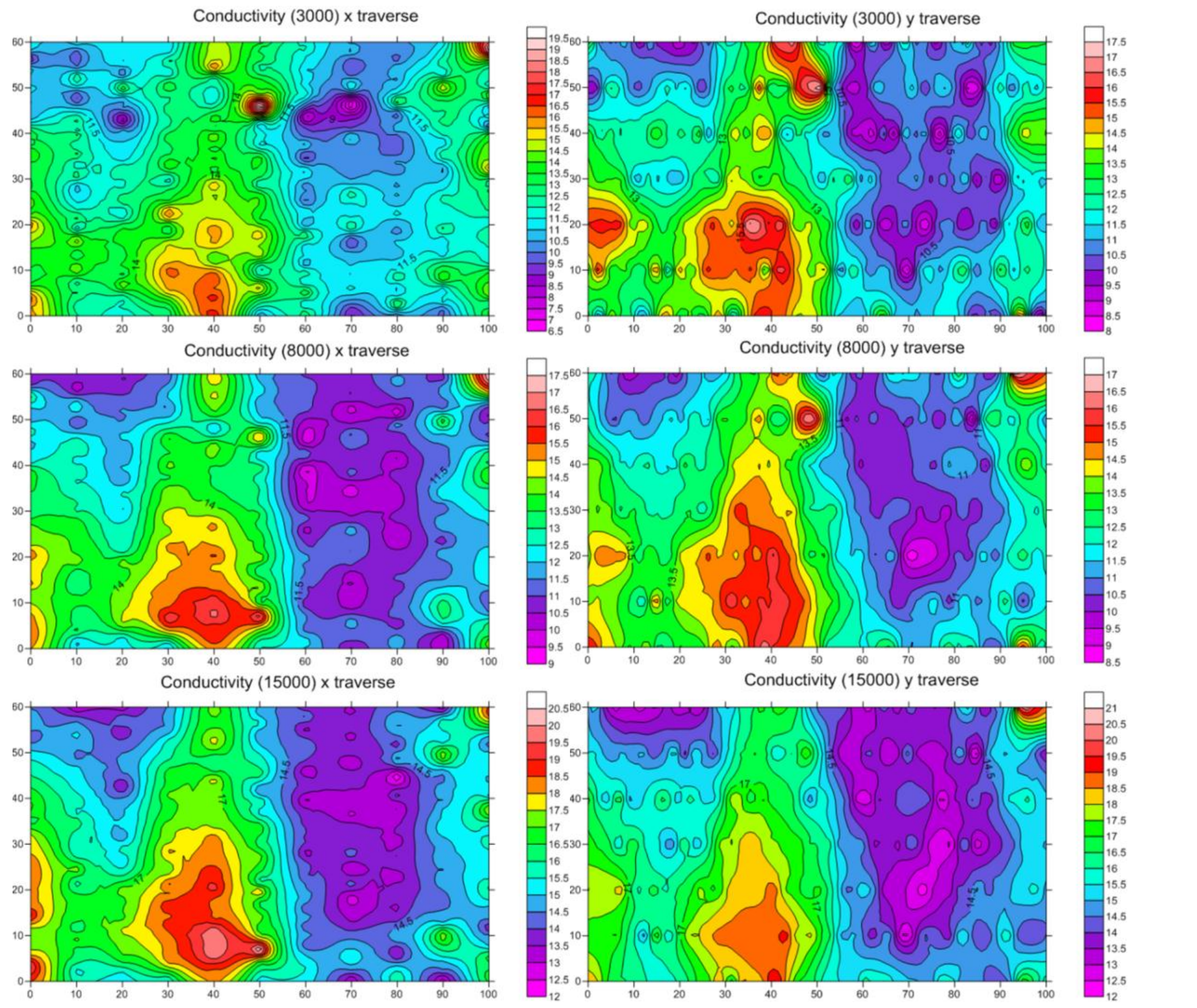

Figure 1. Comparison of EM-derived conductivity fields at 3, 8, and 15 kHz collected in x and y traverse directions.

and Y-oriented traverses. Line spacing was 10 ft. Data was downloaded, quality checked, and a spatial interpolation was performed using the Surfer® software program. A total of six grids were created and compared to check for anomalies and comparability (Fig. 1). A second survey was collected from a linear traverse along the length of the channel. The 15kHz, Y-traverse grid and the in-channel EM measurements were compared to maps of K_v for the same area (Fig. 2).

<u>Initial findings</u>

Conductivity fields collected at various frequencies and along different traverse directions are generally comparable (Fig. 1). Three main zones can be identified in all grids: a low conductivity zone right (east) of center, a high conductivity zone left (west of center), and a low conductivity zone in the upper left (northwest). The 3 kHz signal produced higher variability and larger range of values than the 8 and 15 kHz signals. The three conductivity zones were less well-defined in the 3 kHz grid than in the 8 or 15 kHz grids. Traverse direction has only a minor effect on the shape of the conductivity zones and can be considered negligible.

The north-south orientation of the conductivity zones corresponds to the direction of channel-bend and point-bar migration through time, as manifest in historical photographs dating to the 1940's (Fig. 2a). The relationship between K_v and conductivity, however, is not clearly inverse as was predicted. The K_v field, as determined by in-situ measurement just below the water table, suggests some zonation on the northern point bar that can be compared to the

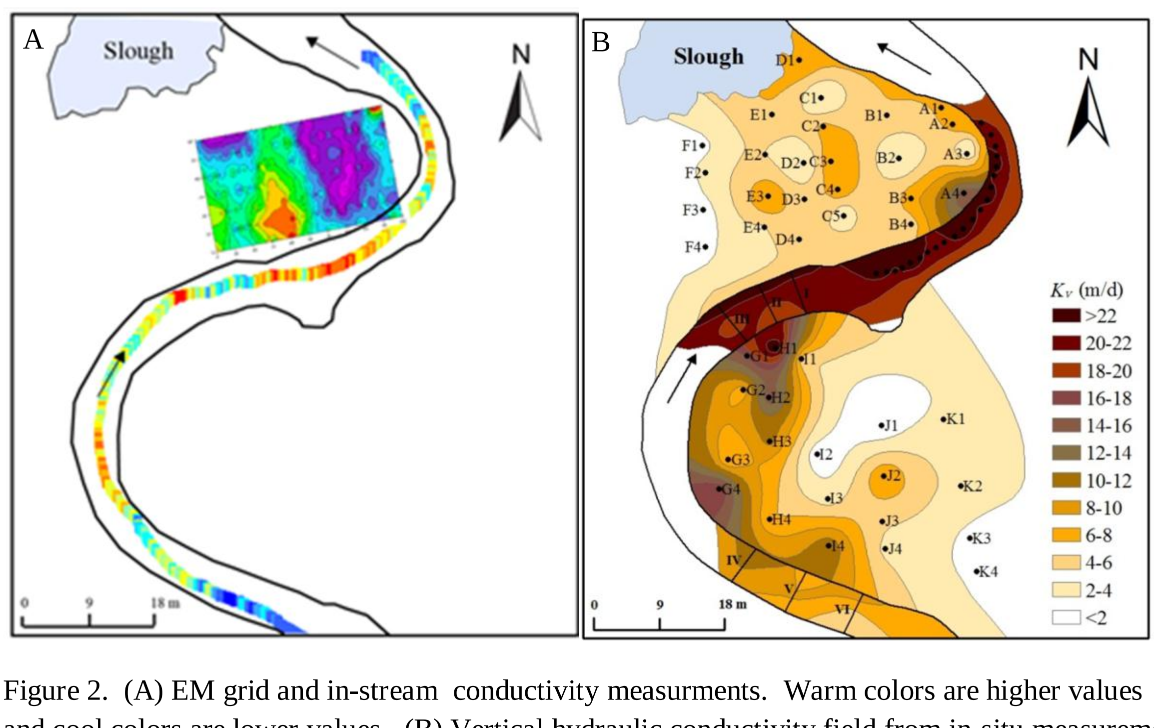

Figure 2. (A) EM grid and in-stream conductivity measurments. Warm colors are higher values and cool colors are lower values. (B) Vertical hydraulic conductivity field from in-situ measurement at the water table or in the streambed.

electrical conductivity field (Fig. 2b). In general, zones of high conductivity correspond to zones of higher K_v, but this relationship can only be established loosely. A zone of higher K_v exists near the center of the point bar at C2, C3, and C4, which corresponds to the area of high conductivity. This zone is surrounded by zones of slightly lower K_v at B1, B2, D2, and D3, which generally correspond to zones of lower conductivity. The zone of highest conductivity, however, corresponds to an area low K_v at C5, and the area of lowest conductivity corresponds to an area of high K_v at B3.

Comparison of the in-stream K_v and conductivity transect shows a good correspondence between the area of high conductivities and the area of high K_v near the northern meander bend. The southern meander bend is characterized by low conductivity and low $K_{v.}$

Overall, the general correspondence between K_v and conductivity is promising, but the lack of a well-defined inverse relationship as predicted suggests that additional field work is needed. Future efforts will aim to 1) collect data over a larger area, 2) resolve differences in sample density, and 3) resolve differences in the degree of vertical averaging. Additional data may need to be collected to investigate the effects of other physical parameters on conductivity, such as organic content, clay content, soil moisture, and water chemistry. Furthermore, data will need to be collected at several different sites to examine the effects of local geology. This study will continue through September, 2012.

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Analysis of Potential Groundwater Trading Programs for Nebraska

Basic Information

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Principal Investigators:	Karina Schoengold

Publications

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"Predicting Groundwater Trading Participation in the Upper Republican Natural Resource District"

By: Nicholas Brozovic, Elizabeth M. Juchems, and Karina Schoengold

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Title: Analysis of Potential Groundwater Trading Programs for Nebraska

PI(s): Karina Schoengold and Nicholas Brozovic @ U of IL

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Executive Summary: While surface water trading has occurred regularly throughout the western United States and the rest of the world for decades, the use of any type of groundwater trading has been very limited. However, groundwater is increasingly under stress from overuse and many areas are starting to regulate groundwater use. Groundwater trading can help move water from low-value to high-value areas of use. Empirical analysis of groundwater trading is a new area of research due in part to the lack of recorded usage, trade data and binding constraints on groundwater use by landowners.

Unlike many other groundwater-dependent areas across the nation, the Upper Republican Natural Resource District (URNRD) has had metering and use restrictions in place for over 30 years. Two of the tools available to producers include creating pools and formally trading water. Formal water trading occurs when the irrigation rights are permanently transferred from one field to another field. When multiple fields are combined to create a pool, a producer can temporarily move a water allocation from one field to another field in the same pool. Within-pool transfers are conducted when fields under the same owner aggregate their total allocations into a pool and then redistribute the water to each field at the owner's discretion. These within-pool transfers have much lower transaction costs than the formal trades due to less time and money spent finding a trading partner and gaining board approval. Therefore, the URNRD provides a great opportunity to better understand the impacts of allowing some restricted trading.

Our study examines formal trading and within pool transfers using thirty years of water use and field level characteristics provided by the URNRD. Specifically, we want to determine if standard economic criteria based on the cost of pumping groundwater and the expected yield are useful indicators in determining trading behavior. To measure some of the relevant economic criteria we use a marginal abatement cost (MAC) curve that is calculated based on well-level characteristics and average market prices. The MAC curve measures the lost profit of reducing water use for a particular well.

Results show that larger fields and larger operation sizes were more likely to participate in a formal trade. Fields with a higher pumping rate and depth to groundwater are less likely to participate in formal trades. Presumably a well with a higher pumping rate has greater capacity to meet its own water needs without trading for additional water. Also, an individual who has invested in a high pump capacity well may be less likely to want to sell some of his water after making the capital investment. Similar results were found with the analysis of within-pool transfers.

In addition to examining participation in trades and transfers we also considered the direction of those trades/transfers. The MAC curves are used to predict whether a field was a net buyer or seller of water during trades/transfers. With formal trading, fields that are predicted to be sellers are 30 percent more likely to actually be sellers. This result shows that the variables that measure the expected profit loss from reduced water use are significant in determining behavior. This result shows that we can have some confidence that predictions of trading behavior in a newly developed market will be accurate. We also find no evidence of any additional stream depletion due to trades; in fact, the overall effect of the water trades that have occurred is a reduction in streamflow depletion. While this result is not something that can be applied to other basins it does reduce some of the concerns with allowing trading/transfers in the URNRD.

INTRODUCTION

Groundwater resources are increasingly under stress in much of the United States and the rest of the world. On average, Nebraska receives 22.9 inches of precipitation each year with the area of interest for this research, receives only 15.1-20 inches annually (U.S. Department of the Interior, 2005). This limited rainfall during the growing season in the western portion of Nebraska results in the high use of irrigation systems to produce grain crops like corn and wheat, which require about 22 inches of rainfall a growing season to reach high yielding maturity (Corn, Water Requirements, 2008). The primary source of the water used for irrigation is the High Plains Aquifer that are often hydrologically connected to the many rivers and watersheds located in the state. According to the Nebraska Department of Natural Resources Well Registry Database, as of May 2012 there were over 122,000 wells registered for use for irrigation.

Upper Republican Natural Resource District

The Upper Republican Natural Resource District (URNRD) covers the three counties in the far southwest corner of Nebraska: Perkins, Chase and Dundy (see Figure A.2 in the appendix). The total land area is 2,697 square miles, with a population of 8,944 in 2010 (United States Census Bureau, 2012). The majority of the land is used for grain production and cattle ranching. Land used for grain production is either irrigated to produce corn, soybeans or wheat or used for dryland production of wheat and edible beans. The URNRD has a total of 3,179 active irrigation wells servicing a total of 452,395 certified acres (Palazzo & Brozovic, 2012).

As a result of the Final Settlement Stipulation of 2002 regarding the Republican River Compact, the URNRD jointly developed an Integrated Management Plan (IMP) with the Nebraska DNR to promote compliance and further reduction in groundwater usage. Starting in 1978, the URNRD has been actively involved in the management of the groundwater resources within the district through the adoption and enforcement of rules and regulations. Along with monitoring pumping from each well, the URNRD has established correlative irrigation allocation rights. Since the first allocation limits were established in 1983, the annual irrigation allocation has decreased from 22 inches per year to the current allocation of 13 inches per year. These allocations are issued as an aggregate amount to be allocated over five years. Groundwater right holders are then allowed to carry forward the unused portion of their allocation, together with any unused portions from previous years, into succeeding allocation periods. The URNRD also allows for the pooling of well-specific allocations into an aggregate allocation for groups of wells owned by the same person, partnerships, corporations, or other individuals, subject to a signed agreement.

The previous literature on water markets have primarily focused on surface water trading, but interest in developing markets for groundwater trading has begun to increase in recent years. Although the majority of surface water rights use the appropriation doctrine of "first in time, first in right", groundwater rights in Nebraska utilize the correlative rights doctrine that is administered by the local NRD. The correlative rights doctrine ties all appropriated water rights together and assigns equal priority to all rights. This idea implies that when a shortage in groundwater within a NRD has been triggered, all groundwater-rights holders within that NRD have their allocation, or supply, decreased proportionally. Groundwater trading (markets) can allow producers to use water most efficiently under restricted access.

Much research has been done to identify the general requirements for a competitive market system. Dinar et. al. (1997) and Saliba (1987) agree that efficient water markets require: 1) many sellers and buyers with full knowledge of the market institutions and facing similar transaction costs; 2) participation decisions are made independent from other buyers and sellers; 3) outcomes are not affected by the decisions of other participants; 4) participants are assumed to be maximizing profits; 5) completely specified, enforceable, and transferable property rights.

Water markets that have met these requirements will move resources from low value uses to high value uses, resulting in an economically efficient allocation of resources for both individuals and society, so long as the gains in value are large enough to offset the costs of completing the transaction. Because markets move water from low value to high value by allowing compensation for the water sold, they provide an incentive for more efficient use of water and reduce the stress on the water supply from high value uses. A well-designed water market requires the measurement and monitoring of water withdrawals, enforcement of withdrawal rules, and should consider any externalities or third party

However, if high transaction costs persist, differences in marginal water values will continue to influence the market and the prices of water rights will vary between uses. Because detailed water rights are generally heterogeneous, transaction costs tend to be higher as buyers and sellers must engage in market searches that fulfill the institutional regulations on legal and hydrological characteristics of the water rights involved. High transaction costs reduce the level of profitable transactions but are not necessarily a sign of an inefficient market. Saliba (1987) concluded that water markets are in fact functioning well where the economic incentives for transfers outweigh the transaction costs involved and where the policies regarding markets are not costless, but are necessary.

DATA SOURCES AND METHODS

The data used in the analysis includes publicly available well data from the Nebraska DNR and proprietary data collected and provided by the URNRD.

The Nebraska DNR well database for the Upper Republican NRD contains technical information on each well, including pump depth, pump rate, date of drilling, and the current status of the well. Specific geographic location information and well ownership information is also included within the file. There are 4,604 wells listed in the DNR database; however, only 3,274 are used for active irrigation after reconciling with the data provided by the URNRD.

The URNRD provided many datasets to help analyze the trades within the district. An important dataset contained records of formal allocation transfers of groundwater for irrigation beginning in 2006 and ending in 2011. This file was used to identify 35 formal trades involving 100 unique field IDs and was verified using the allocation adjustment data set. A map of the participating wells is provided in the appendix as Figure A.3.

Using the historical usage data provided by the district, 1,974 fields were identified as participating in pools during 2005-2007 and 1,914 during 2008-2012. Due to a change in the pool naming convention that made it difficult to track the history of the pools to those that existed before the name change, the decision was made to use only the pools in the two recent allocation periods in this analysis.

The URNRD also provided a dataset identifying the owner(s) and operator(s) of each field with certified irrigation acres. Also provided was the District's key to matching the DNR well IDs to the field IDs used within their datasets. When reconciled, the final dataset contained 3,179 unique field IDs and their owner/operator information for the certified acres in 2011. The final dataset provided by the URNRD is a large file tracking historical use from 1980 to 2012 for 3,346 unique field IDs. The file contains the annual certified acres, crops planted, pool ID (if applicable), beginning allocation, and county ID.

Price and quantity values used to generate the marginal abatement cost, MAC, indicators were generated by Palazzo and Brozovic (2012). The model utilized well-characteristic data available through the Nebraska DNR and results from the use of the Water Optimizer program developed at University of Nebraska-Lincoln (Martin, Supalla, McMullen, & Nedved, 2007). The initial abatement cost points were calculated as the difference in the well's profits under unconstrained and constrained pumping. Each point in the MAC curve is determined by calculating the change in the profit associated with increasing the stringency of the pumping constraint. To determine the value of the MAC indicator used, the relationship of the curves was modeled, as shown in Figure A.4 in the Appendix. In the figure below, Well A is shown in red while Well B is shown in blue. To determine the direction of trade predicted by the MAC curves for these two wells, the quantity was set at two inches—or equal to the average transfer size in the district. For example, at two inches of water abated, the cost or price of abatement calculated by mentally calculating the integral is lower (~$0/year) for Well A than Well B (~$9.00/year). This indicates that cutting back water use is less expensive for Well A and it would be a net seller to Well B, where reducing allocations is more expensive.

The stream depletion factor is not a deciding factor in the approval under the current trading rules; however the URNRD understands the importance of the measure on maintaining compact compliance and are seeking to add the requirement in the near future. The variable was included in the models to test if producers were already considering stream depletion as a motivator for trade participation. Research in hydrology has shed light on the potential negative impact of groundwater pumping on nearby streams through the process of stream depletion. Kuwayama and Brozovic utilized the time path of stream depletion caused by a unit for groundwater pumping by a specified well to define a transfer function that expresses the stream depletion factor, SDF, as a proportion of the volume of water that was pumped by the wells in the past. The function can be considered a density function that ranges from 0 to 1 that characterizes how the lagged effect of pumping in one year is distributed across future years (Kuwayama & Brozovic, In Press).

Owner/Operator Descriptive Statistics

According the owner/operator information provided by the URNRD, there are 524 unique operators in the district who manage the 3,179 fields. The average number of fields per operator is 6.05 fields, with a maximum of 94 fields and minimum of one field. Examining the unique owner data, 731 different individuals or entities were identified as owning the 3,179 fields in the district. The average number of fields per unique owner is 4.35 fields, with a maximum of 94 fields and minimum of one field.

Among the 49 unique operators who participated in formal trades, the average number of fields is 14.27 fields per operator. Even with omitting the largest operator,[1] the average number of fields among operators participating in trades is 13.31 fields, over twice as large as the district average. This indicates that larger operations are more likely to participate in the trade process. A similar pattern emerged when examining the 52 unique owners participating in formal trades. The calculation of the average number of fields per unique owner that participated in formal trades resulted in an average of 9.52 fields, or 2.18 times larger than the district average.

Among unique operators, 53% participated in the pooling process while 50% of the unique owners participated in pools. These significantly higher participation rates for informal trades within pools leads to the hypothesis that there is demand for formal trading, but current rules and procedures are limiting the participation level.

Usage Trends

Examining usage trends within each county and the aggregate district was a way to study whether the current allocation restrictions were binding[2]. Looking at overall trends of usage plotted in Figure A.4 in the Appendix, usage appears to fluctuate around 11 inches from 1980-1999. From 2000-2003 the average usage jumped up significantly; however, this is primarily due to drought conditions in two of the four years. Between 2004 and 2011, the usage showed a distinct downward trend—well below the allocation restriction—with a significant spike in usage in 2012, again the result of drought conditions.

Methodology

The sample was used to create six different models to examine informal temporary trade participation and direction for the two most recent allocation periods, and formal permanent trade and direction model. The entire sample of fields used for irrigation was used to create a permanent trade participation model. The informal trade participation models used the same sample, with those fields participating in single field pools reclassified as those not participating in informal trading. The trade direction models used subsets of the full sample of only those who

[1] There is one large operator in the URNRD who manages 60 fields. This is an anomaly in the district as the next largest operation has 49 fields.

[2] More detailed information regarding the exploration of usage trends is available. See Juchems, E. M. (2013). *Predicting groundwater trading participation in the Upper Republican River Natural Resource District*. (Master's thesis).

participated to predict the probability of being a buyer or seller in the trade relationship. The decision to use two models for informal trade participation and direction, respectively, was motivated by a change in the pool naming convention (begun in 2005) that made it difficult to track pools further back, and by the change in allocation limits in 2008, which triggered the dissolution and reformation of pools generating multiple observations for one field. The variables used in the various models are summarized in Table 3.4, which includes the variable's name, definition, and unit of measure.

Table 3.1 Definitions of Variables.

Dependent Variable Name	Definition
Trade	= 1 if participated in a trade
Seller	= 1 if a seller
Independent Variable Name	Definition
Acres	field size in certified irrigation acres
GPM	pumping rate in gallons per minute at the time of drilling
PWL	the distance from the soil surface to the water level during pumping, measured in feet at the time of drilling
Useavg07	field average water use from the first year of use until 2007, in inches
Percorn07	percentage of years field was in corn production from the first year until 2007
Useavg12	field average water use from the first year of use until 2012, in inches
Percorn12	percentage of years field was in corn production from the first year until 2012
Ownop	= 1 if the owner is also the operator
Opsize	total number of fields owned by the field owner
Medium	= 1 if soil is of medium soil type
Coarse	= 1 if soil if of coarse soil type
Unksoil	= 1 if soil if of unknown soil type
Perkins	= 1 if the field is in Perkins County
Dundy	= 1 if the field is in Dundy County
Avgusetrade	field average water use from the first year of use until the year before trade occurred
Percorntrade	percentage of years field was in corn production from the first year until the year before trade occurred
SDF	ranges from 0 to 1; impact on stream flow as a proportion of the volume of water that was pumped by the wells in the past
MAC	= 1 if the modeled MAC curve relationship indicates a seller
Tradesize	size of the permanent trade, in acres transferred
Transfersize	size of the temporary trade within a pool, in inches
MAC2	= 1 if the MAC curve relationship indicates a seller at 2 inches abated

Constr	= 1 if the pool is constrained by allocation limit
MAC2*constr	interaction variable between MAC2 and constr

Variable Description

The dependent variable for the formal and informal trade-participation models is the decision to participate in a trade (*Trade*). This variable is a binary indicator that equals one if an individual field participates in a formal trade and zero if it did not participate in a trade. The formal and informal trade-direction models use the binary variable (*Seller)* to predict the probability of the field being a buyer or seller in the permanent trade. The variable equals one if the field is a seller and zero if the field is a buyer.

The decision to use the following variables was motivated by previous research on irrigation technology adoption. Negri and Brooks (1990), as well as a technical bulletin published by the Agricultural Research Service of the USDA (1962), included many of the variables for which we had measurements, including acres irrigated, well depth, and soil type. They also included measurements of energy costs, precipitation and soil productivity. Many of these additional variables do not vary enough across the URNRD to be included directly in the models, but were used in the calculation of the MAC prices and quantities through the use of Water Optimizer.

With a few exceptions, most of the variables are unique to a specific field. The size of the irrigated fields *(acres)* is unique to each field observation and is a continuous variable. Our expectation is that this variable will be a significant positive indicator of formal trade participation as the trades are permanent and alter the amount of land that can be irrigated by the participating wells. For the direction models, the sign is also expected to be positive since larger fields are likely to have excess acres that the current irrigation system cannot cover efficiently.

The well technical variables used in the participation models measure the pumping rate *(gallons per minute)* and the distance from the soil surface to the water surface during pumping *(pumping water level)*, and are continuous variables. They are used to compare the cost of pumping and are similar to the technique applied by Negri and Brooks (1990). Our expectation is that when it becomes more expensive to pump, producers will look for ways of increasing their efficiency by participating in trades. Thus, pumping-rate effect is expected to be negative because if the pumping rate increases, it becomes less expensive to pump and less desirable to trade. When the pumping water level increases, the water must be moved a longer distance to the surface, becoming more expensive. The expectation of sign, therefore, is positive as the cost increases with increasing the pumping water level and encouraging participation in trades.

Unique to this study is the availability of water usage records for over thirty years, which allows for the continuous variables for average use (*useavg07*, *useavg12* and *avgusetrade*). The models use the appropriate usage measurement based on the time-frame examined. We expect average use to be positively related to trade participation as those fields that use more are more likely to be reaching their allocation limits and are motivated to find ways to increase the efficiency of their production in the face of decreasing allocation allotments. The sign of the average usage is expected to be negative for the trade direction models because fields that have higher average use are more likely to be constrained by the allocation limit and therefore are more likely to be a net buyer in the trade.

The percentage of corn grown (*percorn07, percorn12,* and *percorntrade*) is a measurement of crop type, which the technical bulletin (1962) identified as an important variable in determining irrigation adoption. The percentage of corn grown was calculated as the number of years corn was planted in the field divided by the number of years usage was recorded. This allows the variable to be continuous between zero and one. Our expectation of sign for the formal participation is negative because participants also includes sellers of water where corn production is less efficient and the producers seek to grow other crops that require less water, freeing up the allocation for sale. The sign for the informal trade participation is expected to be positive as those fields that grow water-intense crops, like corn, are looking for ways to temporarily increase their water allowances to finish a crop. The sign is expected to be negative for the trade direction models, as those fields that grow more corn are likely to be net buyers of water due to the higher water needs of corn compared to beans or wheat.

The land tenure indicator *(ownop)* is equal to one when the owner of the field is also the operator, based on the 2012 URNRD data. The sign of this variable in the participation models is expected to be positive as a land owner is more likely to take the time to participate because he can continue farming the field to recover the transaction cost, whereas a renter may not have the expectation of continuing to farm the field the next year. We expect a positive sign for the formal-direction models as rented land is less likely to be net sellers of water. When the operator is the same as the owner, the decision to sell is less complicated than when dealing with two decision makers that may have different goals.

The operation size (*opsize*) is unique for an entire operation and is based on data from 2012. Through the previous exploration of owner characteristics indicating that larger operations are more likely to participate in trades, the sign is expected to be positive for the participation models. The sign is expected to be negative for the formal-trade direction models as the larger operations are more likely to be net buyers of water due to their increased access to capital. Due to URNRD regulations on pool formation, the operation size does not vary significantly and provides no inference power.

The soil type indicators (*medium*, *coarse*, and *unksoil*) are binary variables that measure the soil's ability to retain water after an irrigation cycle. We expect the medium and coarse variables to be positive based on farmer comments during visits to the URNRD. They noted that their goal was to increase efficiency of their operation by moving water from sandy or coarse soils to field that have better water retention.

The county indicator variables (*Perkins*) and (*Dundy*) capture differences between the three counties in the URNRD. The variables are included in the participation models only as the trade regulations restrict the movement of water beyond the floating township, creating little variation in the county within a trade. These variables equal one when the field is in either Perkins or Dundy, respectively, and zero otherwise. Our expectation of the sign is negative because the majority of eligible fields for trade participation are located in Chase County, indicating that fields in Perkins or Dundy are less likely to participate given the current regulations on water movement.

For the direction models, only the trade size (*Tradesize* and *transfersize*) is common to all fields that participate in a formal and an informal trade, respectively. In some cases this is only two fields (a buyer and seller), but in other cases multiple fields have aggregated rights to trade a water allocation. We expect the sign to be negative as the MAC curves indicate that it is less expensive for fields to cut back a little than to cut back a large amount, indicating that smaller transfer sizes are sellers.

The stream depletion factor *(sdf)* used in the formal trading model was calculated using the methods developed by Kuwayama and Brozovic (in press) and is continuous from zero to one. It measures the impact on stream flow as a proportion of the water that is pumped by wells in the past. Our expectation is a positive sign, indicating that water is moving away from high stream depletion wells to those that have less of an impact on stream flow.

The marginal abatement cost indicators (*MAC* and *MAC2*) are used in the formal and informal trade direction models, respectively. These indicators use curves developed by Palazzo and Brozovic (2012) to determine how the curves would predict buying and selling behavior. The variable equals one when the curves predict a seller and zero if predict a buyer. We would then expect the sign to be positive, reflecting that the field behaves similarly to what theory expects.

The final variables apply only to the informal trade direction models. The first is the indicator of constraint (*constr*), which equals one when the pool is constrained by the allocation limit and zero when it is not. We would expect the sign to be positive because of the convex shape of the MAC curves within the typical transfer size. The final variable is an interaction variable between the *MAC2* and *constr*, which is used to capture any differences when a pool is constrained since we decided to run one model for each period based on the evidence below.

Testing for Heterogeneity among Pools

To test if constrained pools behaved the same as unconstrained pools the data was divided and coefficients tested using the hypothesis that the coefficients were the same across groups. The category is based on the average annual water use in all fields associated with the pool. The earlier dataset was divided at 13.4 inches to catch those pools that are close enough to the limit to be constrained, while the later dataset was divided at 12.9 inches for the same reasons.

In the ‘05-‘07 allocation period, 552 fields were members of a constrained pool while 1,422 were members of unconstrained pools. While for the ‘08-‘12 allocation period, only 247 fields were members of a constrained pool, leaving 1,667 in unconstrained pools. The test of the coefficients for each of the models after applying the method set forth by Allison (1999). The results indicate that constrained pools behave similar to unconstrained pools and the data can be grouped together for one model. Descriptive statistics for the variables in each of the six final models are listed in the Table 3.11 in the appendix.

Probit Model Estimation

The use of binary or limited response variable models, such as probit and logit, have grown in popularity for modeling choice behaviors similar to groundwater trading— such as irrigation and rainwater harvesting adoption (He, Cao, & Li, 2007)—and for determining irrigation technology choice (Negri & Brooks, 1990). Probit models were selected as the best-fitting models to show the factors that affect the likelihood of participating in a formal and informal trade as well as to predict the direction of trade between participants. The response variables for each model are binary variables equal to one or zero. A probit model is of the form:

$$(1)\ P\ (y = 1|x) = G(\beta_0 + \beta_1 x_1 + \ldots + \beta_k x_k) = G(\beta_0 + x\beta)$$

Where G is a standard normal cumulative distribution function taking on values strictly between zero and one: $0<G\ (z)<1$ for all real numbers z (Wooldridge, 2003). This functional form of G (z) requires that estimated response probabilities of the model are strictly between zero and one and will not result in a negative probability or a probability greater than one. For the general probit model, as well as those used here, a standard normal distribution for the error term, ε, is assumed. The model estimations and tests are all done in the STATA software package.

The resulting sign of the coefficients in each model can be interpreted as the individual influence of each explanatory variable on the response probability of the model, ceteris paribus. The statistical significance of each variable is determined by whether we can reject H_0: $B_j=0$ at a sufficiently small significance level. To find the magnitude of effect of a one unit change in an explanatory variable, holding all other variables fixed, the marginal effect of that change is of the form:

$$(2)\ G[\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k(c_k + 1)] - G(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k c_k)$$

There are two widely accepted measures of goodness-of-fit for binary response models discussed in econometric literature. The first is known as the percent correctly predicted. This method first estimates the probability that the predicted value ($\hat{y}_i$) takes on the value of one for each i. The predicted probabilities must then be converted to binary values of one or zero for comparison with the observed values (y_i). A pitfall of this measure is that it is possible to get high percentages of correctly predicted observations without the model being of much use when the sample contains a high proportion of one value to the other, which is why it is important to report the percent correctly predicted for each of the two outcomes (Wooldridge, 2003).

The second measure of fit is the reported pseudo R-squared value for binary response. A pseudo R-squared is similar to the R-squared value for an OLS model, which is a measure of how closely $\hat{y}_i$ is to y_i. The value for the pseudo R-squared is not expected to be as high as a conventional OLS R-squared because it is unlikely that the predicted values of the probit model will be exactly one or zero; they are more likely to be found somewhere in between (Wooldridge, 2003).

RESULTS

Using STATA 12.1, each of the probit models was executed and the results are reported below. For each model, the sign of the coefficients were compared to expectations, marginal effects were interpreted, and the overall fit of the models were evaluated.

Model 1: Informal Trade Participation 2008-2012

The model applied to the informal trade participation for temporary transfers of groundwater during the 2008-2012 allocation period is as follows:

(3) $P(Trade = 1) = G(\alpha + \beta_1{*}acres + \beta_2{*}\ useavg12 + \beta_3{*}percorn12 + \beta_4{*}\ ownop + \beta_5{*}opsize + \beta_6{*}\ medium + \beta_7{*}coarse + \beta_8{*}unksoil + \beta_9{*}Perkins + \beta_{10}{*}Dundy + \beta_{11}{*}gpm + \beta_{12}{*}pwl)$

Due to the comparative ease of creating an informal trade versus a formal trade, participation in the informal trading market is much more prolific. During the most recent allocation period, 1,914 of the 3,179 observations participated in pools. The marginal effects, which provide the most interpretational power, are available in Table 1. The majority of the variables included in the model prove to be highly significant, which aligns with expected significance provided by previous literature on irrigation technology adoption and water trading. The model exhibits similar results of coefficient signs and significance as the previous allocation period model[3], but this paper will focus primarily on the most recent allocation period.

The most significant variables include the field's average groundwater use, operation size, soil type, location in the district, and pumping water level. Although the two periods were separated to account for the change in allocation limits, the sign, significance, and magnitude of

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[3] The results of the earlier allocation period are available in Juchems, E. M. (2013). *Predicting groundwater trading participation in the Upper Republican River Natural Resource District*. (Master's thesis).

the marginal effects are similar to the earlier model and allow for the same conclusions to be drawn.

The marginal effect of increasing average use by one inch indicates a 3.9% increase in the probability of participating in an informal trade. This result is consistent with expected producer behavior and with comments during interviews in which producers said that one of their major goals is to efficiently manage their water allocations. By participating in an informal trade within a pool, a producer is able to increase average use on more efficient fields by cutting back use on less efficient fields, where it may be more expensive to pump or less productive land. Given the current district rules resulting from the Compact settlement, if the producer's goal is to increase average use, their options include participating in a relatively easy-to-form pool or complete the more time-intensive formal trade process, which has proved to be a less popular management choice.

The size of the operation is highly significant when predicting informal trade participation. The marginal effects indicate that increasing the operation size by one field increases the probability of participating by 0.34%. Traditionally, most pools have been formed with one owner for all the participating fields, indicating that in order to form an efficient pool, an operation must have at minimum two fields. Restrictions on the distance water can be moved further restrict the access of pool formation for small operations that may have multiple fields but do not fulfill the distance limitation rules. Thus larger operations have greater opportunities to participate in informal trades.

Compared to fine soils, both medium and coarse soil-type fields are more likely to participate in informal trading. Their marginal effects indicate fields with medium soil-types are 10.4% more likely to participate and fields with coarse soil-types are 13.6% more likely to participate than fields with fine soil-types. Medium or coarse (sandy) soil-types have poorer soil water retention rates than fields with fine soil, which may direct fields with the former soil-types to consider all management options for increasing water use efficiency, including participating in informal trading pools.

Similar to the formal trading model, the informal trading model shows that fields in Perkins and Dundy counties are less likely to participate. The negative coefficients are consistent with the fact that the majority of fields eligible for pooling are located in Chase County. The marginal effect of having a field in either Perkins or Dundy County decreases the probability of participating by 14% and 17.6% respectively.

The coefficient of pumping water levels exhibits a negative sign and is highly significant. The pumping water level measures the distance from the soil surface to the water surface during pumping. When this number increases by one foot, pumping become more expensive as it requires more energy to move the water over a greater distance. The marginal effect of the one foot increase indicates that the probability of participation decreases by 0.04%. This result was inconsistent with prior hypothesis, that producers with higher-expense wells would be looking for ways to increase efficiency of allocated water by moving the water to wells were it is less expensive to pump. This may indicate that other factors that influence cost of pumping—such as pumping rates, irrigation system types and weather patterns—have a greater influence on pumping decisions than the pumping water levels.

Other variables just missing the standard 10% level of significance cut-off include the percentage of years in corn production, the land tenure indicator, and the technical measure of pumping rates. When a field increases the percentage of years in corn by one percent, the marginal effect indicates an increase in participation probability by 7.4%. This is consistent with the expectation that producers are making management decisions that help them increase efficiency, such as creating pools, when producing water-intensive crops like corn.

When the land is owned and operated by the same producer, the marginal effects indicate an increase in participation probability of 3%. This is consistent with expectations that operators are more likely to file the paperwork and form a pool when they are also the land owner. It is difficult for a renter, who may not be managing the farm the following year, to realize the full benefits of forming a pool, and thus they are less likely to incur the time cost of applying for the pool formation.

The final variable of slight significance is the pumping rate measure, and it has the expected negative sign. When a field increases its pumping rate by one gallon per minute, the probability of participating in a pool decreases by 0.002%. Although the marginal effect is very small, the positive sign is consistent with expectations that fields with higher pumping rates are less likely to participate in pools because they are able to efficiently pump the needed amount of water from the single well.

Evaluating the fit of the informal trade participation models will help determine if the directional model can be applied across the entire district or only to small portions of the district. The pseudo R-squared value for the most recent allocation period is 0.099, slightly lower than the model for the previous allocation period. The goodness-of-fit returned similar results as the previous model by correctly identifying 2,140 of the 3,179 observations or 67.3%. Once again the model over-predicted participation but did a better job of predicting participation than non-participation by misidentifying 323 participants and 716 non-participants.

Table 1 Marginal Effect Estimates for Model 1.

Margins	dy/dx	Delta-method Standard Error	Z-Score
Field size, acres	0.0000	0.0001	0.12
Average use through 2012	0.0390***	0.0038	10.27
Percentage of years planted to corn through 2012	0.0740	0.0480	1.54
Owner = operator indicator	0.0300	0.0197	1.53
Operation size	0.0034***	0.0006	5.22
Medium soil type	0.1044***	0.0236	4.42
Coarse soil type	0.1364***	0.0248	5.49
Unknown soil type	0.0905	0.0776	1.17
Perkins	-0.1406***	0.0267	-5.26
Dundy	-0.1756***	0.0254	-6.92
Gallons per minute	0.0000	0.0000	-1.6
Pumping water level	-0.0004***	0.0002	-2.74
Single, double, and triple asterisks (*,**,***) denote statistical significance at the 10%, 5% and 1% levels, respectively			

Model 2: Informal Trade Direction 2008-2012

The model applied to the informal trade direction for the 2008-2012 allocation period pools is as follows:

(4) $P(\text{Seller} = 1) = G(\alpha + \beta_1{*}\text{acres} + \beta_2{*}\ \text{useavg07} + \beta_3{*}\text{percorn07} + \beta_4{*}\text{medium} + \beta_5{*}\ \text{coarse} + \beta_6{*}\ \text{unksoil} + \beta_7{*}\ \text{transfersize} + \beta_8{*}\ \text{MAC2} + \beta_9{*}\ \text{constr} + \beta_{10}{*}(\text{MAC2}{*}\text{Constr})\ )$

For those fields that participated in pools during the ‘08-‘12 allocation period, 945 transferred water to 969 recipients. The marginal effects are presented in Table 2. The model focused on those variables that are unique to the field to better model the decision process faced by the producer. The model exhibits similar results of coefficient signs and significance as the previous allocation period model[4].

The variables of greatest significance are average field use, the MAC2 indicator, the constrained pool indicator and their interaction term. Similar to the model for the previous allocation period, fields with higher average use over the five years are less likely to be net sellers of water. The marginal effects indicate that when average use increases by one inch, the field is 8.6% less likely to be a net seller of water.

When the pool is constrained, the probability of the field being a seller is significantly higher—regardless of the MAC2 prediction—which is consistent with expected producer behavior when facing convex marginal abatement cost curves. Over the average transfer size for the allocation period of approximately 2.12 inches, the MAC curves are convex, which leads to the conclusion that it is less expensive for multiple fields to cut back a little than to have one field cut back a significant amount. Plotting several MAC curves for the dataset also revealed than many fields experience zero cost for cutting back one inch or less. The constrained variable margins indicate that when the MAC2 indicates a buyer, the field is 10.6% more likely to be a seller when the pool is constrained. However, when the pool is constrained and the MAC2 predicts a seller, the field is 7.3% more likely to be a seller. The positive marginal effects, regardless of MAC2 prediction, indicate that producers, although showing consistent behavior with the curve shape, focus on different factors when making trade decisions in the informal trade market. This is a potential result of the temporary timeframe used for decision-making, unlike making a permanent sale or purchase. Although the sign of the MAC2 coefficient is inconsistent with what was expected when the pool is not constrained, the variable is not significant at any reasonable level. The marginal effect of MAC2 when the pool is constrained is significant and results in a field being 4.3% more likely to be a seller, which is once again consistent with the convexity of the curves.

[4] The results of the earlier allocation period are available in Juchems, E. M. (2013). *Predicting groundwater trading participation in the Upper Republican River Natural Resource District*. (Master's thesis).

The lack of significance of the other variables in either allocation period trade model indicates that the MAC curves—calculated using well specific characteristics, production costs and average weather conditions—are better suited for predicting the probability of being a seller or buyer in the informal market.

Evaluating the overall fit of the model was done using the pseudo R-squared measure and the goodness-of-fit method. The pseudo R-squared for the model is 0.1197, which is slightly higher than the model for 2005-2007. The goodness-of-fit evaluation determined that the model correctly predicted 1,276 of the 1,914 observations or 66.7%. Of those correctly predicted, 47.3% were predicted to be sellers and 52.7% were predicted to be buyers, which is consistent with the data sample percentage of sellers and buyers.

Table 2 Marginal Effect Estimates for Model 2.

Margins	dy/dx	Delta-method Standard Error	Z-Score
Field size, acres	0.0003	0.0002	1.6
Average use through 2012	-0.0859***	0.0055	-15.69
Percentage of years planted to corn through 2012	-0.0024	0.0694	-0.03
Medium soil type	-0.0354	0.0311	-1.14
Coarse soil type	0.0174	0.0331	0.53
Unknown soil type	0.0767	0.1165	0.66
Transfer size	0.0023	0.0060	0.38
MAC2 at			
constrained = 0	0.0435*	0.0250	1.74
constrained = 1	0.0097	0.0584	0.17
Constrained at			
MAC2 = 0	0.1063**	0.0442	2.4
MAC2 = 1	0.0725*	0.0441	1.65
Single, double, and triple asterisks (*,**,***) denote statistical significance at the 10%, 5% and 1% levels, respectively			

Model 3: Formal Trade Participation

The model applied to the formal trade participation for the permanent transfers of groundwater is as follows:

$$(5)\ P(Trade = 1) = G(\alpha + \beta_1{*}acres + \beta_2{*}\ useavg12 + \beta_3{*}percorn12 + \beta_4{*}\ ownop + \beta_5{*}opsize + \beta_6{*}\ medium + \beta_7{*}coarse + \beta_8{*}unksoil + \beta_9{*}Perkins + \beta_{10}{*}Dundy + \beta_{11}{*}gpm + \beta_{12}{*}pwl)$$

Due to only recent interest and rule allowances, 100 of the 3,179 observations have participated in trades, skewing the distribution severely to non-participation. The marginal effects are presented in Table 3. Although the number of observations for participating is very small, the model reveals results that are generally consistent with expected behavior. The variables with the highest levels of significance include the size of the field, the amount of corn grown, and the county location of the fields.

While the model indicates a high level of significance for the size of the field, the marginal effect is very small and designates that a one acre increase in field size increases the probability of participation by only 0.01%. This small marginal effect may be the result of the lack of variation in field size for those that did participate in formal trades. The positive sign implies that larger fields are more likely to participate in formal trades, indicating that with the current time-intensive trading process, larger fields are more likely to benefit from the effort of participating in trades.

The percentage of years the field was in corn production is highly significant in the model, and the marginal effect indicates that increasing the variable by one percent decreases the probability of participating in a formal trade by 3.4%. This is consistent with expectations as participants include both buyers and sellers of water. Those fields where it is less efficient to produce corn, thus those field that grow it less often, are more likely to seek out a trade opportunity to get the benefits of their pumping rights since they are not being fully utilized under the current management practices.

The final significant variables are the county indicator variables for Perkins and Dundy counties. Both variables exhibit negative coefficients and their marginal effects indicate that a Perkins County field is 2.8% less likely to participate and a Dundy County field is 2.4% less likely to participate, compared to a field located in Chase County. These results are as expected due to the high concentration of irrigated fields in Chase County and the current regulations restricting the distance water can be traded.

Evaluating the formal trade participation model has implications regarding the application of the trade direction model explained below. With the lack of participating field observation points, the model did not do a very good job of fitting the data—the pseudo R-squared value is 0.0634—and so caution must be used when applying the formal trade direction model to the entire district. The goodness-of-fit calculation reports that the model correctly predicted participation for 3,072 of the 3,179 of the observations or 96.6% but was unable to correctly identify any of the fields that actually participated in the formal trading process.

Table 3 Marginal Effect Estimates for Model 3.

Margins	dy/dx	Delta-method Standard Error	Z-Score
Field size, acres	0.0001**	0.0000	2.47
Average use through 2012	-0.0012	0.0010	-1.27
Percentage of years planted to corn through 2012	-0.0348***	0.0132	-2.63
Owner = operator indicator	-0.0024	0.0056	-0.43
Operation size	0.0002	0.0002	1.23
Medium soil type	0.0103	0.0066	1.56
Coarse soil type	-0.0059	0.0078	-0.75
Unknown soil type	0.0161	0.0188	0.86
Perkins	-0.0280***	0.0087	-3.22
Dundy	-0.0243***	0.0072	-3.35
Gallons per minute	0.0000	0.0000	-1.43
Pumping water level	-0.0001	0.0000	-1.37
Single, double, and triple asterisks (*,**,***) denote statistical significance at the 10%, 5% and 1% levels, respectively			

Model 4: Formal Trade Direction

The model applied to the formal trade direction for the permanent transfers of groundwater is as follows:

(6) $P(\text{Seller} = 1) = G(\alpha + \beta_1\text{*acres} + \beta_2\text{* avgusetrade} + \beta_3\text{*percorntrade} + \beta_4\text{* ownop} + \beta_5\text{*opsize} + \beta_6\text{* medium} + \beta_7\text{*coarse} + \beta_8\text{*tradesize} + \beta_9\text{*MAC} + \beta_{10}\text{*sdf})$

Of the 100 fields that have participated in permanent trades, 46 were net sellers of water and 54 were net buyers of water. The marginal effects can be found in Table 4. The model utilizes those variables that vary on the field level and are consistent with irrigation adoption literature and anecdotal interviews with the producers in the research area.

The model reveals that the most significant factors in determining the direction of trade for permanent transactions are the average field size, the field's average use until the time of the trade, and the MAC prediction of the relationship. Although the field size is statistically significant, the marginal effect is relatively small and indicates that increasing the field size by one acre increases the probability of being a seller by 0.19%. The positive sign of the effect is consistent with expectations as fields with more certified acres have potentially more excess certified acre allocations available to sell. Larger fields are also less likely to be buyers due to the pivot irrigation limitations of existing technology employed in the area.

The significance level of average field use up to the time of trading is the highest of the model and exhibits the expected negative sign. The variable captures the use-history prior to the trade and indicates that fields with higher average use are less likely to be sellers of water, which is consistent with expectations. When average usage increases by one inch, the marginal effect decreases the probability of being a seller by 6.5%.

The final significant variable is the MAC indicator variable, which was created by examining the curve relationships within the convex portion of the curves of the fields involved. The ability of the MAC to accurately identify a field's role in the trading scheme helps to validate its mathematical calculation and appropriateness for predicting permanent trade possibilities in the research area. When the MAC variable predicts a seller, the marginal effect indicates an increase in the probability of being seller by 30.8%.

Although the stream depletion factor is not highly statistically significant, it exhibits the expected sign and has a large positive marginal impact. When the sdf increases by 0.01, the marginal effect indicates that the field is 29.2% more likely to be a seller of water. This is great news for the URNRD water managers as it indicates that the pumping is moving away from areas where pumping has a larger impact on stream flow and is helping the district stay in compliance with the Compact.

This model performs the best of the six and is highly correct in predicting the direction of permanent formal trades according to the two measure of evaluation. The pseudo R-squared for this model is 0.2656, which is the highest among the three trade direction models. The goodness-of-fit method also resulted in the correct prediction of 75 of the 100 observations, or 75%. Of those predicted accurately, 33 or 44% were net sellers and 42 or 56% were net buyers.

Table 4 Marginal Effect Estimates for Model 4.

Margins	dy/dx	Delta-method Standard Error	Z-Score
Field size, acres	0.0019**	0.0009	2.18
Average use until trade	-0.0654***	0.0201	-3.26
Percentage of years planted to corn until trade	0.3299	0.3007	1.1
Owner = operator indicator	-0.0231	0.1266	-0.18
Operation size	-0.0044	0.0055	-0.81
Medium soil type	-0.0425	0.1310	-0.32
Coarse soil type	0.0053	0.1985	0.03
Trade size	-0.0021	0.0016	-1.28
MAC	0.3080**	0.1287	2.39
Stream depletion factor	0.2923	0.2612	1.12
Single, double, and triple asterisks (*,**,***) denote statistical significance at the 10%, 5% and 1% levels, respectively			

CONCLUSION

Water trading literature states that there are significant economic gains to be achieved by moving water from areas of low efficiency to areas of higher efficiency within a region. This study utilized probit models and marginal effects analysis of factors that help predict the probability of participating in formal and informal trades, as well as the direction of trade among participants, in an effort to achieve the aforementioned economic gains and to better predict the impacts of groundwater pumping. The participation models used field-level variables and provided insight into the participation decision process. The trade direction models relied on some of the same factors as participation, but also those factors that are unique to the field and the trade it participated in. The results of the model support our previous trading behavior hypothesis and can be used to guide ex-ante evaluation of groundwater trading in other regions.

The focus on the trade participation models is crucial for determining if it is appropriate to apply the direction of trade models to the entire district or only subsections. For example, can the models be applied to operations of all sizes? The results of the participation models indicate that operation size has a positive marginal effect and is significant for informal trade participation. This indicates that larger operations are more likely to participate in trading, but the significance may be exaggerated and is in fact an artifact of the rules set forth by the URNRD to restrict the distance water is moved. Overall, the participation models do not indicate that separate models are needed and that the general participation models can be applied to the entire district.

A major constraint to the formal trade participation is limited data on formal participation in trades and results in low accuracy model. However, the large participation in informal trades is an indicator that there is, in fact, substantial interest in trading water but that there are currently barriers preventing more formal trading. Once a pool is formed, the marginal cost of trading water is effectively zero, whereas the marginal cost of formal trading under the current process is significantly higher. The large participation in informal trading is a sign of potential economic gains for the District from reducing the transaction costs associated with formal trading. If the marginal cost of participating in a formal trade were reduced—through the aid of an online trading platform to find potential trading partners, for example—participation in formal trades would be expected to increase substantially.

Expanding the formal trading market to include annual use trades (leases of water) in addition to the permanent trades also has the potential to open the market and allow for more observations for the formal participation model. The ranking of the MAC curves are not stationary under different precipitation and crop price scenarios, indicating that buyers and sellers may switch roles under different production situations. The ability for buyers and sellers to switch roles, as predicted by their MAC relationship, indicates that annual leases would provide an additional risk management tool for producers by generating flexibility in the field's annual allocation.

The trade direction models' results perform the best and provide insight into producer behavior and decision-making when it comes to water management in water-short areas. The models indicate that for both formal and informal trading producers behave rationally and generally as expected. By improving the accuracy of trade direction probability, the models for pumping impacts are also improved and are able to more accurately predict the direct and indirect effects of groundwater pumping for irrigation.

Caution must be taken before applying similar models to other areas because field or well level usage data is critical as it is a significant variable in nearly every model. As explained in much of the literature on water markets, there are specific components that must be fulfilled before a water market can exist. These include the installation of meters to record usage (at least annual records of usage), explicit water rights, and enforcement of restrictions. Once these requirements for a groundwater trading market are met, the collection of usage information needed for these models becomes much easier.

The MAC curves are a critical component of the trade direction models and did a good job in helping predict the probability of being a buyer or seller of water. If designed appropriately for the different regions, the curves and MAC indicators can be used to predict direction and ultimately the impacts of groundwater trading in an area. The Water Optimizer program, a key factor in the generation of the MAC values, can be used to model any area of Nebraska, and with slight modification, can be applied to other regions outside Nebraska.

For the URNRD specifically, the large positive marginal effect of the stream depletion factor indicates that formal trading does align with their policy goal of reducing the negative impact on stream flow in the Republican River by groundwater pumping in the District. The marginal effect shows that water is moving from wells that have a large impact on the stream flow (as a percentage of their pumping) to wells that have lower impact on the stream flow. Relaxing the rules on formal trades and increasing participation with lower transaction costs may still be consistent with URNRD goals and more economically desirable.

This research is one of the first to empirically study groundwater trading and provide model results. The results from this research can be used ex-ante to prepare similar models for other areas, include using data from the other Republican River NRDs and expanding to other water-short regions. The main obstacle for application of this research is the lack of usage measurements that will take time to gather as more and more regions are looking to apply meters and enforce restrictions.

Future plans to improve the URNRD models include the generation of more refined measures of relative soil type and other characteristics to separate average characteristics from field characteristics so as to capture more detailed differences at the field level. Creating and conducting a survey of producers would provide additional information about factors that influence the decision-making process, such as education levels, operation structure, and other field characteristics (such as productivity). A major goal of improving this study for future journal publications includes attempting a matching technique for the formal participation model to generate a higher accuracy model than the current method applied. Testing for selection between informal and formal trades will allow for further insight into the behavioral decision of trade participation.

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APPENDIX

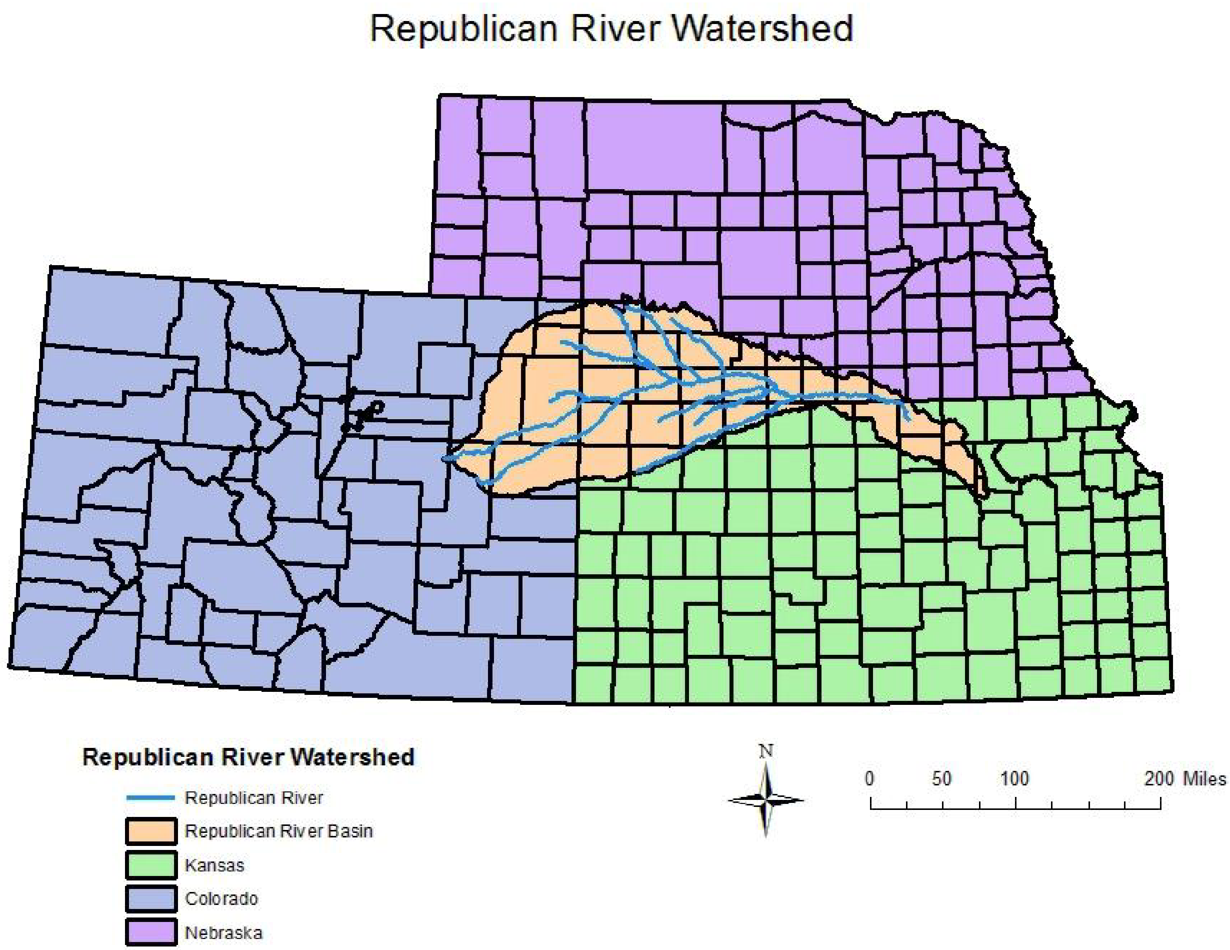


Figure A.1 Map of Republican River Watershed.

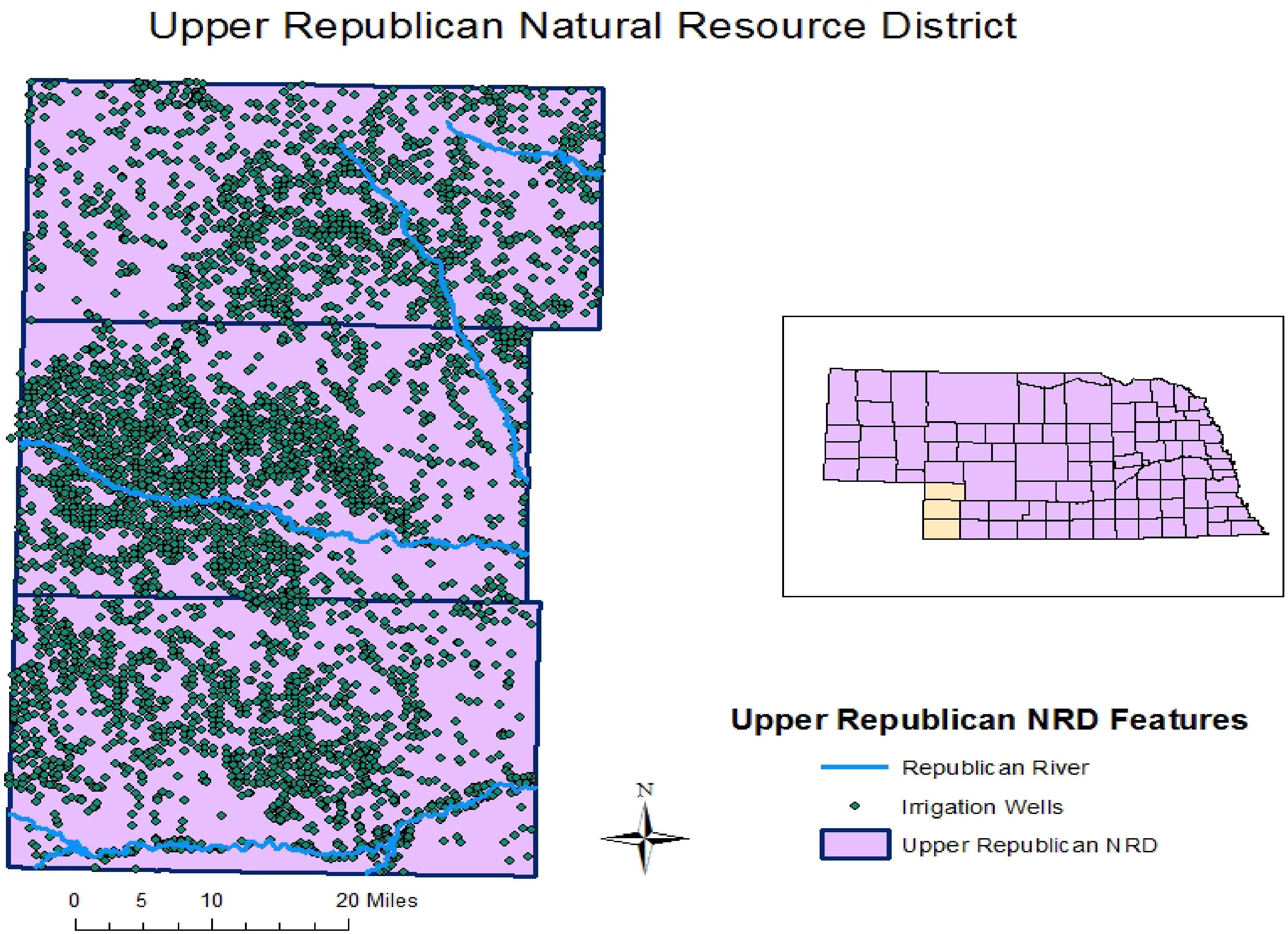


Figure A.2 Map of Upper Republican Natural Resource District

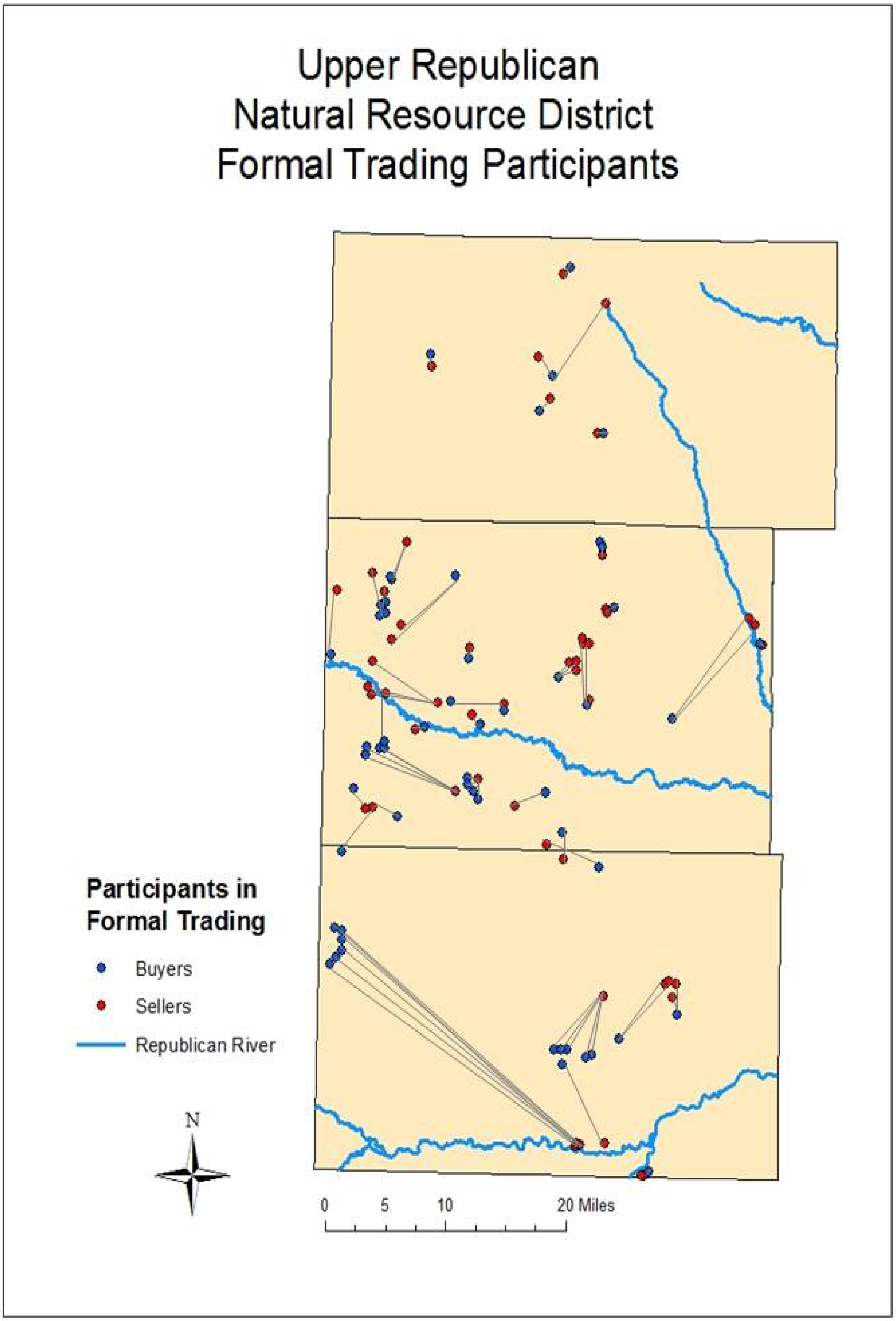


Figure A.3. Location and Direction of Formal Trades.

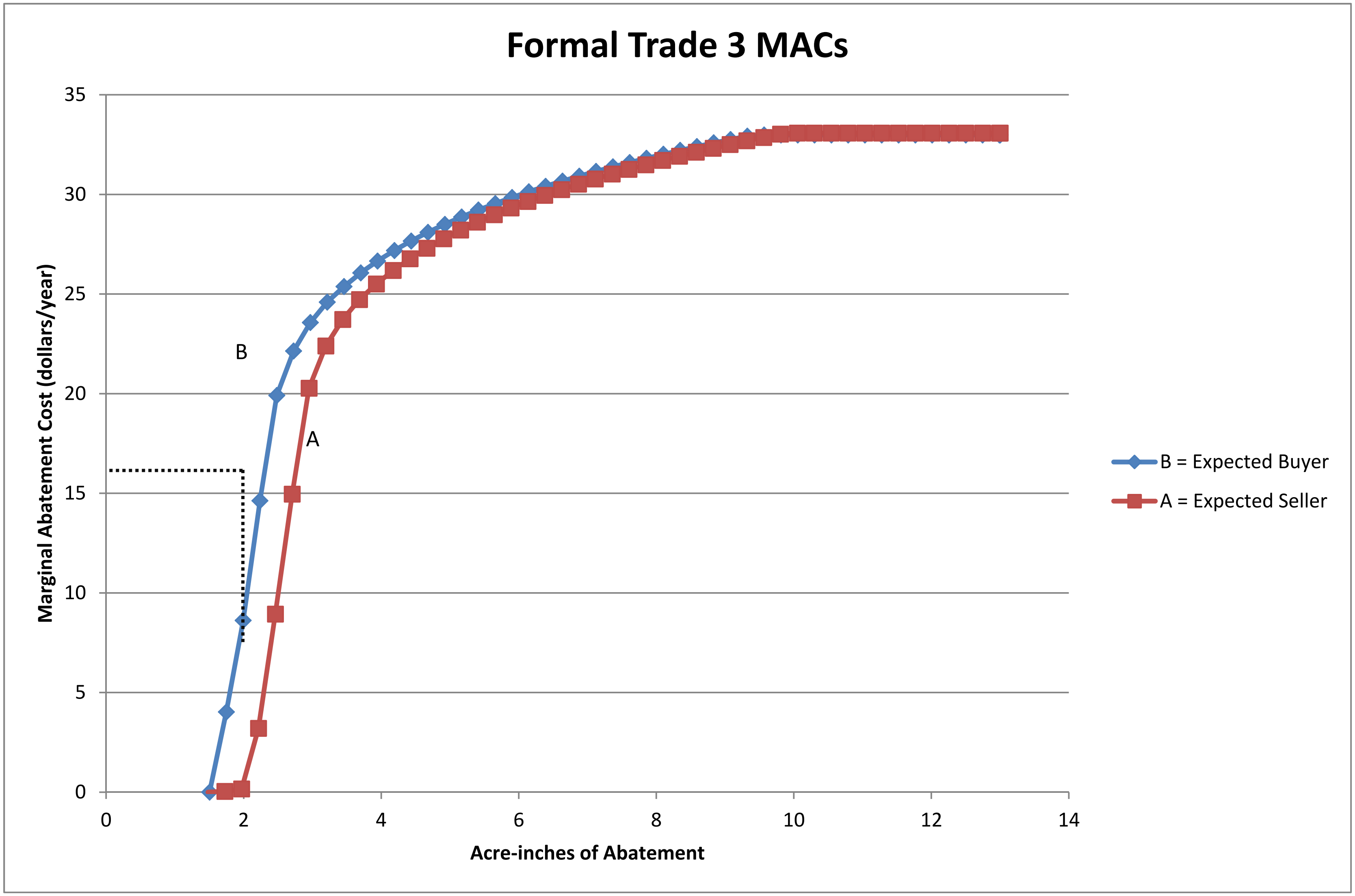


Figure A.4. Graph of Marginal Abatement Cost Curves and Expected Trade Direction

	Formal Trade Participation		'05-'07 Informal Trade Participation		'08-'12 Informal Trade Participation		Formal Trade Direction		'05-'07 Informal Trade Direction		'08-'12 Informal Trade Direction	
Variables	n=	3179	n=	3119	n=	3122	n=	100	n=	1974	n=	1914
Continuous	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
acres	155.966	67.194	155.469	66.320	155.470	66.330	167.439	76.301	155.835	64.646	156.155	65.259
gpm	1512.162	757.449	1508.731	756.953	1508.672	756.879	1489.140	711.857	1538.196	769.472	1540.356	770.243
pwl	136.192	73.506	136.511	73.518	136.504	73.535	113.473	57.704	125.953	66.620	125.778	66.229
opsize	13.383	18.519	13.461	18.600	13.460	18.594	14.030	13.841	16.038	20.467	16.013	20.481
useavg12	12.008	2.976	-	-	12.007	2.985	-	-	-	-	12.706	2.709
percorn12	0.743	0.220	-	-	0.743	0.220	-	-	-	-	0.761	0.196
useavg07	-	-	12.061	3.139	-	-	-	-	12.808	2.860	-	-
percorn07	-	-	0.725	0.233	-	-	-	-	0.746	0.207	-	-
avgusetrade	-	-	-	-	-	-	11.363	4.360	-	-	-	-
percorntrade	-	-	-	-	-	-	0.642	0.263	-	-	-	-
SDF	-	-	-	-	-	-	0.583	0.234	-	-	-	-
Tradesize	-	-	-	-	-	-	34.759	37.547	-	-	-	-
transfersize	-	-	-	-	-	-	-	-	2.120	2.172	2.060	2.297
Binary =1	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**
ownop	1,812	57	1,778	57.01	1,782	57.08	53	53	1,105	55.98	1,083	56.58
medium	1,069	33.63	1,048	33.6	1,050	33.63	46	46	692	35.06	665	34.74
coarse	1,007	31.68	991	31.77	992	31.77	21	21	718	36.37	699	36.52
unksoil	50	1.57	49	1.57	49	1.57	-	-	27	1.37	26	1.36
Perkins	913	28.72	908	29.11	907	29.05	-	-	-	-	-	-
Dundy	909	28.59	887	28.44	889	28.48	-	-	-	-	-	-
MAC2	-	-	-	-	-	-	-	-	1,009	51.11	977	51.04
constr	-	-	-	-	-	-	-	-	552	27.96	247	12.9

Table A.1 Descriptive Statistics

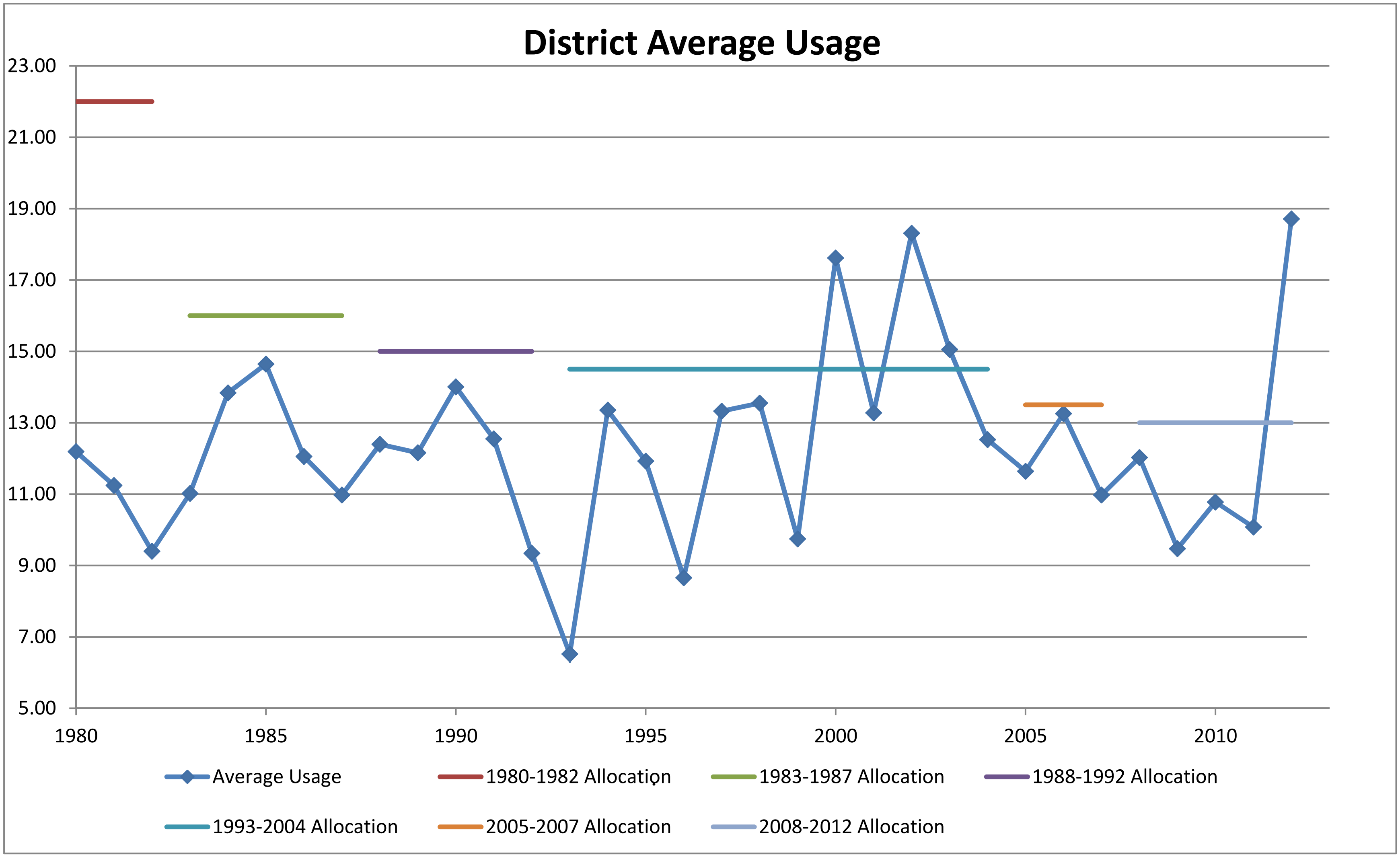


Figure A.5 District Average Use and Allocation Limits

Developing a two-tier screen to evaluate the health of Nebraska's wetlands

Basic Information

Title:	Developing a two-tier screen to evaluate the health of Nebraska's wetlands
Project Number:	2012NE227B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	NE-002
Research Category:	Water Quality
Focus Category:	Wetlands, Toxic Substances, Surface Water
Descriptors:	None
Principal Investigators:	Alan S Kolok, Craig R Allen, Paul H Davis

Publications

1. Alan S. Kolok, Marlo K. Sellin-Jeffries, Lindsey A. Knight, Daniel D. Snow, & Shannon L. Bartelt-Hunt, The hourglass: A conceptual framework for the transport of biologically active compounds from agricultural landscapes. Journal of the American Water Resources Association (In Review).
2. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, The spring runoff in Nebraska's Elkhorn River watershed and its impact on two sentinel organisms. Environmental Toxicology and Chemistry (In Press).

WRRC 104B Project Annual Report

Project # 2012NE227B

(Activities during the period March 1, 2012 through February 28, 2013)

Project Title:

DEVELOPING A TWO-TIER SCREEN TO EVALUATE THE HEALTH OF NEBRASKA'S WETLANDS

Principal Investigators:

Alan S. Kolok[1], Paul H. Davis[1], & Craig R. Allen[2]

[1]Department of Biology
University of Nebraska at Omaha
6001 Dodge Street
Omaha, NE 68182

[2]School of Natural Resources
University of Nebraska-Lincoln
3310 Holdredge Street
Lincoln, NE 68583

Contents

Executive Summary

The goal of this project was to develop a two-tier screening tool that could be used to evaluate the agrichemical contamination of ephemeral wetlands. In achieving this goal, two specific objectives were proposed: 1) the development of genetic biomarkers in the northern leopard frog (*Lithobates pipiens*) so that it can become an environmental sentinel organism for ephemeral wetland environments and 2) the successful completion of a field trial in which the two tier screening approach using atrazine test strips would be tested. A number of useful outcomes were generated from this work. In order to develop genetic biomarkers for use in *L. pipiens,* the entire *de novo* transcriptome for *L pipiens* was assembled. This resource allowed for the completion of multiple genetic biomarkers for use in *L. pipiens* as an environmental sentinel. Additionally, the massive genetic dataset generated by the transcriptome will assist researchers in fields utilizing this model organism where previously genetic resources were previously lacking. In developing the two-tier screen, the atrazine test strips were assessed in two separate field trials. The two-tier screen was shown to be a useful tool in identifying periods of agrichemical runoff within a single watershed. Using this screen, the biomarker responses of *L pipiens* were verified following exposure to water during an agrichemical pulse. In the second field trial, atrazine test strips were used to screen multiple wetland sites for atrazine contamination. While the two-tier screen was successful in monitoring a single watershed, the use of atrazine tests strips across multiple sites warrants greater investigation due to inconsistencies in the test results.

Project Status

Publications

1. Alan S. Kolok, Marlo K. Sellin-Jeffries, Lindsey A. Knight, Daniel D. Snow, & Shannon L. Bartelt-Hunt, The hourglass: A conceptual framework for the transport of biologically active compounds from agricultural landscapes. Journal of the American Water Resources Association (In Review).

2. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, The spring runoff in Nebraska's Elkhorn River watershed and its impact on two sentinel organisms. Environmental Toxicology and Chemistry (In Press).

Presentations

1. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, May 2013, A Tale of Two Species: Do Fathead Minnows and Northern Leopard Frogs Respond Similarly to Agrichemical Runoff? Dakota Amphibian and Reptile Network (DARN) Annual Meeting, Vermillion, SD.
2. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, April 2013, A Tale of Two Species: Do Fathead Minnows and Northern Leopard Frogs Respond Similarly to Agrichemical Runoff? 4th Annual UNO Biology Research Symposium, Omaha, NE.
3. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, April 2013, Development of the Northern Leopard Frog as an Environmental Sentinel of Nebraska's Wetlands, Nebraska Herpetological Society, Omaha, NE.
4. Matthew K. Christenson, Andrew J. Trease, Steven V. Ready, Lindsey A. Knight, Alan S. Kolok, and Paul H. Davis, April 2013, The Transformation of the Sentinel Organism Lithobates pipiens, The Nebraska Academy of Sciences 123nd Annual Meeting, Lincoln, NE.
5. Matthew K. Christenson, Andrew J. Trease, Steven V. Ready, Lindsey A. Knight, Alan S. Kolok, and Paul H. Davis, March 2013, From Frog to Knight: The Rise of the Sentinel Organism Lithobates pipiens, 5th Annual Student Research and Creative Activity Fair at the University of Nebraska-Omaha, Omaha, NE.

6. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, March 2013, A Tale of Two Species: The Fathead Minnow and Northern Leopard Frog as Environmental Sentinels of the Elkhorn River. 5th Annual Student Research & Creative Activity Fair, Omaha, NE.
7. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, March 2013, A tale of two species: Do fathead minnows and northern leopard frogs respond similarly to agrichemical runoff? Abstract accepted at the American Water Resources Spring Specialty Conference, St. Louis, MO.
8. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, December 2012, A Comparison of Fathead Minnows and Northern Leopard Frogs as Environmental Sentinels. Midwest Amphibian Interest Group, Omaha, NE.
9. Lindsey A. Knight, Matthew K. Christenson, Andrew J. Trease, Paul H. Davis, & Alan S. Kolok, November 2012, Poster: The Elkhorn River Atrazine Pulse: Does it Cause Reproductive Effects in Aquatic Organisms? Water: Science, Practice and Policy 2012, Lincoln, NE.
10. Matthew K. Christenson, Andrew J. Trease, Steven V. Ready, Lindsey A. Knight, Alan S. Kolok, and Paul H. Davis, April 2012, De novo assembly and characterization of the Northern Leopard Frog's transcriptome, 2nd Annual Beta Beta Beta National Biological Honor Society Undergraduate Research Symposium at the University of Nebraska-Omaha, Omaha, NE
11. Matthew K. Christenson, Andrew J. Trease, Steven V. Ready, Lindsey A. Knight, Alan S. Kolok, and Paul H. Davis, February 2012, Nebraska's farmland amphibian springs from river bank to GenBank, 4th Annual Student Research and Creative Activity Fair at the University of Nebraska-Omaha, Omaha, NE.

Students Supported by 104b Funds

1. Lindsey Knight, Graduate Student, expected graduation, Fall 2013

1. Project Description

A recent national reconnaissance conducted by the USGS tested 139 waterways in 30 states for the occurrence of organic wastewater contaminants (Kolpin and others, 2002). The compounds were detected in approximately 80% of the waterways tested with the median number of contaminants at any given site being eight. When extrapolated to the United States as a whole, these data suggest that a vast environmental monitoring program may be necessary to adequately assess the current state of the nation's waterways. Given the cost of analysis and personnel necessary to quantitatively collect and analyze water samples for trace organic contaminants, it is apparent that the current resources available to assess the nation's water quality may not be up to the task. In fact, recent papers have suggested that a tiered approach to studying contaminants over a large area, in which small-scale and citizen scientists can be successfully used to conduct first-tier screening for environmental contaminants (Kolok and Schoenfuss, 2010; Kolok and others, 2011).

In Nebraska and throughout the Midwestern United States, the most important trace organic contaminants may prove to be pesticides and veterinary pharmaceuticals, as they are widespread and have well documented, though perhaps somewhat controversial, endocrine disruptive activities (Kolok and others, 2007). In addition, the waterways in Nebraska that may need to be tested may prove to be the numerous and often ephemeral wetlands and potholes that dot the landscape. Concentrations of agrichemicals are likely to reach their highest levels in these ephemeral ponds, and water from these ponds will ultimately contribute to the permanent surface and ground water.

There are two vexing problems associated with sampling these small wetlands. The first is simply the sheer number of these wetlands, as there are thousands scattered across eastern and central Nebraska. The second is the lack of a well characterized, sentinel organism that can be used to accurately assess the relative severity of the agrichemical contamination within these small wetlands. The goal of this project is to develop a two-tier screening tool that can be used to evaluate the agrichemical contamination of these small wetlands while simultaneously addressing the two vexing problems listed above. To achieve the goal of developing a two-tier screening tool to evaluate the agrichemical contamination of ephemeral wetlands, two specific objectives have been proposed:

1. Develop genetic biomarkers in the northern leopard frog (*Lithobates pipiens*) so that it can become an environmental sentinel organism for ephemeral wetland environments.
2. Successfully complete a field trial in which the two tier screening approach will be field tested.

2. Research Objectives

2.1 Objective 1: Biomarker Development in *Lithobates pipiens*

The first objective of this project was to develop genetic biomarkers in the northern leopard frog (*Lithobates pipiens*) so that it can become an environmental sentinel organism for ephemeral wetland environments*.* Characteristics of L*. pipiens* suggest it may provide an ideal model organism for the study of agrichemical contaminants in ephemeral wetland environments. First, ephemeral wetlands are commonly used as breeding grounds for *L. pipiens.* The amphibious nature of the organism causes them to lay eggs in ponds that are dry most other seasons of the year. These ponds have been demonstrated to have extremely elevated levels of pesticide concentrations (Hayes and others, 2003). Second, much previous work exists detailing this species and the impacts of pesticides on their reproductive capabilities (Hayes and others, 2010).

Development of *L. pipiens* as an environmental sentinel first requires the design of quantitative real time polymerase chain reaction (qPCR) primers that can be used to assess the expression of gene transcripts that are expected to vary upon exposure to endocrine disrupting compounds, such as agrichemicals. EDC exposure biomarkers include genes for steroid receptors (androgen receptor, estrogen receptor-α), genes for transporter proteins (steroidogenic acute regulatory protein), genes involved in steroidogenesis (3b-hydroxy steroid dehydrogenase; 17a-hydroxylase; 17b hydroxy steroid dehydrogenase and aromatase), as well as genes involved in follicle maturation (vitellogenin). The development of additional housekeeping genes is also necessary (such as helicase or actin) to provide increased normalization power (Vandesompele and others, 2002).

One hurdle with regard to the development of genetic markers for *L. pipiens* is the absence of complete genome sequences. The first frog genome, *Xenopus tropicalis*, was only published in 2010 (Hellsten and others, 2010). It was expected that *X. tropicalis* would share significant sequence homology with *L. pipiens,* allowing *X. tropicalis* to serve as a source for initial sequence information based on 5 randomly sampled homologs found to share between 74-99% identity. Despite initial screening of sequence homology, initials attempts to utilize degenerate primer design techniques were unsuccessful. Therefore, the *de novo* transcriptome assembly using isolated *L. pipiens* RNA was necessary to generate complete transcript sequences information for use in the development of qPCR primers. Following primer design, the responsiveness of genetic biomarkers in *L. pipiens* was later tested during field trials as described below.

2.2 Objective 2: Field Trials

The second objective of this project was to successfully complete a field trial in which the two-tier screening approach would be tested. Atrazine test strips were adopted as the test method to assess for agrichemical contamination of ephemeral wetlands. Atrazine test strips provide an inexpensive and rapid alternative to analytical chemistry, producing an immediate ordinal response at the US EPA safe drinking water standard of 3 ppb. Atrazine was specifically targeted due to atrazine's widespread agricultural use, minimal loss experienced during transport, and the commercial availability of atrazine test strips. These characteristics suggest atrazine is an ideal surrogate chemical to screen for contaminated wetlands. Furthermore, atrazine has been implicated as having endocrine disrupting activity leading to the feminization of exposed male frogs (Hayes and others, 2003; Hayes and others, 2010).

The wetlands of Nebraska span the home range of the two sister species, the northern leopard frog (*Lithobates pipiens*) and the plains leopard frog (*Lithobates blairi*). While *L. blairi* has historically been located south of the Platte River and *L. pipiens* located north of the Platte River, modern ranges of these two species allow them to overlap in their distribution where they may hybridize in co-localized regions. As such, it was not possible to make an *a priori* estimate of the relative numbers of each species and hybrids that would be collected. Therefore, the *a priori* assumption was made that the response of the two species to atrazine (i.e., feminization) would be similar.

Within Nebraska, eleven distinct wetland complexes formed the focus of this research project (Figure 1). Initially, one hundred and twenty wetlands across Nebraska were selected to be sampled for the presence of atrazine. Wetlands testing positive for atrazine (i.e. above 3ppb) using atrazine test strips were to be sampled for frogs. Collected frogs were to then be transported to the University of Nebraska to assess the relative expression of EDC-responsive biomarkers. However, in order to better utilize available resources, two smaller-scaled field screening trials were performed to better focus sampling efforts.

The first trial utilizing the two-tiered screening approach was performed within a single watershed, the Elkhorn River. Analysis of the Elkhorn River using atrazine test strips allowed atrazine strip results to be assessed within a site where agrichemicals are commonly detected and known endocrine disruption has previously been observed (Soto and others, 2004; Kolok and others, 2007; Sellin and others, 2009). Because previous endocrine disruption has been associated with Elkhorn River waters, exposure of frogs to Elkhorn River water following atrazine screening allowed for the biomarker responses of *L. pipiens* to be tested and verified.

Figure 1. The eleven distinct wetland complexes found within Nebraska.

The second field trial utilized atrazine test strips within a subset of wetland sites in Nebraska. Atrazine testing and sampling of frogs occurred at wetland sites within the Rainwater Basin and Eastern Saline wetlands (Figure 1). The wetland field trial provided a means to assess the two-tier screen across multiple sites utilizing the atrazine strips to screen for impacts of agrichemical exposure in leopard frogs.

3. Materials and Methods

3.1 Biomarker Development in *Lithobates pipiens*

3.1.1 RNA Isolation. All procedures involving experimental animals adhered to the University of Nebraska and Institutional Animal Care and Use Committee (IACUC) guidelines. Several adult male and female *L. pipiens* were sacrificed and their organs, including liver, gonad, kidney, brain, bone marrow, spleen, and skeletal muscle tissues were isolated as well as whole tadpoles of two developmental stages, according to IUCAC guidelines. In order to preserve the integrity of the RNA, the isolated tissues were stored in RNA*later* (Qiagen) and placed at -80°C. Next, male liver and gonad, female liver, gonad, kidney and brain, and the two tadpole stages were removed from the RNA*later* preservative and separately homogenized using Buffer RLT Plus (Qiagen) and Tissue-Tearor rotary homogenizer (BioSpec). Total RNA was isolated from the homogenates using the RNeasy Plus kit and protocol (Qiagen). The integrity of the RNA in each sample was verified using a Bioanalyzer and the 28S and 18S ribosomal RNA peaks, which is represented by the RNA integrity number (RIN score). The high quality RNA samples were converted into cDNA libraries using the TruSeq RNA Sample Prep Kit and protocol (Illumina).

3.1.2 Transcriptome Assembly and Analysis. RNA was sequenced using the state-of-the-art Illumina GAIIx sequence analyzer at the University of Nebraska Medical Center High-Throughput DNA Sequencing and Genotyping Core Facility. In total, the seven libraries were run on three sequencing lanes (Lane 1: Library A – Male Gonad, Library B – Male Liver, Library C – Female Gonad, and Library D – Female Liver; Lane 2: Library E – Female Brain and Library F – Female Kidney; and Lane 3: Library G – Two Tadpole Stages) with 100 bp paired end reads. A complete assembly was then done with all seven cDNA libraries. To facilitate a fast and accurate transcriptome assembly, the raw sequencing reads were first processed using the *trim by quality score*, *sequence complexity*, and *minimum length* features of the PRINSEQ program (-trim_qual_right 10 -trim_qual_left 10, -lc_method dust -lc_threshold 40, and -min_len70) to remove errors that occurred during sequencing (Schmieder and Edwards, 2011). Next, read variations, redundancies, and errors were reduced by digital normalization using the program Khmer (-C 30 -k 20 -N4 -x 2e9) (Brown and others, 2012). The processed reads were then assembled using the *de novo* sequence assembly programs Velvet (Version 1.2.07) and Oases (Version 1.2.07) in a multi-k fashion in which several single k-mer assemblies were then merged into one final cumulative assembly (Zerbino and Birney, 2008; Schulz and others, 2012). Individual k-mers of 45, 51, 53, 55, 57, 59, 61, 63, and 69 were chosen as a range closely surrounding an experimentally determined "sweet spot," the point at which the greatest N50 statistic was achieved (single k-mer: velvetg –cov_cutoff 5 –exp_cov 13 –scaffolding no –read_trkg yes; multi-k: *velvetg* –conserve Long yes –read tracking, *oases* –merge yes).

Basic transcriptome statistics, including: mean, N50, etc., were obtained using a local Perl script (courtesy of Keith Bradnam). The full *L. pipiens* transcriptome was then compared to the NCBI non-redundant (nr) database (retrieved December 12, 2012) and *Xenopus tropicalis* proteome (retrieved October 13, 2013) using blastx of the BLAST+ program (Version 2.2.27) and an *E*-value of ≤1e-5 (MCAT_REF5 and MCAT_REF6). A representative transcriptome was obtained by using blastx of the BLAST+ program (Version 2.2.27) and an *E*-value of ≤1e-5 to compare the full *L. pipiens* transcriptome against an alias database representing all Reference Sequence (RefSeq) protein sequences belonging to *Metazoan* spp. within the NCBI nr database (retrieved December 15,2012) (Altschul and others, 1990; Altschul and others, 1997). Transcripts that closely matched a given Metazoan protein were counted and in the case of multiple query matches to a single subject protein, the transcript with the highest bit-score was selected.

Multiple functional analyses were performed on the representative *L. pipiens* transcriptome to assess the putative functions of the *L. pipiens* transcripts and validate its completeness against the *X. tropicalis* RefSeq nucleotide entries on NCBI (retrieved March 5, 2013). First, transcripts were translated in silico into gene products by OrfPredictor (Min and others, 2005). These results were aligned using RPS-BLAST from the program BLAST+ (version 2.2.26), with an *E*-value of ≤1e-5, to the Eukaryotic Orthologous Groups (KOG) database (version 1.0) available at NCBI (Tatusov and others, 2003). Next, transcripts were compared to the Eukaryotic and Amphibian GENES Kyoto Encyclopedia of Genes and Genomes (KEGG) datasets using the KEGG Automatic Annotation Server (KAAS) (Version 1.66x) (Moriya and others, 2007).

3.1.4 Primer Design. A preliminary assembly was done using only Libraries A-D and G, livers and gonads of both sexes and the tadpole stages, to aid the development of *L. pipiens* specific steroidogenesis genes primers. The raw sequencing reads were first processed using the trim by quality score, sequence complexity, and minimum length features of the PRINSEQ program (-trim_qual_right 10 -trim_qual_left 10, -lc_method dust -lc_threshold 40, and-min_len50) (Schmieder and Edwards, 2011). The *de novo* sequence assembly programABySS (abyss-pe k=25 n=10) (Version 1.3.4) was then used to assemble the processed reads (Birol and others, 2009; Simpson and others, 2009; Robertson and others, 2010). The steroidogenesis gene transcripts were detected in the preliminary transcriptome using blastx of the BLAST+ program (Version 2.2.27) and an *E*-value of ≤1e-5 and a custom database containing *X. tropicalis* steroidogenesis genes (Retrieved July 18, 2012) (Altschul and others, 1990; Altschul and others, 1997). The sequences demonstrating a significant homology to the *X. tropicalis* steroidogenesis genes were then used as templates for designing primers with which to probe the genes in real-time quantitative PCR (RT-qPCR) using NCBI Primer-Blast and Integrated DNA Technologies PrimerQuest program (Ye and others, 2012). Primers were verified with RT-qPCR analysis followed by gel electrophoresis (2% agarose/TAE) to verify size.

3.2 Elkhorn River Field Trial

3.2.1 Atrazine as a surrogate chemical for agrichemical runoff. In the Elkhorn River, atrazine test strips were used to define a period when a pulse agrichemical runoff was likely to occur. Strip testing of the Elkhorn River began on March 31, 2012 and was repeated every three days until the first recorded positive atrazine test result on May 4. Two consecutive positive strip results (May 4 and May 5) were used to signify the beginning of the pulse (Figure 2).

3.2.2 Water Collection and POCIS Deployment. During the agrichemical pulse, polar organic chemical integrative samplers (POCIS) were deployed in the Elkhorn River to provide an assessment of agrichemicals present in the Elkhorn River. Atrazine strip testing of the Elkhorn River was used to determine when POCIS would be deployment as well as when water collection for laboratory exposures would occur (Figure 2). Following the detection of atrazine, POCIS were deployed on May 5 with retrieval of the POCIS occurring 14 days later on May 19. Water was collected for laboratory exposures with *L. pipiens* from May 7 to May 13 and transported to the University of Nebraska laboratory. The water collected was taken directly from the Elkhorn River at the Elkhorn River Research Station test site located approximately 10 km from the confluence of the Elkhorn and Platte Rivers. Atrazine strip tests were performed daily throughout the week. From May 13 to July 14, interval strip testing was again conducted every 3 days. The last positive atrazine test strip was recorded on June 3. POCIS were deployed during a post-pulse period starting on June 30, with retrieval of the POCIS occurring 14 days later on July 14.

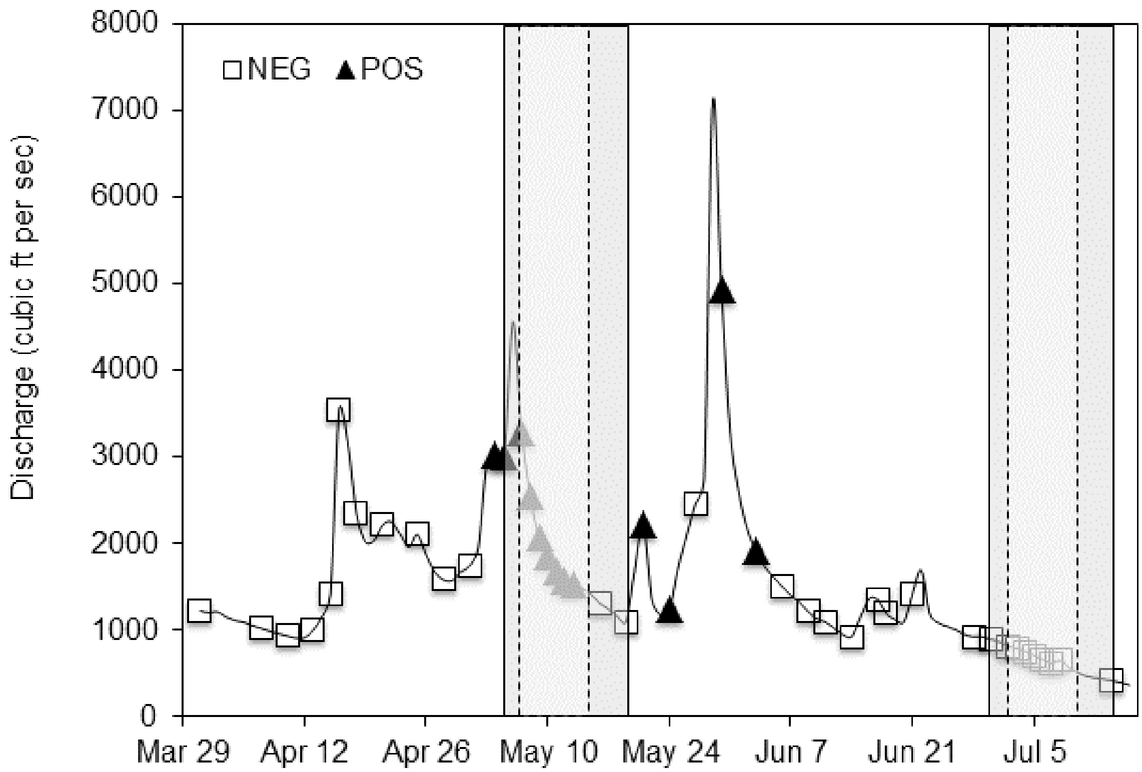


Figure 2. The temporal occurrence of atrazine was determined using atrazine test strips. Elkhorn River hydrograph data was used to highlight the use of atrazine as a surrogate to predict the occurrence of agrichemical runoff. Positive atrazine test strip results are shown as closed triangles while negative test results are represented as open boxes. The pulse and post-pulse POCIS

deployment periods are highlight by the dark grey bars. The 7 day exposure periods during which water was collected from the Elkhorn River are denoted by the light grey bars within the dashed lines. *Figure adapted from Knight et al. (2013).

3.2.3 POCIS and Chemical Analyses. POCIS were obtained from Environmental Sampling Technologies and were stored at -20 °C until their field deployment. After the 14 day deployments, POCIS were removed from their deployment canisters, rinsed briefly, and stored at -20 °C until analysis. The POCIS were analyzed by the University of Nebraska Water Sciences Laboratory for 17 steroids and 21 pesticides (Table I) following previously described protocols [3, 26]. Steroid concentrations were determined by LC/MS/MS using previously published methods (Bartelt-Hunt and others, 2011; Snow and others, 2013), while pesticide concentrations were determined using GC/MS (Cassada and others, 1994; Sellin and others, 2009). The instrumental method detection limits for steroid hormones and pesticides analyzed were estimated at 0.5 ng. Recovery of analytes in laboratory fortified blanks analyzed at the same time as the POCIS extracts averaged 71 ±11% for steroid hormones and 102±19% in the pesticide method. No analytes were detected in laboratory reagent blanks analyzed at the same time as the extracts. Amounts of pesticides and steroids in POCIS extracts were quantified as ng per extract (ng/POCIS). The mass of detected pesticides and steroid hormones in ng/POCIS were then converted to a time weighted average (TWA) concentration (ng/L) using previously determined sampling rate coefficients (Mazzella and others, 2007; Bartelt-Hunt and others, 2011). The TWA concentration was determined by dividing the mass (ng/POCIS) by the sampling coefficient (L/d) and the total number of days deployed (d).

3.2.4 Sentinel Organisms. All procedures involving experimental animals adhered to the University of Nebraska and Institutional Animal Care and Use Committee (IACUC) guidelines. All animals were maintained on a 16:8 light dark cycle at ambient room temperature (20±1°C) throughout all experimental procedures. Laboratory water to which frogs were exposed prior to the start of experiments consisted of de-chlorinated Omaha tap water.

Sexually mature male and female northern leopard frogs (*Lithobates pipiens*) were obtained from a commercial supplier (Connecticut Valley Biological) 4 days prior to the start of the exposures. Frogs were segregated by sex and then randomly assigned to exposure units where they were maintained on de-chlorinated water until the start of the experiment. . Tanks contained 7 L of static water at room temperature (20±1°C). Frogs were fed mealworms and crickets every other day. Complete water changes were performed in both the exposed and unexposed treatments daily.

Unexposed frogs received laboratory water only, while exposed frogs received daily water changes from Elkhorn River water.

Table I. Pesticides and steroid hormones analyzed for in extracts of the polar organic chemical integrative samplers (POCIS) deployed at the Elkhorn River Research Station.

Pesticides		Steroid Hormones	
Acetochlor	Norflorazon	Androsterone	17 α-Hydroxyprogesterone
Alachlor	Pendimethalin	4-Androstenedione	Melengesterol Acetate
Atrazine	Permethrin	Epitestosterone	Testosterone
Butylate	Prometon	17a-Ethynyl Estradiol	α-Trenbolone
Chlorthalonil	Propachlor	α-Estradiol	β-Trenbolone
Cyanazine	Propazine	β-Estradiol	α-Zearalanol
Deethylatrazine	S-Ethyl-dipropylthiocarbamate	Estriol	α-Zearalenol
Deisopropylatrazine		Estrone	β-Zearalenol
Dimethenamid	Simazine	Progesterone	
Metolachlor	Telfluthrin		
Metribuzin	Trifluralin		

** Table originally published in Knight et al. (2013)

3.2.5 Elkhorn River agrichemical exposures. Male and female leopard frogs were exposed to Elkhorn River water collected during the agrichemical pulse (May 7-May 14). Unexposed animals were maintained on de-chlorinated laboratory water. For the Elkhorn River exposed treatment groups, water was bucketed daily from the Elkhorn River into two 100L polyethylene water storage containers during the exposure regimes. Water was replaced in the exposure tanks with collected river water within 2 hours of field collection. One female was removed prior to the start of the experiment due to illness, and one male frog mortality was observed on day 1 in the agrichemical exposure treatment group. No other frog mortalities were observed during the agrichemical pulse exposure. At the end of the exposure period, frogs were pithed and body mass and snout-urostyle lengths (SUL) were recorded.

3.2.6 Gene Expression. Following dissection, liver and gonad tissues from frogs were weighed and flash frozen in liquid nitrogen. A Gonadosomatic index (GSI) and hepatosomatic index (HSI) for each individual was generated by dividing the mass of the tissues by the total body mass and multiplying by 100. Tissues from six animals were selected for quantitative real-time PCR (RT-qPCR) from each treatment group. Animals were selected for analysis such that animals across treatment groups were as consistent in mass, GSI and HSI as possible. In cases where the number remaining was greater than six, animals selected for PCR were chosen randomly.

RNA was extracted from the liver tissue of selected animals using the SV Total RNA Isolation System (Promega Corp). RNA was re-suspended and stored in nuclease free water at -80°C until analysis. RNA purity and concentration was assessed by Nanodrop (NanoDrop Technologies). First strand cDNA synthesis was performed with 1µg total RNA using the iScript cDNA Synthesis Kit (Bio-Rad) per manufacturer recommendations.

RT-qPCR reactions were performed using the iQ SYBR-Green Supermix (Bio-Rad) per the manufacturer's protocol. Briefly, 2 µL of diluted cDNA template was added to 300 nM forward and reverse primers in a 15 µL volume containing 1x SYBR-Green Supermix. The RT-qPCR negative control consisted of the primer and SYBR-Green Supermix with 2 µL of nuclease free water in place of the template. Dilution of a single DNA template served as the standard curve by which the samples were quantified.

Two estrogen responsive genes, vitellogenin (Vtg) and estrogen receptor-α (ERα), were selected for analysis in *P. promelas* and *R. pipiens*. Primer sequences for *P. promelas* Vtg, ERα, and ribosomal protein L8 (L8) were obtained from Kolok et al. [3]. Primer sequences for ERα and L8 for *R. pipiens* were obtained from Hogan et al. [32]. The *R. pipiens* Vtg gene sequence was generated by using next-generation RNA sequencing (Table III).

3.2.7 Data Analysis. Expression of the housekeeping gene, ribosomal protein L8, was selected in both species as an internal control as it did not vary significantly across treatment groups in any case. As such, all gene expression data was expressed as the relative mRNA expression normalized to L8 mRNA expression. Data from one female fish was excluded from mRNA expression analysis in the pulse unexposed treatment group due to non-amplification of the cDNA template. Significant differences in mass, HSI, GSI, and relative mRNA expression between exposed and unexposed male and female animals were tested using a single *t*-test (JMP 9.0.1). Statistical significance was assumed at $p \leq 0.05$.

3.3 Wetland Field Trial

3.3.1 Wetland Sites. To focus sampling efforts, the pilot wetland field trial was narrowed to a subset of wetlands within the Rainwater Basin and Eastern Saline wetlands. The Rainwater Basin is an area of shallow marshes and wetlands south of the Platte River in south-central Nebraska.

Wetlands in the Basin are largely rain-filled and many are dry by late summer. Nine sites were selected using the National Wetland Inventory database and a public lands GIS layer. Selected sites Waterfowl Production Areas (WPAs) under the management of the U.S. Fish and Wildlife Service, Wildlife Management Areas (WMAs) under the management of the Nebraska Game and Parks Commission, and city parks under the management of Lincoln Parks and Recreation. In the interest of sampling frogs from wetlands well buffered from agriculture and therefore unlikely to contain atrazine, reference sites were selected from the Salt Valley in north Lincoln, Lancaster County, Nebraska. All sites were visited and tested with an Abraxis atrazine test strip which detects atrazine at concentrations of 3ppb or higher. When atrazine status at a wetland was confirmed an informal aural and visual survey was performed to detect presence of the target species the Plains leopard frog (*Lithobates blairi*). If leopard frogs were heard or seen the site was included in the pilot study.

3.3.2 Collection of frogs. All procedures involving experimental animals adhered to the University of Nebraska and Institutional Animal Care and Use Committee (IACUC) guidelines. All sites determined to have *L. blairi* were visited at night for collection. The males of many anuran species including the Plains leopard frog vocalize attraction calls from bodies of water during the breeding season. Nighttime collection allowed targeted collection of males of the species and reduced incidental capture of females. Frogs were collected by hand or by net from the water's edge, from within the wetland, from adjacent vegetation, or while crossing nearby roads. Collected individuals were placed in individual plastic containers with air vents and labeled before being placed on ice for transfer. If there were successful captures at any site the water was retested that night to determine the current status of atrazine in the wetland.

3.3.3 Tissue Collection. Frogs collected at field sites were transported to the University of Nebraska and immediately dissected. Frogs were pithed and body mass and snout-urostyle lengths (SUL) were recorded per IACUC guidelines. Following dissection, liver and gonad tissues from both frogs and fish were weighed and flash frozen in liquid nitrogen. A Gonadosomatic index (GSI) and hepatosomatic index (HSI) for each individual was generated by dividing the mass of the tissues by the total body mass and multiplying by 100. Tissues were stored at -80°C.

4. Results

4.1 Biomarker Development in *Lithobates pipiens*

4.1.1 Transcriptome Assembly and Analyses. Sequencing of seven cDNA libraries, male liver and gonad, female liver, gonad, kidney and brain, and the two tadpole stages, yielded 1.166 billion paired end reads totaling 116.6 Gb in length and 286.6 GB of information (Table II). These reads were assembled into a transcriptome containing 650,307 transcripts with a mean, median, and N50 of 1,372 bp, 652 bp, and 2,853 bp, respectively. In addition, >35% of the transcripts of the full assembly yielded significant hits to the NCBI nr and *Metazoan* databases, with 40,842 unique *Metazoan* proteins, and demonstrated an >66% coverage of the *X. tropicalis* proteome. The representative transcriptome had a mean, median, and N50 of 2,272 bp, 1,662 bp, and 3,530 bp, respectively. Moreover, the KOG and KEGG functional analyses of the representative transcriptome revealed putative functions of the *L.* pipiens transcripts, as well as the overall completeness of the transcriptome. That is, the *L. pipiens* and *X. tropicalis* patterns in the KOG and KEGG outputs are nearly identical in all categories, suggesting the completeness of *L. pipiens* transcriptome as compared to the model organism *X. tropicalis* (Figure 3 and 4).

4.1.2 Primer Design. To develop *L. pipiens* into a sentinel organism, a total of 12 *L. pipiens* genes*,* including steroidogenic pathways genes and a number of "housekeeping" genes to be used as stably expressed references, were chosen as biomarkers. The development of these biomarkers relies heavily on reliable primers for which to measure potentially subtle deviations in gene expression. The primers designed to probe the *L. pipiens* steroidogenic were subjected to a two-tiered verification approach. Primarily primers were tested using a *L. pipiens* cDNA template in a RT-qPCR reaction to verify that amplification occurred and that it was specific. Amplification occurred for all designed primers (data not shown), and further analysis was carried out to select a single primer set per biomarker gene. Criteria for qualifying a primer as high included the presence of only a single amplicon which was matched the size predicted by the primer design program, as well as robust amplification. To verify a primer set produced only one amplicon a melting curve analysis was conducted for each of the primer sets of each target biomarker series, a single peak on the curve indicates that only one amplicon was present, as in the case of vitellogenin (Figure 5A). Primer sets that indicated via melting curve analysis to have only a single amplicon were separated on agarose gel to determine if the amplicon was of the predicted size, the vitellogeninin primers were all found to have an amplicon corresponding to the same size as predicted by the primer design program (Figure 5B). The primer set selected as the most robust for each biomarker was that which produced the greatest amplification and met both the single amplicon and fragment size criteria. High quality and reliable primers were created for each of the 12 target genes (Table III).

Table II. Statistics of the paired end reads for the seven *Lithobates pipiens* cDNA libraries, male liver and gonad, female liver, gonad, kidney and brain, and the two tadpole stages. In total, 1,166,235,238 reads were obtained totaling 286.6 GB of data and a length of 116.6 Gb.

Lane	Library	Sample Name	Paired End	Number of Reads	Total Length of Reads (Gb)	Data Size (GB)
1	A	Male Gonad	R1	32,771,881	3.3	8.1
1	A	Male Gonad	R2	32,771,881	3.3	8.1
1	B	Male Liver	R1	35,791,829	3.6	8.8
1	B	Male Liver	R2	35,791,829	3.6	8.8
1	C	Female Gonad	R1	77,777,594	7.8	19.1
1	C	Female Gonad	R2	77,777,594	7.8	19.1
1	D	Female Liver	R1	35,527,804	3.6	8.7
1	D	Female Liver	R2	35,527,804	3.6	8.7
2	E	Female Brain	R1	111,972,214	11.2	27.5
2	E	Female Brain	R2	111,972,214	11.2	27.5
2	F	Female Kidney	R1	108,243,825	10.8	26.6
2	F	Female Kidney	R2	108,243,825	10.8	26.6
3	G	Tadpole	R1	181,032,472	18.1	44.4
3	G	Tadpole	R2	181,032,472	18.1	44.4
-	-	Total	-	1,166,235,238	116.6	286.4

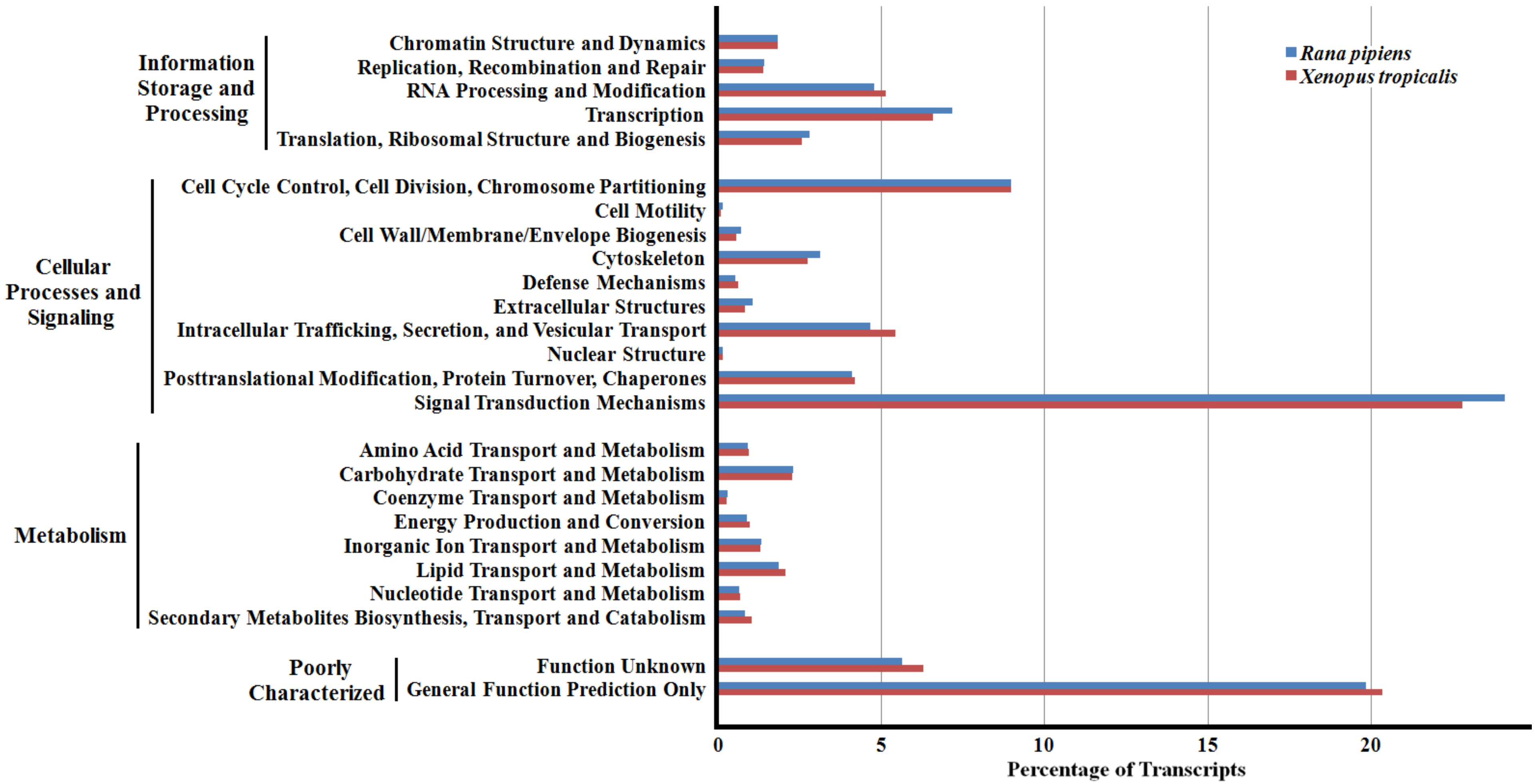
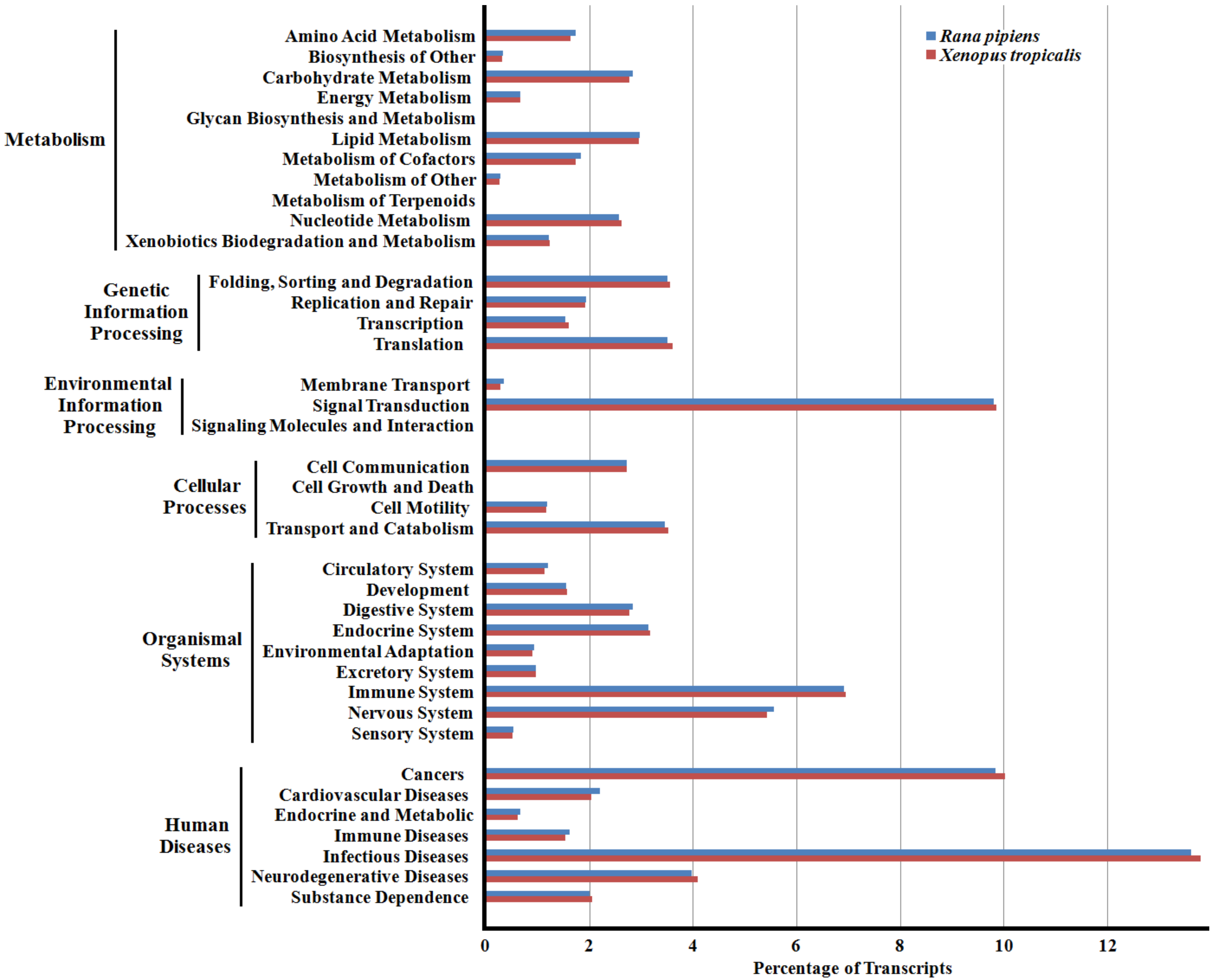


Figure 3. Eukaryotic Orthologous Groups (KOG) characterization of the *Lithobates pipiens* representative transcriptome. Representative transcripts were analyzed using RPS-BLAST against the NCBI KOG database of proteins. The largest group was “Signal Transduction Mechanisms” in both *L. pipiens* and *Xenopus tropicalis*, and little variation was observed been *L. pipiens* and *X. tropicalis* across all groups.



Rana pipiens
Xenopus tropicalis
Metabolism
Amino Acid Metabolism
Biosynthesis of Other
Carbohydrate Metabolism
Energy Metabolism
Glycan Biosynthesis and Metabolism
Lipid Metabolism
Metabolism of Cofactors
Metabolism of Other
Metabolism of Terpenoids
Nucleotide Metabolism
Xenobiotics Biodegradation and Metabolism
Genetic Information Processing
Folding, Sorting and Degradation
Replication and Repair
Transcription
Translation
Environmental Information Processing
Membrane Transport
Signal Transduction
Signaling Molecules and Interaction
Cellular Processes
Cell Communication
Cell Growth and Death
Cell Motility
Transport and Catabolism
Organismal Systems
Circulatory System
Development
Digestive System
Endocrine System
Environmental Adaptation
Excretory System
Immune System
Nervous System
Sensory System
Human Diseases
Cancers
Cardiovascular Diseases
Endocrine and Metabolic
Immune Diseases
Infectious Diseases
Neurodegenerative Diseases
Substance Dependence
0
2
4
6
8
10
12
Percentage of Transcripts

Figure 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the *Lithobates pipiens* representative transcriptome. The *L. pipiens* representative transcripts were compared to Kyoto Encyclopedia of Genes and Genomes (KEGG) Eukaryotic and Amphibians datasets and K-numbers were assigned using KEGG automated annotation serve*L.* Overall, only minor variations can be seen in the percentage of sequences between *L. pipiens* and *X. tropicalis* across all groups.

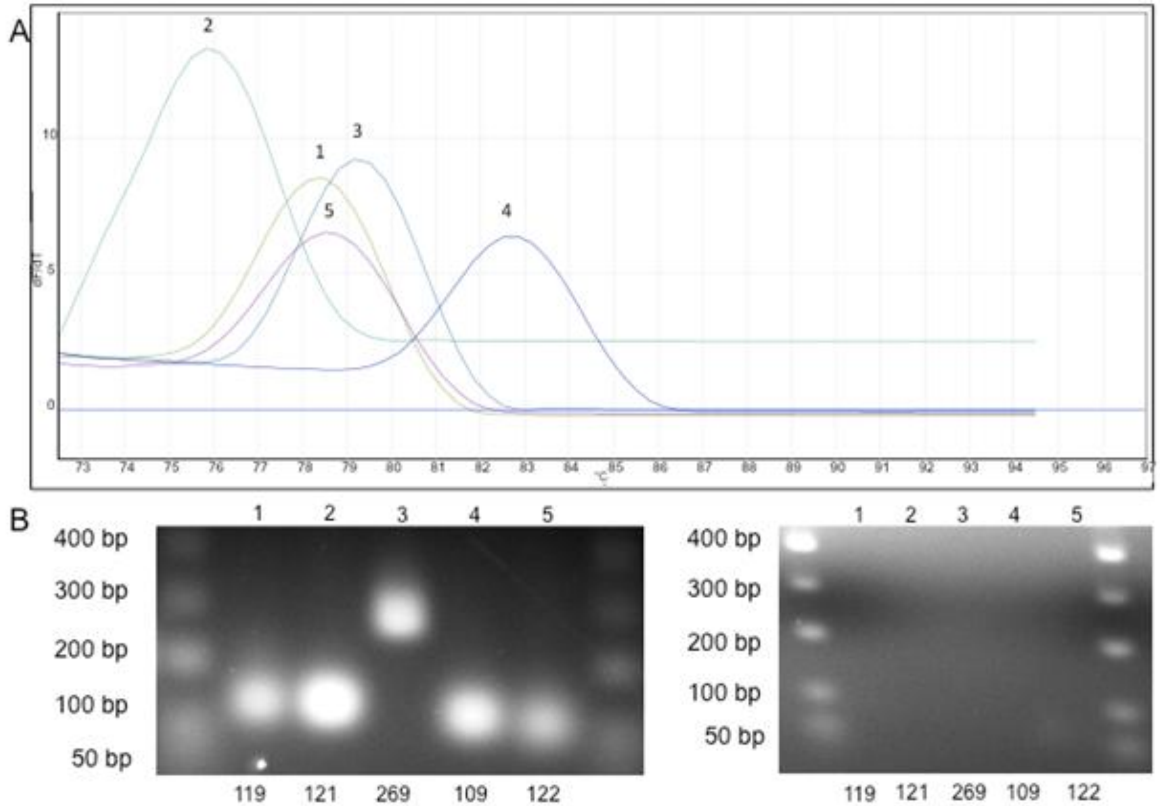


Figure 5. Confirmation of *Lithobates pipiens* specific RT-qPCR primers. A) Melting curve analysis of a series of primers designed for vitellogenin using sequences assembled by ABySS. B) Gel electrophoresis of the RT-qPCR products of the same series of vitellogenin primers as well as the no cDNAcoutnerparts, integers placed across the lower edge of the gels are expected size based on information provided by the primer design program.

Table III. *Lithobates pipiens* specific primers for steroidogenesis and housekeeping genes obtained from the preliminary *L. pipiens* transcriptome. All primers were confirmed using melt curves and gel electrophoresis.

Gene Name	Amplicon Size (bp)	Primer Sequence (5'-3')	
		Forward	Reverse
3-β-hydroxy steroid dehydrogenase (3-β-HSD)	131	ATTACCACCAGCGCTATGACCCTT	TGGATGGTGTCAGTGATAGCCCAA
11β-hydroxylase (Cyp11b1)	89	GGTAACTCGCTCTTCGCACT	CTAGTGGCGGGTTTGTCACT
17-α-hydroxylase (Cyp17a1)	175	AATGTCATGGCTCCCCAGTC	GGAAGCCTGCATGTGGGTAA
Androgen receptor (AR)	119	GCACTACCCTTCATAACTACTCCT	GGTAGCAAAGCGATATGCAACTAA
Aromatase (Cyp19a1)	113	TACTTTCAGCCATTTGGTTTTGGT	AGGGTTTGCACCTTGTATCTTTTC
Estradiol 17-β-dehydrogenase 12-B (DH12B)	149	AGCTGGAAACCTTCCACACGATGA	ACTTGGCTTCAGGGAAGTGATGGA
Glucocorticoid receptor (GR)	155	GTGCCCATGGACTACCCTTT	ATGTGACCTGTCAACCCAGC
Hypoxia inducible factor (HIF)	98	ATGCTCGCAAAGCATGGTGGTTAC	TTCACACAGACGATGCATTGTGGC
Ribosomal protein L13 (RiboL13)	96	TAGAGGGATTCAAGGGGAACAGAA	GGAGAAGAAACATATCGGTCGAAG
Steroidogenic acute regulatory protein (StaR)	88	ATCAACCAGGTTCTCTCCCAGACA	AGCAGAGAGACACATTGGAGGACA
TATA-box-binding protein (TBP)	61	AAGTTCGAGCCGAGATCTATGAAG	CGAAAGCCCTTCAAAATTGGGTAT
Vitellogenin (VTG)	121	AAGTCCACTAATCCCATTCTCCTG	ACCAAAAGACCTGTCAGAGACTAC

4.2 Elkhorn River Field Trial

4.2.1 Atrazine strips. Atrazine strip results were collected from March 31, 2012 until July 18, 2012 (Fig. 1). No positive hits occurred earlier in the season than May 4, nor did any positive hits occur after June 3. Between these two dates, the positive hits tended to be clustered immediately after rainstorm events indicated by the peaks of increased discharge (Figure 2). The occurrence of atrazine greater than 3ppb is consistent with field application dates resulting in the pulse of atrazine.

4.2.2 POCIS. POCIS extracts contained both pesticides and steroids (Table IV). For pesticides, the pulse and post-pulse periods had similar chemical signatures, although pesticides were found in much greater proportion during the pulse. Acetochlor and simazine had the greatest fold change with a decrease in concentration of 281 fold and 48 fold respectively, during the post pulse. Atrazine, dimethenamid, and Metolachlor were 28, 26, and 23 fold lower in pesticide concentration, respectively, during the post-pulse. Pesticide concentrations that showed the least temporal variability were prometon, deethylatrazine, deisopropylatrazine, alachlor, and propazine which ranged from 1.6 to 11.38 fold lower during the post pulse relative to the pulse. Very few steroids were present in the POCIS extracts. Of the steroids analyzed for in Table I, only 4-androstenedione was detected at both time periods, while both estrone and progesterone were detected only during the post-pulse (Table IV).

4.2.4 Elkhorn River agrichemical exposures. Frog morphometrics are shown in Table V. No significant differences were detected in mass, HSI, GSI, or SUL. The expression of Vtg and ER-α was unaffected in female northern leopard frogs (Figure 6A - 6B, respectively; $p > 0.37$ in both cases). Although the mean Vtg mRNA expression in exposed male northern leopard frogs was 2.5 fold greater than unexposed males, this difference was not statistically significant (Figure 6C; $p = 0.0547$). However, expression of ER-α transcripts was significantly increased in the male pulse exposed leopard frogs relative to the control (Figure 6D; $p = 0.0055$).

Table IV. A comparison of pesticides and steroid hormones detected in polar organic chemical integrative samplers (POCIS) deployed in the Elkhorn River in 2012.[a] Concentrations determined as ng/POCIS were measured in duplicate and then converted to ng/L using published time weighted average (TWA) rate uptake coefficients from Bartelt-Hunt et al. [27] unless otherwise specified. Concentrations are shown as M(±SD).

Pesticides and Steroids	Pulse (ng/L)	Post-pulse (ng/L)
Acetochlor[c]	3460.4 (101.3)	12.3 (0.6)
Alachlor	2.5 (0.1)	0.3 (0.02)
Atrazine	4812.9 (45.4)	170.3 (4.4)
Deethylatrazine	382 (7.0)	67.0 (0.3)
Deisopropylatrazine[c]	736.5 (13.2)	119 (3.9)
Dimethenamid	150.9 (8.0)	5.7 (0.2)
Metolachlor	514.2 (20.3)	22.6 (1.1)
Prometon	6.3 (0.2)	3.9 (5.5)
Propazine	45.5 (0.8)	4.0 (0.8)
Simazine[c]	19.3 (0.05)	0.4 (0.5)
4-Androstenedione	0.11 (0.06)	0.20 (0.01)
Estrone	ND	0.69 (1.0)
Progesterone	ND	0.13 (0.2)

* Figure adapted from Knight et al. (2013).

[a] Pesticides and steroid hormones that were analyzed for but not detected are not shown.

[b] Concentrations in ng/L determined by rate coefficients published in Mazzella et al. [30]

[c] ND = below limit of detection

Table V. Morphometrics of exposed frogs. Values are represented as the M(±SD).

	Male		Female	
	Control	Exposed	Control	Exposed
Mass	34.8(0.83)	33.7(1.6)	39.3(1.4)	43.8(1.6)
HSI	3.6(0.30)	3.4(0.23)	3.4(0.43)	3.3(0.28)
GSI	0.2(0.04)	0.2(0.03)	16.1(1.8)	15.4(0.94)
SUL	66.6(1.1)	65.1(1.3)	65.6(1.4)	69.1(1.1)
n	8	7	7	8

* Figure adapted from Knight et al. (2013).

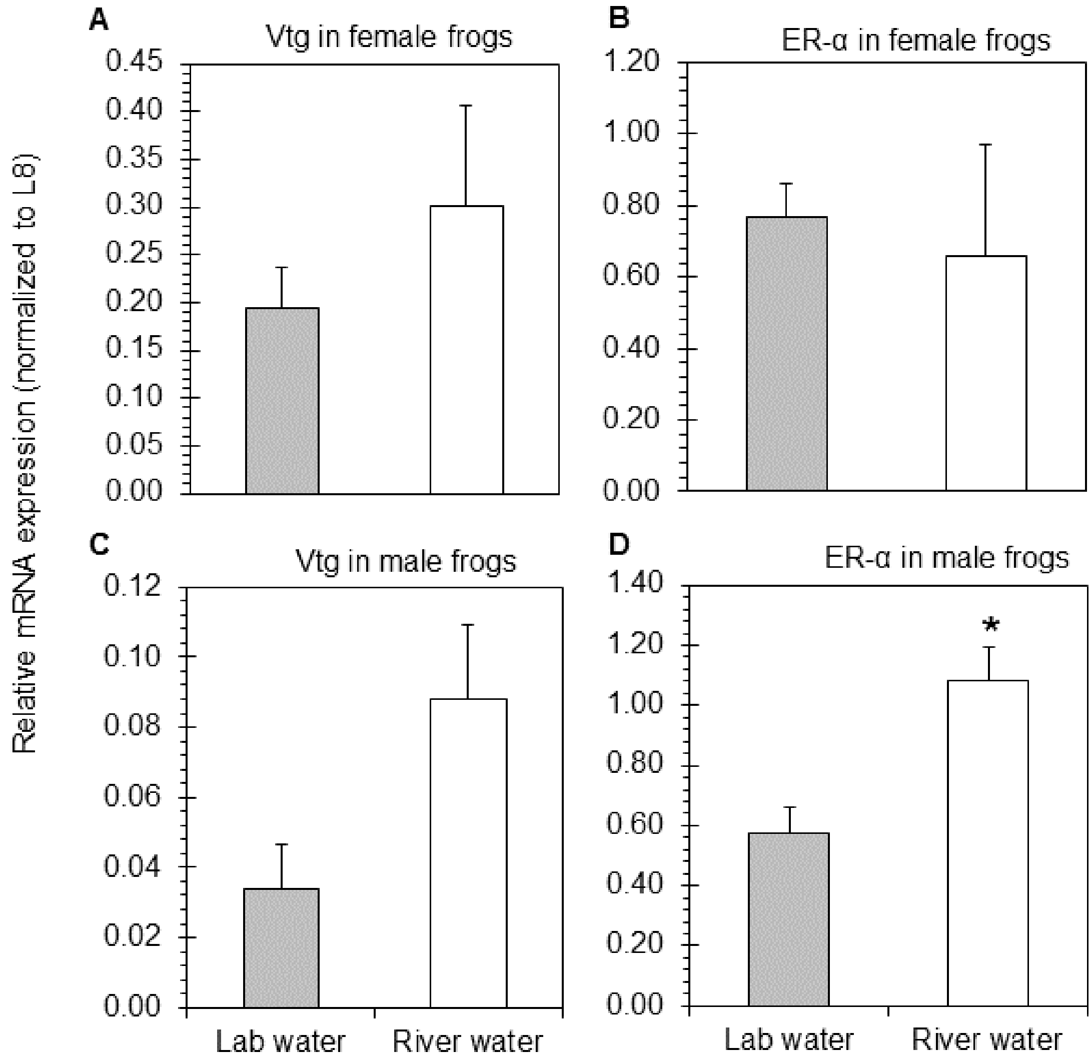


Figure 6. Relative hepatic mRNA expression in unexposed (lab water) and pulse exposed (river water) northern leopard frogs. A) Vtg and B) ERα mRNA expression in female frogs. C) Vtg and D) ERα mRNA expression in male frogs. Significant differences are denoted by an asterisks (*p* < 0.05 in all cases). * Figure adapted from Knight et al. (2013).

4.3 Wetland Field Trial

Six wetlands tested positive for atrazine during the sampling season. Frog collection occurred on six nights. A total of 63 frogs, 41 male, 22 female were collected (Table VI). Atrazine test results are shown in Table VII. All frogs were transported to the University of Nebraska-Omaha for tissue removal and further analysis. While primer analysis of *L. pipiens* has been completed, no gene expression analysis has been performed with *L. blairi* tissues as qPCR primers are currently under development.

Table VI. Morphometrics and distribution of field collected *Lithobates blairi.* Values are represented as the M(±SD).

	Atrazine +		Reference
Males	Tamora	North Lake Basin	Arbor Lake
Mass	23.5 (6.2)	21.6 (7.8)	35.7 (4.8)
HSI	2.3 (0.3)	2.2 (0.5)	1.6 (0.4)
GSI	0.73 (0.1)	0.87 (0.3)	0.81 (.06)
SUL	55.3 (3.8)	53.5 (6.4)	61.9 (5.3)
n	11	27	3
Females			
Mass	24.0 (9.4)	19.0 (10.4)	53.5 (7.3)
HSI	1.5 (0.3)	2.2 (0.4)	1.9 (0.2)
GSI	11.4 (8.6)	3.3 (4.0)	9.1 (3.1)
SUL	54.9 (5.7)	50.3 (8.1)	68.5 (2.9)
n	6	11	5

Table VII. Wetlands included in the 2012 pilot study. Six Rainwater Basin sites tested positive for atrazine at or above 3ppb and three Salt Creek Valley wetlands tested negative. Frogs were collected from two atrazine positive and one atrazine negative site. [a-d]

				Initial Testing		5/8		5/9		5/14		5/22		5/24		6/12	
Site #	**Name**	**Longitude**	**Latitude**	**ATZ**	**Date**	**ATZ**	**Frogs**	**ATZ**	**Frogs**	**ATZ**	**Frogs**	**ATZ**	**Frogs**	**ATZ**	**Frogs**	**ATZ**	**Frogs**
2	Tamora WPA	-97.2155	40.8854	1[a]	5/8/2012	1	N[c]	.	.	1	Y[d]	1	Y	.	.	.	.
5		-97.3306	40.8850	1	5/8/2012	1	N	.	.	.	.	.	.	.	.	.	.
7	North Lake Basin WMA	-97.3409	40.9155	1	5/14/2012	.	.	.	.	1	Y	0	Y	.	.	1/0	Y
9		-97.3943	40.8588	1	5/8/2012	1	N	.	.	.	.	.	.	.	.	.	.
10		-97.4049	40.8617	1	5/8/2012	1	N	.	.	.	.	.	.	.	.	.	.
13		-97.2348	40.8688	1	5/8/2012	1	N	.	.	.	.	.	.	.	.	.	.
20	Whitehead	-96.6792	40.8802	0[b]	5/9/2012	.	.	0	N	.	.	.	.	.	.	.	.
21	Arbor Lake	-96.6790	40.9012	0	5/9/2012	.	.	0	N	.	.	.	.	0	Y	0	Y
22	Shoemaker Marsh	-96.6832	40.9043	0	5/9/2012	.	.	0	N	.	.	.	.	.	.	.	.

[a] 1 indicates positive for atrazine

[b] 0 indicates negative for atrazine

[c] N indicates no frogs were collected

[d] Y indicates frogs were collected

5. Conclusions and Significance

The overall goal of this research proposal was to develop a two-tier screening tool that could be used to evaluate agrichemical contamination of ephemeral wetlands. To achieve this goal, two main research objectives were proposed: 1) to develop genetic biomarkers in the northern leopard frog (*Lithobates pipiens*) so that it can become an environmental sentinel organism for ephemeral wetland environments, and 2) successfully complete a field trial in which the two tier screening approach using atrazine test strips would be field tested.

In order to develop genetic biomarker, the complete *de novo* assembly of the *L. pipiens* transcriptome was first necessary. This achievement is significant for two reasons. First, it allowed for the successful development of genetic biomarkers for EDC responsive genes in the northern leopard frog (*Lithobates pipiens*). This achievement provides a significant step towards developing *L. pipiens* as an environmental sentinel in which the responses of EDC sensitive genes can be evaluated. Second, the transcriptome is significant simply due to the overwhelming lack of genetic sequence information available for amphibians. While amphibians are unique in that they are the only vertebrate taxa that represent both terrestrial and aquatic species, they have remained relatively overlooked with regards to genomic resources. The first frog genome, *Xenopus tropicalis*, was only published in 2010 (Hellsten and others, 2010). When such genetic resources are inadequate or only available for a few select species, researchers may be severely limited when attempting to develop new tools such as the genetic biomarkers utilized in this present research. The assembly of the *L. pipiens* transcriptome can therefore serve as an important template to develop new tools for amphibian research.

The second objective of this research was to complete a field trial in which the two tier screening approach would be tested. This field testing was broken down into two smaller scale field trials. In the Elkhorn River field trial, atrazine strip results revealed the temporal variations in the occurrence of atrazine that can be associated with field application dates and subsequent rainfall (Figure 2). While atrazine was observed occurring following discharge spikes due to rainfall, not every discharge event resulted in positive atrazine hits. This finding highlights the utility of the two-tier screen in which the immediate atrazine test results provided a means to focus sampling in the Elkhorn River during periods when agrichemical contamination are likely highest. Chemical analyses clearly indicate pesticide levels were significantly elevated during the atrazine pulse compared to the post-pulse period (Table IV) further indicating the utility of the two-tier screen.

In addition to the screening approach using atrazine test strips, the biomarker responses of *L. pipiens* was also assessed in the Elkhorn River field trial. Exposure of *L. pipiens* during the Elkhorn River field trial confirmed that *L. pipiens* is indeed sensitive and responsive to water where endocrine disruption has been previously observed (Soto and others, 2004; Kolok and others, 2007; Sellin and others, 2009). Furthermore, the responses observed in the exposed male frogs are indicative of feminization. Atrazine has previously been implicated in the feminization of frogs (Hayes and others, 2010), suggesting the gene responses observed may provide useful biomarkers in future field trials of atrazine exposed frogs.

In the second field trial, agriculturally impacted wetlands in the Rainwater Basin (Figure 1) were sampled using the two-tier atrazine test strip screen. Initial results reveal a number of potential shortcomings. While the atrazine test strips are low-cost and provide an immediate response, considerable effort may still be required to sample multiple areas over multiple days. A number of discrepancies become apparent when looking at the data presented in Table VII. Although only a small subset of wetlands was selected to sample, a lack of research volunteers made consistent sampling difficult. This may simply highlight the potential usefulness of recruiting citizen scientists which can utilize atrazine test strips to canvas large areas at regular intervals, a task which can be virtually impossible for small research groups (Kolok and Schoenfuss, 2010; Kolok and others, 2011).

In addition to the lack of regular atrazine test results at all sites, the atrazine test results were also found to be inconsistent within sites. For example, on one occasion the North Lake Basin wetland was observed to be negative in one pool of water, while a connected pool in the same site was found to be positive. This may suggest that the hydrology of wetlands is complex and atrazine test strips may not be an adequate tool for screen for agrichemical contamination. The lack of consistent sample data make it difficult to adequately assess the use of atrazine test strips in screening wetland areas, and further investigation is warranted in this regard.

In conlusion, a number of a number of useful outcomes were derived from this work. The development and assembly of the *L. pipiens* transcriptome will provide a significant resource for researchers in multiple fields utilizing this model organism. Furthermore, the development of EDC-responsive gene primers allows *L. pipiens* to be utilized as an environmental sentinel of ephemeral wetlands areas. Lastly, atrazine test strips were shown to be a useful tool in two-tier screening of a single watershed, but requires additional investigation for use in screening multiple sites.

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Direct Monitoring of Knickpoint Progression

Basic Information

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2013 Project Report: Direct Monitoring of Knickpoint Progression

MS Student: Clark Kephart[1]
PI: David Admiraal[1]

[1]Department of Civil Engineering, University of Nebraska, Lincoln, NE 68588-6105

Abstract

Channel degradation is of growing concern in areas of the Midwestern United States where there are large deposits of loess soils. Channel degradation occurs as a result of disturbances to the sediment load, bed soil configuration, and hydraulic characteristics of the stream (i.e. discharge, channel slope and geometry). These changes may occur suddenly or slowly over time. A given disturbance may be due to a large rain event, channel adjustments (i.e. dredging and straightening), and changes in land use around the stream (Chen et al. 1999).

A knickpoint is an abrupt drop in stream bed elevation over which water plunges and scours the downstream bed. The plunging water may lead to intense bed degradation and subsequent upstream migration of the knickpoint, often causing stream banks to become unstable and unsafe. Knickpoint migration problems have been particularly prevalent in the loess soil regions of western Iowa and eastern Nebraska as a result of wide spread channel straightening projects in the region. The goal of this project is to monitor the migration of an active knickpoint. Analysis of the knickpoint consists of: (1) acquiring time lapse images of the knickpoint every thirty minutes from a camera installed at the site, (2) periodically collecting detailed survey data of the stream channel and knickpoint, (3) collecting Large-scale Particle Image Velocimetry (LPIV) videos of the flow for a variety of high and low flow conditions, and (4) estimating discharges in the channel using the LPIV results.

The time lapse images provide a frame-by-frame depiction of the upstream movement of the knickpoint. The images allow us to assess when the knickpoint has migrated and if its migration is associated with a particular storm event. The less frequently collected survey data provide a more accurate assessment of knickpoint position and can also be used in conjunction with LPIV videos to establish velocity distributions and a rating curve for the knickpoint. LPIV videos have been converted to bitmaps and rectified for analysis using Particle Image Velocimetry (PIV) software. They are then used to examine depth, discharge, and velocity conditions for different flow events.

Background

Knickpoints are natural or man-induced formations that occur frequently in streams and rivers all over the world. A knickpoint is manifested as a sudden drop in channel bed elevation that may resemble a river rapid or (at a larger scale) a waterfall (Brush and Wolman, 1960). Knickpoints tend to induce large scale channel degradation that consequently causes headward (upstream) migration of the knickpoint. The headward migration of the knickpoint results in steepened side slopes, bank failures, and channel widening downstream of the knickpoint. These degradation processes introduce safety concerns for people and structures adjacent to the stream channels. It is important to develop a deeper understanding of the migration processes of knickpoints in order to properly assess the condition of a given stream channel and to insure the proper design of stream crossings and surrounding structures.

Objectives of Research

Extensive laboratory and flume studies concerning the behavior of knickpoints have been carried out over the years; however little research has been conducted on actively migrating knickpoints in the field. This study centers on an active knickpoint located within the deep loess area of western Iowa. The knickpoint has induced large amounts of channel degradation, and is approaching a bridge crossing upstream of the knickpoint. The end goal of this study is to acquire unique field measurements of the migration process of an active knickpoint that can help assess the condition of this and other streams containing headward progressing knickpoints. Analysis of the knickpoint consists of the following:

1.) Acquiring time lapse images of the knickpoint over an extended period of time. The goal is to create a unique two-dimensional depiction of the headward retreat of the knickpoint front. The images aid in the determination of when the knickpoint has moved, how far it has moved, and if the movement was related to a given storm event.
2.) Periodically collecting detailed survey data of the stream channel and the knickpoint. The elevation data are used to create a series of contour plots that can more accurately assess the position of the knickpoint and visually show the stream morphology over time as a result of the presence of the knickpoint. The surveyed data are also used in conjunction with Large-scale Particle Image Velocimetry (LPIV) to establish velocity distributions and discharge estimates.
3.) Collecting LPIV videos for a variety of high and low flow conditions. These videos are analyzed using Particle Image Velocimetry (PIV) or Particle Tracking Velocimetry (PTV) software that yield surface water velocity distributions. These velocity distributions are then used to estimate the discharge in the channel.

Site Description

The knickpoint selected for this study is in the deep loess area of the Midwestern United Sates, which has loess depths ranging from 50 to 75 feet. It is located on Mud Creek, a tributary stream of the West Nishnabotna River located in northeast Mills County, Iowa. Mills County is located in the Southwest corner of Iowa, and the study site is near the town of Henderson, Iowa. The site was selected because it was readily accessible, because it has a history of active knickpoints, and because it was a part of an ongoing study being conducted jointly with the University of Iowa. Mud Creek has a stream length of 25.75 km with a watershed area of 97.5 km^2.

This location of the Midwestern United States promotes a climate that is characterized by hot summers, cold winters, and wet springs. The average summer temperature in this area is 30^{0}C (86^{0}F) and is accompanied with periodic large thunderstorms. The winter average temperature is -4^{0}C (24.8^{0}F) with significant snow accumulation and periods of freeze and thaw. The average annual precipitation is approximately 846 mm/yr with the majority of the precipitation falling in the spring and summer months (April – September) (Papanicolaou, 2008). Over the course of this study the precipitation was recorded from the Iowa Environmental Mesonet (2013), which can be accessed at <mesonet.agron.iastate.edu>.

The knickpoint that was studied is active, but it is currently approaching a concrete grade stabilization structure just downstream of a bridge. The study site is surrounded by crop land and range land for cattle. Figure 1 shows the knickpoint and the study site.

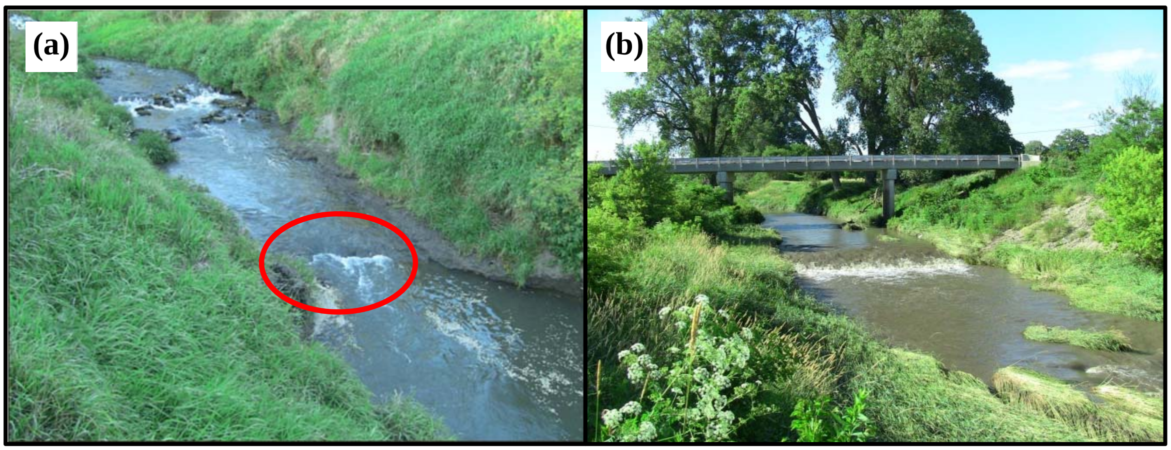

Figure 1: (a) Mud Creek knickpoint. (b) Upstream of the knickpoint showing the grade stabilization structure and bridge.

Methods

A time lapse camera was installed at the Mud Creek study site to continually monitor the headward progression of the knickpoint front over the study period. The images from the time lapse camera were used to create a two-dimensional overhead representation of the knickpoint migration. The camera was installed on the right descending bank of the channel and was programed to capture images of the knickpoint every thirty minutes. It was only possible to collect images during daylight hours. Though it was useful to gather images at 30 minute intervals to capture high-flow events and to allow selection of optimal images, one image per day was determined sufficient for monitoring knickpoint progression. Of the images collected, 499 images have been reviewed from July 14, 2011 to November 28, 2012.

To create an accurate representation of the knickpoint retreat, time-lapse images had to be processed to correct: (1) slight changes in the alignment of the camera when data were downloaded, (2) minor shifts in the camera position from day to day caused by temperature and humidity changes, and (3) the obliqueness of the images. First, the design of the camera made it difficult to return it to its exact previous position when images were downloaded. Slight rotations of the camera were corrected for by applying an oblique correction tool to align images collected after downloading data with those collected before downloading data. Second, minor shifts caused by thermal expansion of the camera mount and changes in moisture content of the soil supporting the camera were corrected by aligning fixed points within the images on consecutive days. The final adjustment that was necessary was to correct the images for obliqueness; that is, the camera was set up at an angle and not directly above the knickpoint. In order to properly determine knickpoint retreat distances, the images were rectified to make them appear as if they were being viewed from directly overhead. These processes are described in more detail by Kephart (2013).

As an example of the image rectification process; Figure 2 shows the oblique, unaligned images downloaded from the time lapse camera for the dates of September 15, 2011, December 3, 2011, and February 12, 2012. The corresponding rectified images are shown in Figure 3.

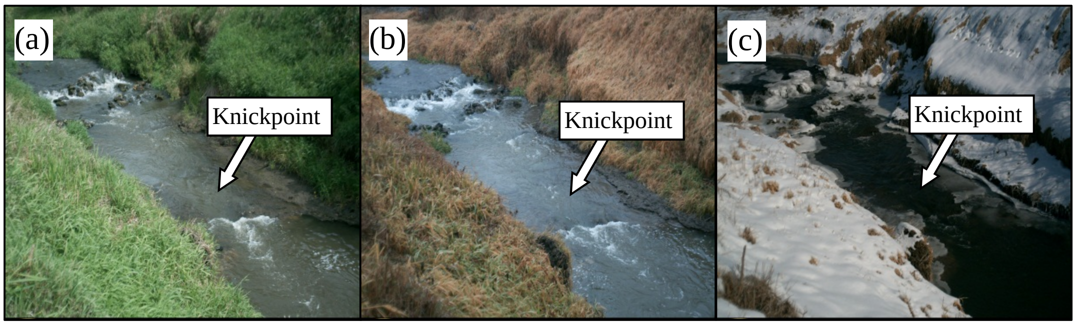

Figure 2: Time-lapse images collected on (a) September 15, 2011, (b) December 3, 2011, and (c) February 12, 2012

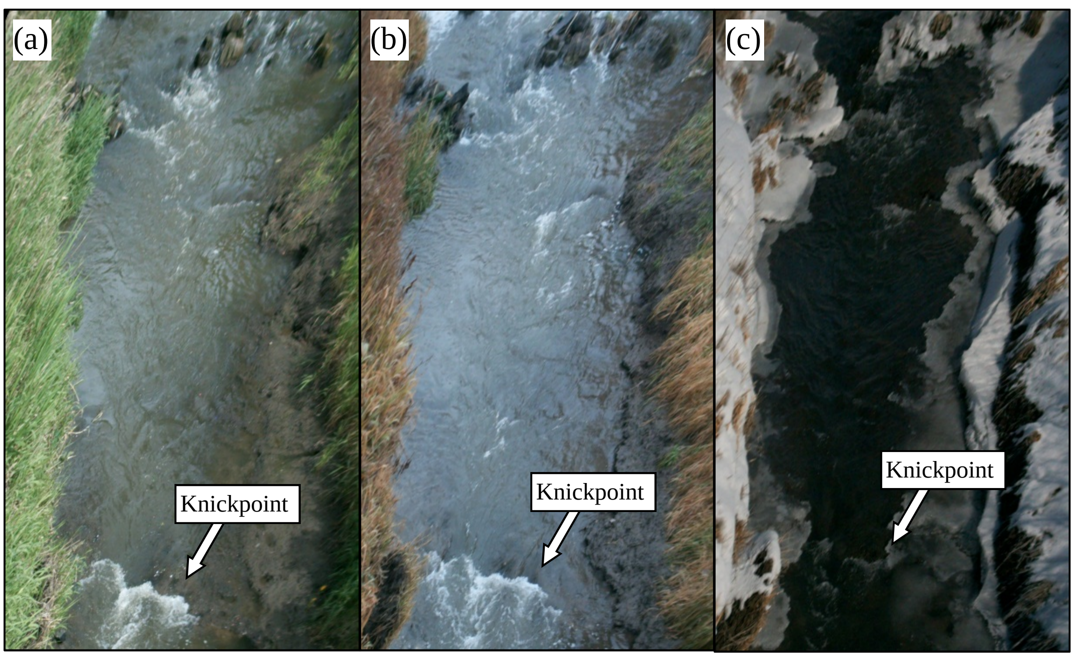

Figure 3: Rectified time-lapse images from (a) September 15, 2011, (b) December 3, 2011, and February 12, 2012

After the time-lapse images were aligned and rectified, the knickpoint front was identified and highlighted in each image. The exact location of the knickpoint front was not always easily identified due to variations in flow rate. The vertical face of the knickpoint is also somewhat three-dimensional, and what is actually observed in each time-lapse image is the location of the hydraulic jump above the knickpoint. However, at low flows, the hydraulic jump occurs at the crest of the knickpoint, and for the majority of the study period, the stream had relatively low flows, making the crest of the knickpoint face easy to identify.

Survey data of the banks and stream were collected during each site visit. The survey data were used to provide calibration points for rectifying Large-scale Particle Image Velocimetry (LPIV) videos and time lapse images. The surveys also provided bathymetric information of the channel. A series of detailed surveys were carried out on the dates of September 27, 2011, March 21, 2012, and September 25, 2012. These detailed surveys contained information about the changes in the stream bathymetry that occurred over the duration of the study.

Surface water velocity distributions upstream of the knickpoint were determined using Large-scale Particle Image Velocimetry (LPIV) techniques. During each visit to the site, a series of videos was taken of the flow. The videos were collected using a digital video camera that was placed on the right descending bank near the time lapse camera mount. Like the time lapse camera, the digital video camera was set up at an angle to the knickpoint and not directly above the knickpoint. The videos were converted into sequences of bitmaps with image separation times of 1/30th of a second. Using surveyed calibration points, the bitmaps were corrected for obliqueness (rectified) as shown in Figure 4.

Figure 4: LPIV images: (a) original image and (b) rectified image

Low flows were seeded with particles (slightly buoyant cereal) to aid in the LPIV analysis. High flows were left unseeded, as there was an abundance of highly visible bubbles present on the water surface. Surface velocities were calculated at a series of cross sections upstream of the knickpoint. The cross sections were developed where detailed elevation data of the stream were known from collected survey information. For each cross section, interrogation points were selected at regular intervals from bank to bank. The average velocities were calculated at each of the interrogation points using image analysis software developed for the project for 100 to 200 pairs of images (see Kephart 2013).

The results of the LPIV analysis in combination with bathymetric information were used to estimate discharges at multiple flow cross sections. Based on the 1/7th power law, the depth averaged velocity for each velocity profile is 7/8th of the surface velocity. The discharge at each cross section was then found using:

$$Q = \sum \frac{7}{8} U_{surf,i} A_i \tag{1}$$

Where Q is the discharge, $U_{surf,i}$ is the surface velocity found by applying LPIV analysis at each subsection of a transect of the channel, and A_i is the cross sectional area of the subsection based on bathymetric information. The total discharge is calculated by summing the subsection discharges over the entire cross section of the channel.

Results

For 499 days the position of the knickpoint front was continually observed using the time lapse images collected at the site. The images were collected between July 14, 2011 and November 28, 2012. The knickpoint fronts were identified and highlighted in each image. Nine of the front locations observed throughout the study period were overlain and placed on a rectified bitmap of the knickpoint (Figure 5). The time spacing between the front locations in Figure 5 was not uniform, but an effort was made to allow significant time between observations (approximately two months) and to present observations in each season of the year. Presenting the front position in this way offers a unique two dimensional representation of the headward retreat of the knickpoint over the observation period. Some of the front locations shown in the image overlap. The knickpoint crest is not always clearly identified because the depth of the water changes over time, and the knickpoint crest can erode downward as well as upstream – downward erosion of the crest can be perceived as downstream migration of the knickpoint due to the oblique angle of the camera.

Upon observing the movement of the knickpoint front over the duration of the study period (Figure 5), it is apparent that most of the time, the migration is very slow, moving only 2 meters over the 1.5-year study period. The data recorded thus far indicate that the migration upstream slows significantly in the fall and winter months, and increases during the spring and summer. Figure 6 graphically shows the progression of the knickpoint upstream over the duration of the study period.

Figure 6 was developed by recording the upstream-most point of the knickpoint lip in each of the time lapse images. There is uncertainty associated with the location of the knickpoint lip. In each of the time lapse images the lip of the knickpoint is submerged by the flow and what is actually observed is the position of the leading edge of the hydraulic jump. As the flow in the stream fluctuates, the position of the hydraulic jump changes slightly, causing positioning errors, and indicating that the knickpoint has migrated downstream in some cases. Nevertheless, Figure 6 clearly illustrates progression of the knickpoint throughout the study period.

Another possible reason for the apparently slower movement of the front may be a change in the mode of knickpoint erosion in combination with the time-lapse analysis technique. Only the upper surface of the knickpoint can be seen in the time-lapse images, and when the location of the knickpoint front is determined, it is the location of the crest. It is possible that the mode of knickpoint migration changes with the onset of the lower stream flows and freezing that take place during the winter months. The lower flows may cause the bulk of the erosion to shift from the knickpoint face to the knickpoint lip (i.e. causing the knickpoint to migrate in a rotating fashion). If this is the case, observing the movement of the knickpoint front in the way shown in Figure 5 does not completely capture the erosion behavior of the knickpoint front. The results may indicate that the knickpoint front is not eroding (or eroding slowly); when in reality the knickpoint front is being undercut during the winter months, and then retreating upstream in the spring and summer when higher flows are present in the channel to carry away loose sediment at the lip of the knickpoint.

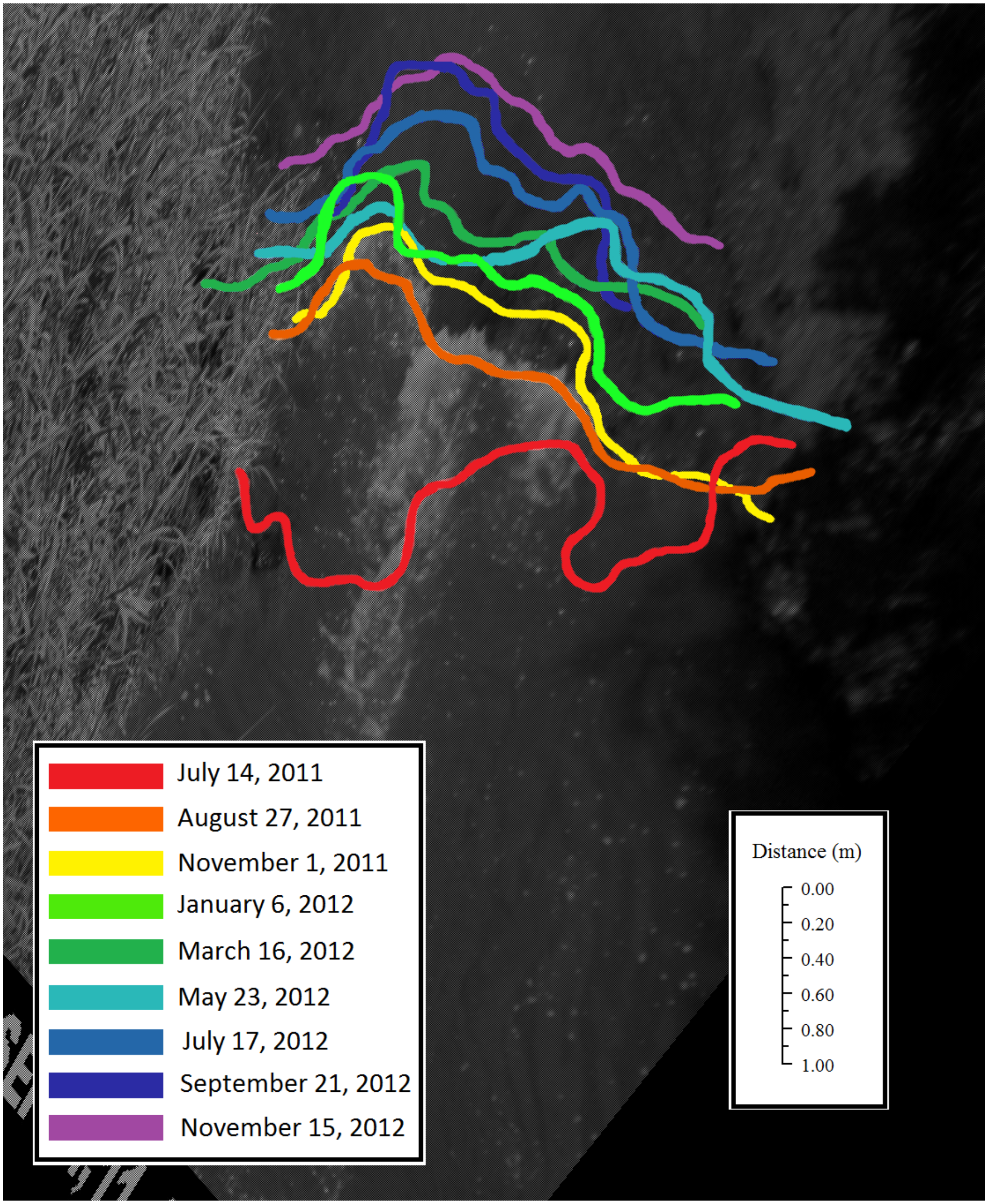

Figure 5: Knickpoint retreat over the duration of the study period.

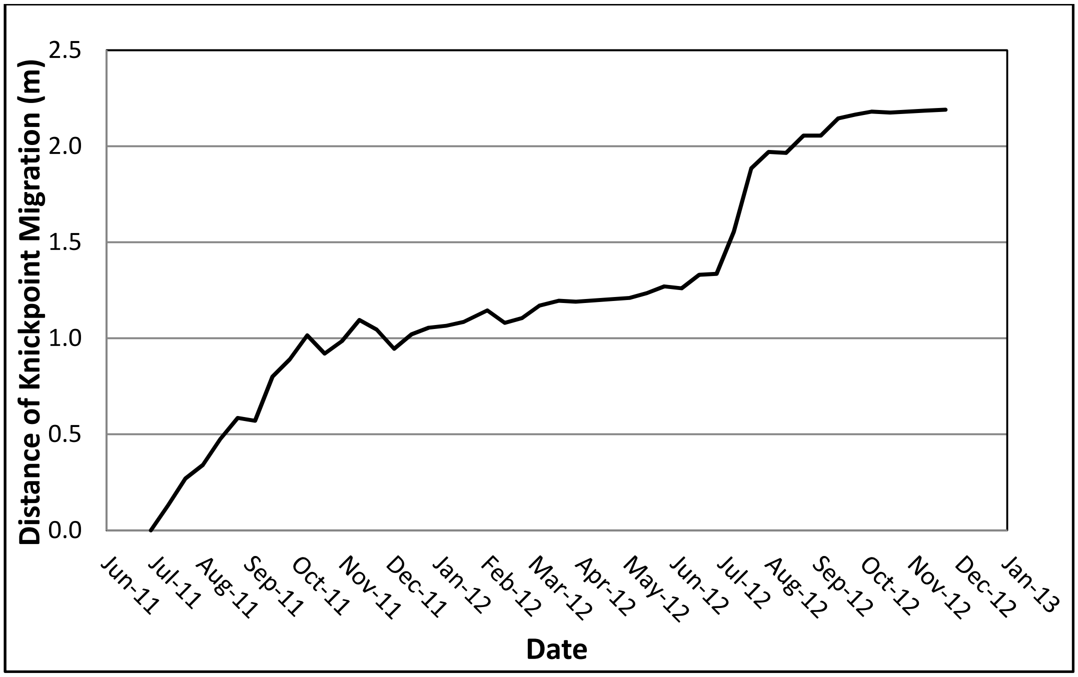

Figure 6: Knickpoint migration of upstream-most point of knickpoint over the study period.

Nevertheless, the knickpoint front has significantly migrated upstream over the course of the time lapse analysis. By examining the highlighted fronts in Figure 5 and the knickpoint progression in Figure 6, it is apparent that the knickpoint front has moved upstream approximately 2.2 meters over the 499 day study period. The most significant front movement appears to have occurred near the beginning of the study period migrating at 0.0102 m/day. The migration upstream then slowed in the winter months to 0.0009 m/day, and speeded up again in the spring and summer. The average migration rate over the duration of the study period was determined to be 0.0044 m/day. Table 1 provides a summary of the varied migration rate of the knickpoint throughout the 499 day study period.

Table 1: Summary of varied migration rates of the knickpoint

Season	**Time Period**	**Migration Rate (m/day)**
Summer and fall	July-Oct (2011) (100 days)	0.0102
Winter and spring	Nov - July (2012) (232 days)	0.0009
Summer and fall	June -Sept (2012) (99 days)	0.0089
Fall and early winter	Oct - Nov (2012) (68 days)	0.0007
All Seasons	Study Period (499 days)	0.0044

To further examine the morphology of the knickpoint front and the stream channel, extensive survey data were collected on the dates of September 27, 2011, March 21, 2012, and September 25, 2012. During the collection of the survey data, two separate sets of survey equipment were used. For the surveys collected on September 27, 2011 and March 21, 2012 a Sokkia Set 5A total station was used to gather the elevation data. For the September 25, 2012 survey a new TopCon GPS based survey system was acquired and used to gather the elevation data. There were some problems with the Set 5A system, and the data collected using the new TopCon system were assumed to be more accurate than those collected using the Set 5A. The inaccuracies in the Set 5A data were observed by comparing data collected at each of the bridge corners, which were points that should not change between surveys. The bridge corner elevations did not match up with the TopCon elevation points; they were considerable lower. To properly align the elevations of the three contour plots some adjustments to the Set 5A elevations were made. Comparison of fixed cross sections collected with the three surveys allowed us to accurately adjust the datums for the Set 5A surveys (see Kephart 2013 for details).

The elevation data collected during these surveys were used to create the series of contour plots presented in Figure 7. The east and north positions given on the x and y axes of the plots are relative to an arbitrary benchmark located at the southwest corner of the Elderberry Avenue Bridge set to (0,0). The elevations given in the contour plots are relative to the World Geodetic System 84 (WGS 84) datum. The same benchmarks were used for all of the contour plots so that the elevations could be directly compared with one another.

The three contour plots were able to illustrate the morphology of the stream channel and knickpoint throughout the period of analysis, as well as to highlight important features of the channel that may be affecting the migration of the knickpoint. To offer context to the three contour plots presented in Figure 7, the flow direction and the face of the knickpoint are shown.

As observed during the time lapse analysis (Figure 5), the contour plots confirm that measurable upstream migration of the knickpoint front has occurred over the duration of the study. However, the contour plots also show significant widening of the knickpoint face and that the downstream plunge pool is deepening and widening as well. Also, upstream of the primary knickpoint, on the left descending bank, a secondary scour hole is forming. Upon further examination of the contour plots, it was determined that the main channel itself is approximately 4.5 meters wide, but there also appears to be a deep, narrow trench forming at the center of the channel . This trench is approximately 1 meter wide and extends upstream from the primary knickpoint to the upstream scour hole. The trench has increased in depth over the study period and appears to have a significant impact on the morphology of the knickpoint and stream channel, as the knickpoint appears to be working its way upstream within the confines of the trench. The trench appears to be a mechanism by which knickpoint migration can occur much more rapidly than by direct erosion of the knickpoint face.

During each visit to the site, a series of videos were taken of the flow surface upstream of the knickpoint. The videos were converted into bitmap images. The Oblique images were then rectified using surveyed calibration points that were gathered at the edges of the water surface. A multi-file minimum quadratic difference algorithm was applied to determine average velocities at interrogation points spaced across a series of cross sections. The chosen cross section locations and processing information varied slightly for each data set. The cross section locations for each LPIV calculation are available in Tables 3, 4, and 5. These locations are relative to the location of the knickpoint.

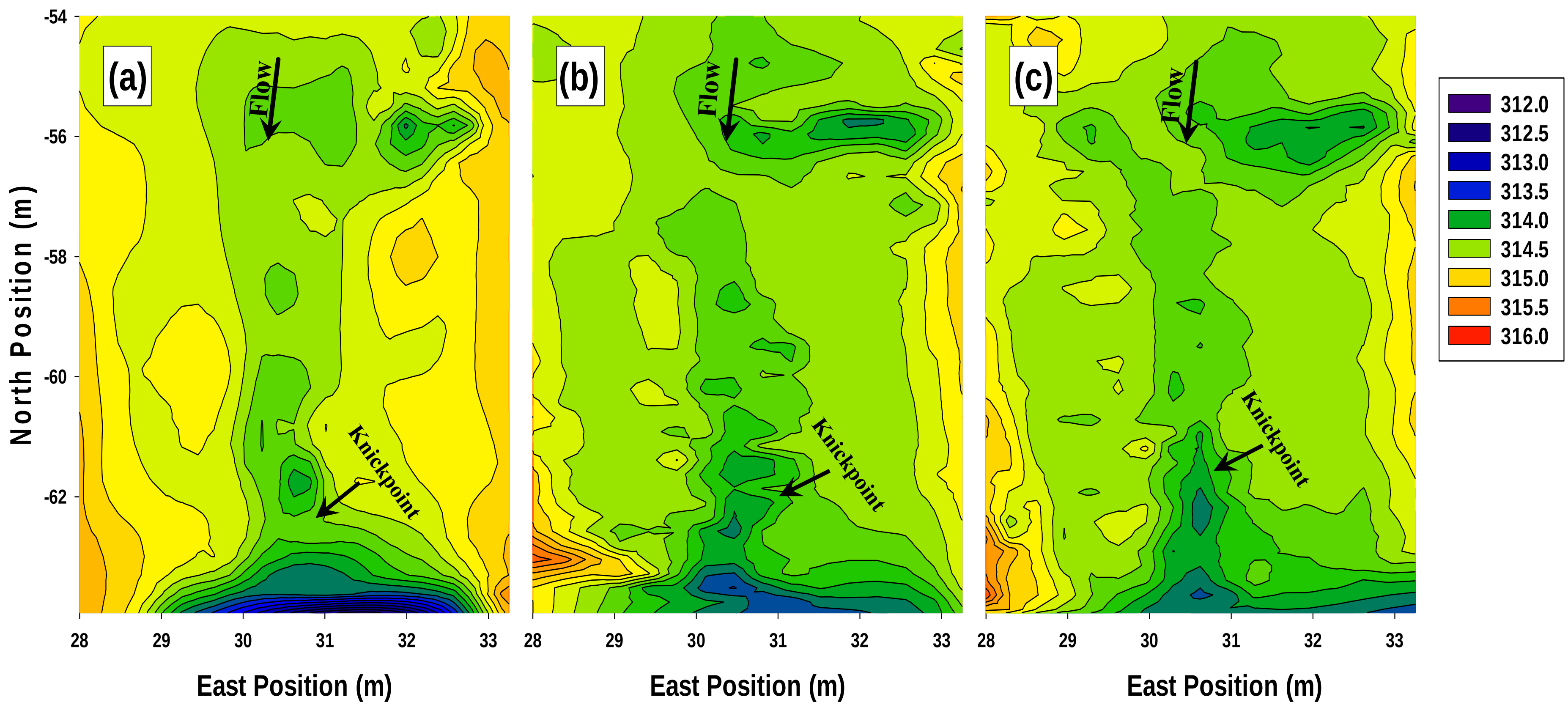

Figure 7: Contour plots of Mud Creek knickpoint: (a) September 27, 2011, (b) March 21, 2012, (c) September 25, 2012

The processing information for the LPIV data sets collected on September 27, 2011 and March 21, 2012 were nearly identical. The image scale was set at 2.0 pix/cm, the image separation time was 0.0333 s, the interrogation area was 16 pix by 16 pix, and the search bounds were set as 16 pix by 27 pix. 50 interrogation points were used across each cross section with a pixel spacing of 18 pix and 20 pix, respectively. For each of these two data sets, 100 pairs of images were used for processing. The processing information for the September 25, 2012 LPIV data set was slightly different than the previous two data sets. This was because the scale of the video taken at the site was considerably larger than for the previous two videos, as the water surface in the stream was very low due to drought conditions. For this reason the image scale for this data set was set to 3.0 pix/cm, providing higher resolution. The separation time remained at 0.0333 s, the interrogation area was set at 16 pix by 16 pix, and the search bounds were set to 16 pix by 27 pix. 100 interrogation points were used across each cross section with a pixel spacing of 7 pixels. For this data set, 200 image pairs were used for processing. Table 2 gives a summary of the LPIV processing information used for each data set collected at the site.

Table 2: Summary of LPIV Processing Information used for each data set

	Sept 27, 2011	**Mar 21, 2012**	**Sept 25, 2012**
Separation Time (s)	0.0333	0.0333	0.0333
Image Resolution (pix/cm)	2.0	2.0	3.0
Interrogation Area (pix^2)	16x16	16x16	16x16
Search Bounds (pix by pix)	16x27	16x27	16x27
Interrogation Points	50	50	100
Pixel Spacing (pix)	18	20	7
Image Pairs	100	100	200

A sample velocity distribution for the LPIV analysis is presented in Figure 8. Figure 8 corresponds to the March 21, 2012 site visit. Although the width of the water surface varies from observation to observation, the highest surface velocities are in the center of the channel. For the lower flows, there are areas of stagnation or flow rotation near the channel banks. This may be as a result of water being diverted into the deeper, narrower trench. The highest velocities for all flows were observed within the bounds of the 1 meter wide region directly above the trench that has developed upstream of the knickpoint. The higher velocities associated with this region result in an increase in shear stress within the trench. It is very likely that the concentrated flows will continue to deepen, lengthen, and widen the trench until a mass failure occurs; At this point the knickpoint front will likely quickly move upstream within the confines of the trench.

Figure 8: Surface water velocity distributions created from LPIV data collected on March 21, 2012 site visit.

Using the average surface velocities obtained from the LPIV techniques, volumetric discharges through each cross section were estimated. The surface velocities were converted into mean velocities using the 1/7th power law. The mean velocities were then multiplied by the subarea associated with the velocity to obtain a discharge flux. Each flux was then summed to get a discharge for the entire cross section. A summary of cross section locations, flow areas, and volumetric discharges for the channel are presented in Tables 3, 4, and 5 for the three flow measurement tests. Transects where the uniform flow assumption is questionable and where discharge calculations are considered to be less accurate are highlighted in the tables.

Table 3 presents the discharge calculations determined from the LPIV data collected on September 27, 2011. The flow condition in the stream appeared to be a typical base flow condition for Mud Creek, since the water covered most of the non-vegetated bed. The discharge at cross section 1A was not calculated because of its proximity to the knickpoint; the hydraulic jump at the knickpoint face prevents accurate LPIV measurements in addition to the flow not being uniform in the vicinity of the face, meaning that the 1/7th power is not valid in this region. The remaining cross sections produced a consistent set of results, aside from the results at cross sections 1B and 1F, which seem a bit higher than the other measurements. Cross section 1B is still located quite close to the knickpoint and cross section 1F is located just downstream of a secondary scour hole. At these transects, rapid changes in the bathymetry make the assumption of uniform flow questionable. The average discharge for this first test was *0.306* m^3/s with a standard deviation of *0.036* m^3/s.

Table 3: LPIV Discharge Calculations and Cross Section Geometry: Sept 27, 2011

Cross Section	**Distance Upstream of Knickpoint (m)**	**Flow Area (m^2)**	**Discharge (m^3/s)**
1A	0.40	-	-
1B	1.60	0.705	0.351
1C	2.15	0.657	0.316
1D	2.85	0.582	0.244
1E	3.73	0.481	0.310
1F	4.45	0.776	0.342
1G	6.75	0.977	0.290
1H	8.85	0.679	0.293

Table 4: LPIV Discharge Calculations and Cross Section Geometry: March 21, 2012

Cross Section	**Distance Upstream of Knickpoint (m)**	**Flow Area (m^2)**	**Discharge (m^3/s)**
2A	0.15	-	-
2B	0.75	0.413	0.448
2C	1.45	0.472	0.332
2D	2.25	0.578	0.412
2E	2.95	0.505	0.401
2F	3.75	0.485	0.336
2G	4.45	0.613	0.396
2H	6.45	0.871	0.539

Table 5: LPIV Discharge Calculations and Cross Section Geometry: Sept 25, 2012

Cross Section	**Distance Upstream of Knickpoint (m)**	**Flow Area (m^2)**	**Discharge (m^3/s)**
3A	0.60	0.180	0.097
3B	1.20	0.237	0.081
3C	1.80	0.218	0.067
3D	2.20	0.224	0.088
3E	3.00	0.260	0.128
3F	3.42	0.302	0.130

Table 4 presents the results from the discharge calculations performed on the LPIV data collected on March 21, 2012. The flow condition in the stream on this date was slightly higher that than the flow calculated previously, as there was a light but consistent drizzle falling throughout the data collection period. Much like the previous calculation, Transect 2B appears to be a bit high because of its proximity to the knickpoint. Transect 2H is located just upstream of the secondary scour hole, and again may make the uniform flow assumption questionable. The average discharge is *0.387* m^3/s with a standard deviation of 0.045 m^3/s.

Table 5 shows the results from the discharge calculations carried out on September 25, 2012. On this date the observed flow in Mud Creek was very low, due to the increased drought conditions in the Midwest during the months leading up to the data collection. In an attempt to avoid inconsistencies in the LPIV data, cross sections that would not be affected by the knickpoint or by the secondary scour hole were selected for analysis. The average discharge for this flow was determined to be *0.098* m^3/s, with a standard deviation of 0.026 m^3/s. As can be seen in the discharge table, for this low flow, the irregular bathymetry of the channel plays a much larger role, resulting in increased relative error. However, as expected the discharge in the stream was significantly lower than the previous two flows because of the extreme drought conditions in the Midwest.

The three flows that were calculated represent a typical base flow, a small rainfall flow, and a low flow condition for Mud Creek. The discharges for the three flow events were 0.098, 0.306, and 0.387 m^3/s with corresponding stage elevation measurements upstream of the concrete weir of 316.17, 316.21, and 316.26 meters. By plotting the stage vs. discharge for these three flow conditions a rating curve for the site was created (Figure 9). The flows in Mud Creek during each data collection period were relatively low, with very little variation. More data should be collected to create a more reliable rating curve for the site. It will be important in the future to gather LPIV data for a high flow event and possibly a large storm event. With the collection of these data, a more accurate hydrologic record of Mud Creek can be created.

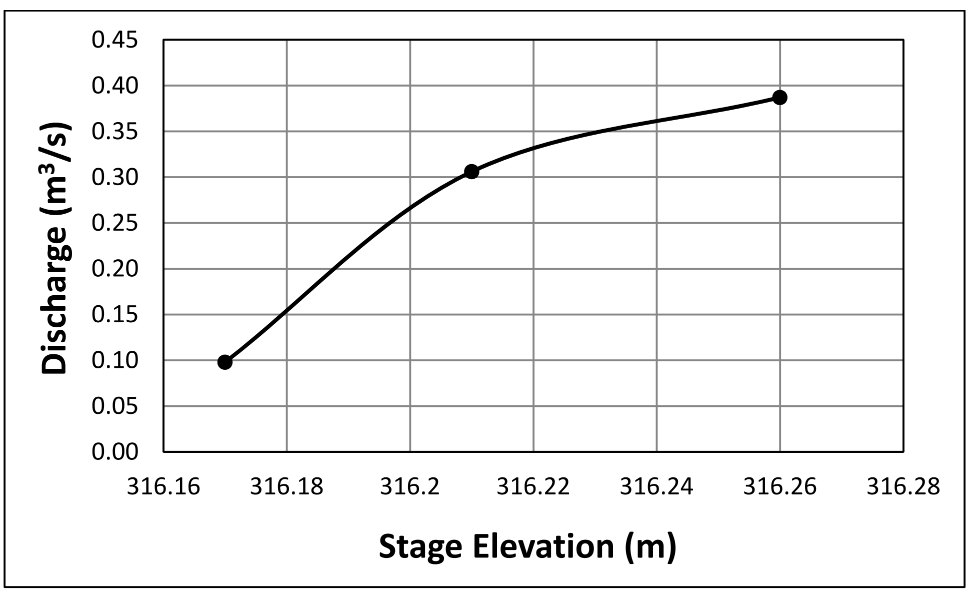

Figure 9: Grade control rating curve of discharge vs. stage for the three LPIV flows

Over the course of the study period precipitation records were continually monitored, but stage data for Mud Creek were available after March, 2012. With the use of stage information and the data in Figure 9, the discharge over the weir was calculated based on a standard weir equation. Discharge calculations performed well for the low flows when compared to the discharges found from the LPIV measurements. However the weir coefficient will need to be verified for higher flows, since the channel width increases at higher stages. A runoff hydrograph for the Mud Creek watershed was created and is shown in Figure 10 along with the relative position of the knickpoint. The hydrograph indicates that this watershed is quite flashy, meaning that with the onset of precipitation a sharp increase in flow occurs.

There were 6 significant flow events in March, April, and May of 2012; and a prolonged period of low stage in Mud Creek during the summer of 2012. The corresponding stage readings ranged from 316.12 m to 317.20 m. The period of low stage coincides with the extreme drought conditions that were documented in the Midwest during 2012; 2012 had only 692 mm of rainfall, which is 154 mm less than the annual average. The discharges in Mud Creek ranged from 0.1 m^3/s to 14.0 m^3/s, with the highest flows taking place in the late summer of 2011 and early spring of 2012 and the lowest flows taking place in mid-summer and winter of both years. Again prolonged drought conditions greatly influenced the stage records, meaning that observation of the stage in the stream will need to be continued for a more accurate assessment of typical knickpoint behavior.

Observing the recorded knickpoint front movement with the calculated flow as shown in Figure 10, the relationship between the migration of the knickpoint front and the discharge in the stream can be examined.

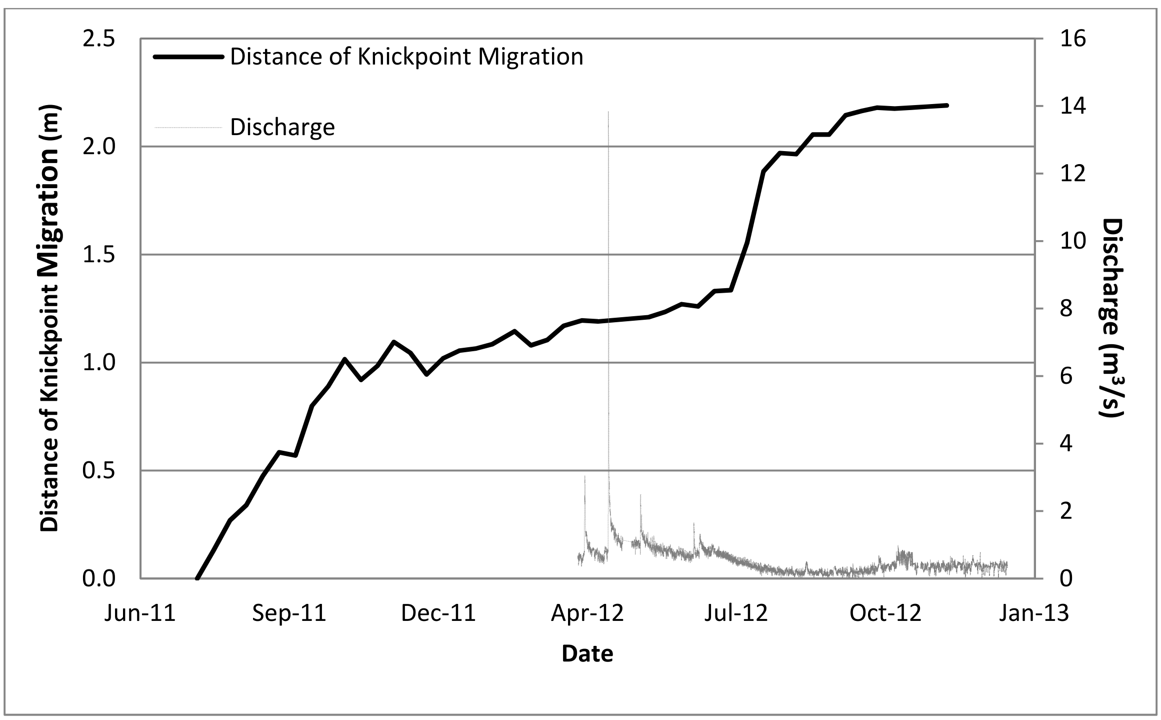

Figure 10: Knickpoint migration compared with the calculated discharge in Mud Creek

The main observation that can be made from Figure 10 is that knickpoint migration rates do not appear to be closely tied to large scale flow events. In general, bank failures occur more frequently following large storms, when saturated soils lose their cohesiveness. In the present case, the knickpoint is always submerged, so changes in the cohesiveness of the soils may be more likely to occur during dry periods, but the increase in erosion rate observed during the late summer in 2011 and 2012 during the driest periods was unexpected. A longer record of data, as is currently being collected at the site, will be helpful.

As mentioned previously, there was an extended period of low flow in Mud Creek during the summer of 2012. During these low flow conditions the majority of the flow was in the aforementioned trench, causing the majority of the erosive stresses from the flow to act within the confines of the trench and on a small portion of the knickpoint face. These concentrated low flows certainly influence the migration behavior of the knickpoint. Evidence of this can be seen as an increase in front moment during the extended period of low flow experienced during the summer of 2012. It is only during high flow conditions that the flow is more evenly distributed across the entire channel, and though the magnitude of the shear stresses acting on the channel may be larger for these high flows, they occur for such a brief period of time that they do not appear to be strongly correlated to the headward progression of the knickpoint. In March, April, and May of 2012 there were periods of increased flow events in Mud Creek, during which, the movement of the knickpoint front showed no increase in migration rate, and even appeared to retreat more slowly.

Conclusions

The results of the time lapse and survey analyses lend some insight into the mode of knickpoint migration. The mode of knickpoint migration changes with the change in season. Slowed headward retreat of the knickpoint front in the fall and winter months observed in this study may indicate that other forms of knickpoint erosion, not observed in the time-lapse photos, are taking place. It is also possible that the 2012 winter was too mild to provide a representative picture of knickpoint erosion. The 2013 winter will provide additional data for comparison with the previous year, but that will not be available for this report.

Over the study period, the study area was in a severe drought, and stream flow was much lower than usual. The hydrographical data provided by the bridge-mounted sensor at the site showed that there were 6 large storms or increased flow events during 2012 that had estimated flow rates ranging from 0.50 m^3/s to 14.00 m^3/s. During the remainder of the year, the base flows were quite low, and ranged from 0.1 to 0.3 m^3/s. During the study time period, the knickpoint migrated 2.2 meters upstream, with the migration rate rising in the spring and summer and slowing in the fall and winter. The average migration rate observed for the 499 day study period was determined to be 0.0044 m/day. Though the migration rate varied from season to season, overall the rate of knickpoint migration was relatively steady over the study period with no large, punctuated knickpoint failures.

Compared with other related studies, the migration of the Mud Creek knickpoint appears to be quite slow. Daniels (1960), observed a headward progressing knickpoint in Willow Creek, IA over a 5-year period. The Willow Creek knickpoint migrated upstream 2,819 meters over the course of the 5-year study, which is much more than the Mud Creek knickpoint which migrated 2.2 meters upstream over a 2-year period. Simon and Thomas (2002) and Simon et.al., (2002) also observed larger migration rates for a series of knickpoints that had developed along the Yalobushaa River in Mississippi. They observed migration rates ranging from 0.4 m/yr (0.001m/day) to 11 m/yr (0.030 m/day), whereas the Mud Creek knickpoint exhibited migration rates ranging from 0.0007 m/day to 0.01 m/day.

Though the Mud Creek knickpoint study observed what appears to be a slowed rate of knickpoint migration, all three studies observed periods of varied migration rates. The Willow

Creek and Yalobussa River studies attributed the quick pulses of the knickpoint upstream to the onset of high flow events. In the case of the current Mud Creek study it appears as though the large flow events are not correlated to expedited movement of the knickpoint. As shown in Figure 10, during the onset of high flow events there was very little headward progression of the knickpoint. It was during the prolonged period of low flow that the larger migration rates were observed. This may be as a result of soil conditions in the area, as well as the dry weather patterns observed during the study period. However, the presence of the deeper narrower trench that has developed upstream of the Mud Creek knickpoint appears to be greatly affecting its migration behavior, by temporarily reducing headward migration at the knickpoint face, but potentially leading to rapid changes in the location of the knickpoint face in the future.

Throughout most of the year, especially during dry periods, the channel flow was mostly confined to a trench that progressed upstream from the knickpoint face. Low flows persisted most of the time, and the bulk of the flow traveled through the trench, causing an uneven distribution of erosion on the bed and on the face of the knickpoint, consequently slowing its headward progression. It was only during larger flow events that the flow was more evenly distributed within the channel and over the knickpoint face. The result was that low flows had a significant impact on the morphology of this particular channel (see Figure 10). The impact was observed as the development of the trench between surveyed contour plots. The trench appeared to be the mechanism that was driving the migration of the knickpoint. Comparing the contour plots in Figure 7, it appears that most of the erosion took place within the narrower region of the channel and in the location where the trench crosses the knickpoint face. Thus, the knickpoint is slowly migrating upstream within the confines of the trench, a process that is slowly deepening, widening, and lengthening the trench, but it is altogether possible that development of the trench will cause a rapid, punctuated mass failure of the knickpoint face to occur. If this happens, the knickpoint will quickly erode upstream until it reaches a location where the bed material is more stable.

This migration process will continue until the grade stabilization structure (the concrete weir upstream of the knickpoint) is reached. When the study began at the site in early 2011, evidence of the trench-based migration behavior of the knickpoint was present, though it was not recognized at the time. Figure 11*Figure* shows evidence of a trench and large plunge pool that existed downstream of the present knickpoint location, and through which the knickpoint had passed previously. It appears as though the knickpoint remained stationary for a long period as the previous plunge pool and trench developed. Then, a rapid mass failure occurred, and the knickpoint quickly retreated upstream within the confines of the trench until it reached more stable bed material. Other than photos like the one given in Figure 11, evidence of the previous trench and plunge pool of the knickpoint are now gone because of mass wasting of the downstream channel banks. The continuing upstream migration of the knickpoint and other knickpoints like it has resulted in a channel that is very incised, leading to extensive mass wasting of the channel banks.

Based on the current development of the secondary plunge pool and trench; we think that it is likely that when a mass failure of the knickpoint face occurs the knickpoint will quickly retreat upstream until it reaches the secondary scour hole. At this point the trench based migration process may begin again.

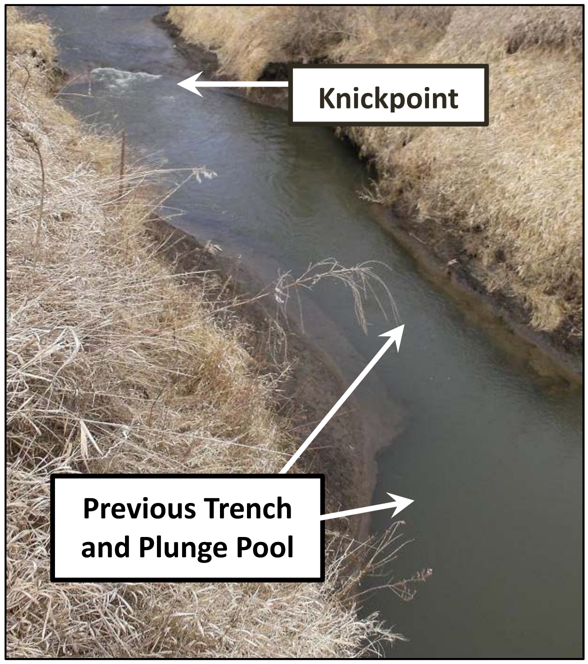

Figure 11: Image of knickpoint on March 18, 2011 with evidence of previous trench and plunge pool downstream of knickpoint.

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USGS Award No. G13AP00006 Development of a National Database of Depreciated Structure Replacement Values for Inclusion with SimSuite/HAZUS and Flood Mitigation Reconnaissance Studies

Basic Information

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End Date:	7/31/2014
Funding Source:	Supplemental
Congressional District:	
Research Category:	Engineering
Focus Category:	Floods, Economics, Management and Planning
Descriptors:	
Principal Investigators:	Suat Irmak, Steven Shultz

Publications

There are no publications.

USGS Award No. G13AP00006 Development of a National Database of Depreciated Structure Replacement Values for Inclusion with SimSuite/HAZUS and Flood Mitigation Reconnaissance Studies

Project #: 2013NE257S

PI's: Suat Irmak and Steven Shultz

Funding Period: January 1, 2013 through July 31, 2014

Project research has not begun as of date and nothing to report at this time.

Information Transfer Program Introduction

The Nebraska Water Center continued active pursuit of its traditional diverse information transfer program in 2012. USGS funding continues to help underwrite a wide variety of public and professional information, public relations and education efforts, including: (1) four quarterly issues of the Water Current newsletter, which are mailed to more than 2,800 subscribers and appears as an online pdf; (2) updated and reprinted Water Center fact sheets and online UNL water faculty directories database; (3) more than 20 press releases reporting on water-related research and outreach programming or promoting Nebraska Water Center and University of Nebraska water-related educational activities; (4) helping to support four internet web sites; (5) publicity and supporting materials for an annual water law conference, public lecture series, water symposium, brown bag lectures, water and natural resources tour; (6) coordinating UNL Extension's largest public display and student recruitment event of the year at the Husker Harvest Days farm show; (7) publication via U.S. Environmental Protection Agency grant funds of a full-color book detailing the Nebraska Department of Environmental Quality's "CLEAR" community lake restoration program; and (8) publication of a full-color University of Nebraska "Water Faculty and Staff Directory" book.

The Nebraska Water Center continues as part of the Robert B. Daugherty Water for Food Institute, a global imitative involving all University of Nebraska water-related faculty and staff with a mission of greater global agricultural water management efficiency.

Information Transfer Plan/Water Education

Basic Information

Title:	Information Transfer Plan/Water Education
Project Number:	2008NE173B
Start Date:	3/1/2011
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Congressional District:	1
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Descriptors:	
Principal Investigators:	Suat Irmak, Steven W. Ress

Publications

1. Newsletter: The Water Current newsletter has a free, subscriber-based distribution of approximately 3,100 copies per issue, more than 95% of which are requested subscriptions. It is published quarterly in a full-color magazine format, and is available online. Water-related research, engagement, education and outreach faculty and water-related professional staff are featured in each issue. Guest columns and articles are encouraged. A director's column is published in each issue. News Releases: The Water Center produces about 30 press releases annually focused on research results or progress, extension programming, educational opportunities, public tours, seminars, lectures, symposiums and conferences, awarding of major research grants and other matters of public impact involving the Water Center and other natural resource-focused UNL entities. These releases support a wide variety of UNL water-related research and outreach that cross departmental and academic disciplines. They focus on public impacts of UNL-sponsored research and programming. The UNL Water Center writes these for many UNL environmental science-related departments and faculty members who do not have a staff communicator available to them. The Water Center coordinates public media requests for information and interviews with sources on any water-related topic of interest to them and devotes significant attention to cultivating long-term relationships with members of the working media. The Water Center has a long reputation as a willing and reliable "source" among local, state and regional media for water and natural resources news. Media calls are frequent and water-related faculty and staff are accustomed to fielding questions from the media, doing radio and television interviews, etc. The Water Center makes wide use of electronic and broadcast journalism sources, as well as more traditional print (newspaper) sources.
2. Brochures and pamphlets: All full color. Produced as needed. These include, but are not limited to, mission and programming of the UNL Water Center, UNL Water Sciences Laboratory, Tern and Plover Conservation Partnership, annual Water and Natural Resources Tour and for other units or programs affiliated with or sponsored by the Water Center. All have online versions, as well.
3. Water Center fact sheets: All full-color, generally one sheet. Used to inform and promote both general themes, such as the Water Center itself, or to announce specific programs, seminars, courses, etc. There are various editions, designed for specific internal and external audiences.
4. Nebraska Water Map: A 24 x 36" full-color map of Nebraska surface and groundwater resources. Includes inset maps, diagrams and photos that describe the basics of water quantity, quality and use in Nebraska. The map is used for educational purposes across the state, and is available online. More than 65,000 have been distributed statewide. A range of publications produced outside the UNL

Water Center, particularly fact sheets, research project results and other print materials from USGS, Nebraska Department of Environmental Quality, U.S. Environmental Protection Agency, U.S. Army Corps of Engineers, local Natural Resources Districts and University of Nebraska-Lincoln Extension, are available through Water Center and School of Natural Resources web sites or in print form. UNL Water Center assists with content, design, editing and production for many of these publications.

5. Electronic Resources: Print materials produced by the UNL Water Center, and other information, are available online. The Water Center co-sponsors, designs and maintains the following related Internet web sites: UNL Water: http://water.unl.edu UNL Water Center: http://watercenter.unl.edu/ Water Sciences Laboratory: http://waterscience.unl.edu UNL School of Natural Resources: http://snr.unl.edu/water/index.asp

Publication

Newsletter: The *Water Current* newsletter has a free, subscriber-based distribution of approximately 2,800 copies per issue, more than 95 percent of which are requested subscriptions. Published quarterly in full-color magazine format and available online as a pdf. One or two water-related faculty and/or professional staff are featured in each issue. Guest columns and articles are encouraged. A director's column/report publishes in each issue. News Releases: The Nebraska Water Center produces, on average, more than 20 press releases annually focused on water-related research results, extension programming, educational opportunities, public tours, seminars, lectures, symposiums and conferences, major research grants and other matters of public impact. These releases support and cross many departmental and academic discipline lines within the University of Nebraska system. The Nebraska Water Center writes these for many faculty members who do not have a staff communicator available to them. The Nebraska Water Center also coordinates many working media requests for information and interviews and devotes significant attention to cultivating long-term relationships with members of the media, locally and nationally. Within media communities, the Nebraska Water Center has a long reputation as being a willing and reliable "source" for water and natural resources news. Media calls are frequent and water-related faculty and staff are accustomed to fielding media questions.

Other Print Resources (distributed free to clientele and public):

Brochures and pamphlets: Produced on an as-needed basis. These include, but are not limited to, mission and programming of the Nebraska Water Center, UNL Water Sciences Laboratory, annual Water and Natural Resources Tour and for other units or programs affiliated with or sponsored by the Nebraska Water Center. All have electronic versions, as well.

Water Center fact sheets: Generally one sheet and produced as needed. Used to inform and promote general themes, such as the Nebraska Water Center and UNL Water Sciences Laboratory, or to announce specific programs, seminars, courses, etc. Used for both specific internal and external audiences.

Nebraska Water Map: A 24 x 36" full-color map of Nebraska surface and groundwater resources. Includes inset maps, diagrams and photos that describe the basics of water quantity, quality and use in Nebraska. The map is used for educational purposes across the state, and is available online. More than 65,000 have been distributed statewide. An updated version is pending.

A range of publications produced outside the Nebraska Water Center, particularly fact sheets, research project results and other print materials from U.S. Geological Survey, Nebraska Department of Environmental Quality, U.S. Environmental Protection Agency, U.S. Army Corps of Engineers, local Natural Resources Districts and University of Nebraska-Lincoln Extension, are available via the Nebraska Water Center web site or in print form. Nebraska Water Center assists with content, design, editing and production for many of these publications.

Electronic Resources:
The Nebraska Water Center co-sponsors, designs and maintains the following related Internet web sites:

UNL Water:

http://water.unl.edu
Nebraska Water Center:
http://watercenter.unl.edu/
Water Sciences Laboratory:
http://waterscience.unl.edu
Robert B. Daugherty Water for Food Institute:
http://waterforfood.nebraska.edu/

Conferences, Seminars, Tours, Workshops, Other Outreach:

Water and Natural Resources Seminars: A longstanding annual series of 12 to 14 free weekly public lectures conducted January to April. The series dates to the early 1970's and includes a broad range of water and natural resource-related topics, including the latest research and programming on irrigation and other agriculture topics, fish and wildlife, drinking water and wastewater, watershed management, modeling, energy, climate change, law, economics, and political science. Individual lectures attract an audience of 60 to 100, including approximately 15 to 20 graduate and undergraduate students enrolled for a one-credit-hour course. Other audience members include faculty, government and organizational employees, policy makers and interested members of the public. News releases, mailings, brochures, posters and web-based information are produced supporting this series. Most lectures are taped and then posted online for viewing.

Water and Natural Resources Tour: The tour is another long-standing Nebraska Water Center activity, dating to UNL "Irrigation tours" first conducted in the 1970's. The 2012 tour explored water and agricultural issues arising from the 2011 flooding of the Missouri River lowlands. Attendees include state legislators, congressional staff, faculty, and water scientists and managers from a wide variety of public and private entities. The event is co-sponsored and co-planned with the Kearney Area Chamber of Commerce, Nebraska Public Power District, Central Nebraska Public Power and Irrigation District, USGS Nebraska Water Science Center and others.

Water Law Conference: A one-day event focused on Nebraska water law issues such as water rights transfers, drainage issues, Clean Water Act enforcement, etc. It is targeted to practicing attorneys but open to all. Typically half those attending are water managers and policy-makers. The program is developed by a committee that includes Nebraska's top water lawyers, and is co-sponsored by the University of Nebraska College of Law. Continuing Legal Education (CLEs) credits are made available in Nebraska, Iowa and Colorado.

Great Plains Climate, Water and Ecosystems Symposium: A one-day event following the water law conference focusing on Great Plains climate, water and ecosystems and showcasing impacts at the intersection of climate change or variability, water and all other disciplines, including infrastructure, design, hydropower, agriculture, ecosystem services, drinking water and many others. Geographic focus was centered on the Great Plains, including research or programming transferrable to the Great Plains.

Mentoring: The Nebraska Water Center prioritizes mentoring newer assistant professors to help them establish successful careers. Newer faculty from the many academic units associated with the Nebraska Water Center attended several brown bag sessions during the year where they could get acquainted and get advice from senior faculty and external partners on topics such as working with stakeholders, multidisciplinary research, and managing large data sets over their careers. In addition to helping link individual faculty members to groups, Nebraska Water Center faculty and staff meet with faculty individually upon their arrival and as needed afterwards. This is an ongoing effort. Weekly funding opportunity emails are sent to all water-related faculty and staff.

Other Outreach: Nebraska Water Center staff routinely provides talks for groups and respond to requests for information. These include requests for water-related presentations from the public schools and special “Sunday with a Scientist” displays at the Nebraska State Museum.

Educational Displays:
The Nebraska Water Center makes frequent public displays in association with conferences, symposiums, trade shows, educational open houses and water and environmental education festivals. Nebraska Water Center staff make presentations and sit on steering committees for such annual educational and informational festivals as “Earth Wellness Festival” and others. Water Center staff superintends UNL research and extension exhibits at “Husker Harvest Days,” one of the largest commercial agricultural expos in the country. More than 50,000 tour UNL exhibits during this three-day event.

Primary Information Dissemination Clientele:
U.S. Department of Agriculture
U.S. Environmental Protection Agency
U.S. Geological Survey
U.S. Bureau of Reclamation
U.S. Army Corps of Engineers
U.S. Bureau of Land Management
Nebraska Department of Natural Resources
Nebraska Department of Agriculture
Nebraska Health and Human Services System
Nebraska Department of Environmental Quality
Nebraska Environmental Trust Fund
Nebraska Association of Resources Districts (and 23 individual NRDs)
Nebraska Congressional delegation
Nebraska State Senators
Public and private power and irrigation districts
The Audubon Society
The Nature Conservancy
Nebraska Alliance for Environmental Education
Nebraska Earth Science Education Network
Other state Water Resources Research Institutes
University and college researchers and educators
NU students Public and parochial science teachers
Farmers
Irrigators
Irrigation districts and ditch companies
Private citizens

Cooperating Entities:
In addition to primary support from the USGS, the following agencies and entities have helped fund communications activities by the UNL Water Center during the past year.
U.S. Environmental Protection Agency
U.S. Department of Agriculture Nebraska Department of Environmental Quality Nebraska Research Initiative
Nebraska Game and Parks Commission
Nebraska Environmental Trust

Nebraska Department of Environmental Quality
National Water Research Institute
Nebraska Public Power District
Central Nebraska Public Power and Irrigation District
Farm Credit Services of America
Kearney Area Chamber of Commerce
Nebraska Association of Resources Districts
UNL Institute of Agriculture and Natural Resources
UNL Agricultural Research Division
UNL College of Agricultural Sciences and Natural Resources
UNL School of Natural Resources
University of Nebraska Robert B. Daugherty Water for Food Institute
NU College of Law
USGS Nebraska Water Science Center
Nebraska Center for Energy Sciences Research

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	0	0	0	0	0
Masters	5	0	0	0	5
Ph.D.	0	0	0	0	0
Post-Doc.	0	0	0	0	0
Total	5	0	0	0	5

Notable Awards and Achievements

Publications from Prior Years