

INDICATOR INVERTEBRATES: DETERMINING CHANGE IN BENTHIC  
MACROINVERTEBRATE COMMUNITIES DUE TO DEPOSITED SEDIMENT IN THE  
NORTHERN GREAT PLAINS

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By

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## ABSTRACT

Excessive sedimentation is a major stressor to ecosystem health in freshwater systems globally. Benthic macroinvertebrates are excellent bioindicators of ecosystem health because they have a range of environmental tolerances and are typically associated with certain substrate types. This study tested the hypothesis that sedimentation is a driver of benthic macroinvertebrate communities by determining their responses to increased deposited sediment levels in the Northern Great Plains using both experimental and survey approaches. In both approaches, the effects of deposited sediment were isolated, the responses of specific indicator invertebrates were characterized and finally, indices that commonly respond to deposited sediment were analyzed for their sensitivity. At the community level, the overall multivariate redundancy model was not significant and deposited sediment accounted for only 0.2% of the total variation in species composition in the river survey. Indicator species analysis identified taxa that were associated with sediment impairment classes in both studies. Index sensitivities indicated that Percent Swimmers responded to sediment and can potentially be used as an index of deposited sediment in this region, however this index was not sensitive to sediment in the landscape-scale survey. Although individual taxa that responded to sediment deposition may be used as bioindicators of sediment impairment in further studies, the relatively small effect of sediment at the community level and on univariate composition metrics suggests benthic macroinvertebrate communities are adapted to deposited sediment in the Northern Great Plains.

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## **Dedication**

This work is dedicated to my guardian angels.

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CHAPTER 1  
1.0 INTRODUCTION

**1.1 Sediment as an Ecological Stressor**

With water demands increasing globally and locally, freshwater resources are becoming increasingly valuable. However, the scientific tools used to measure and ensure sustainable water use are lagging. To support industrial, agricultural, and mining developments in southern Saskatchewan, flows will be altered from controlled reservoirs, changing conditions for the downstream benthic communities. Altering flow, combined with urbanization and industrial development, produces a combination of interacting stressors including stream bank erosion and excessive sedimentation. These major ecological risks are the most widespread of all pollution problems affecting freshwaters globally (CCME, 1999; USEPA, 2000) and therefore warrant attention. Although deposited sediment has the potential to be a threat to stream biota, little is known about how to identify and assess its effects on stream ecosystem health in the Northern Great Plains (NGP).

Deposited sediments are particulate organic and inorganic matter, including clay (particle size  $<4\mu\text{m}$ ), silt ( $<62\mu\text{m}$ ), and sand ( $<2\text{mm}$ ) and gravel ( $<2\text{mm}$ ) that are deposited onto the streambed (USEPA, 2000). Agricultural practices, industrial developments, urban runoff, and engineering projects can change the natural vegetation in riparian areas and change flow regimes leading to accelerated erosion of sediment and subsequent deposition in stream beds and banks. Human-induced erosion can exceed natural erosion by a factor of 10, changing the in-stream conditions for biota (Knox, 2006; Leigh & Webb, 2006).

**1.2 Potential Ecological Damage Due to Excessive Sedimentation**

Excessive sedimentation can have destructive consequences for the physical structure and function of streams, on the biota that depend on those streams, and on ecosystem function

(Bjornn *et al.*, 1977; Wood & Armitage, 1997; Henley *et al.*, 2000). Consequences for the physical structure of streams include changes to habitat complexity and connectivity due to the scouring and smothering of habitats, and altering of foraging, spawning, and refuge habitat affecting stream biota (Waters, 1995).

The chemical and physical characteristics of a stream determine the type and quality of habitat available for organisms, providing a structure within which biological communities develop (Southwood, 1977). The available habitat strongly affects the composition and function of a stream community, therefore a description and assessment of habitat is critical in understanding ecosystem health.

Aquatic communities can be affected by sedimentation at multiple trophic levels including algae, macrophytes, invertebrates, and fish. Algae and macrophytes can be scoured and smothered, with sediment clogging interstitial spaces leading to a reduced habitat for attachment (Griffith *et al.*, 2009; Molinos & Donohue, 2009). In consumers, sediment deposition can lead to altered benthic macroinvertebrate communities indirectly through increased invertebrate drift (Culp *et al.*, 1986), lowered respiration capacity through physical blocking of gills or reduced dissolved oxygen (Lemly, 1982), lowered feeding efficiencies in filterers (Box *et al.*, 1999), grazers (Runde & Hellenthal, 2000), and active predators (Waters 1995), and directly by smothering sensitive species (Suren & Jowett, 2001). Upper trophic predators, such as fish, can have their eggs smothered (Fudge *et al.*, 2008), visual predatory skills reduced (Sweka & Hartman, 2001), habitat complexity altered (Zimmerman *et al.*, 2003), and forage resources reduced (Suttle *et al.*, 2004; Wang, *et al.*, 2007). These consequences at the individual level can affect ecosystem function, shifting the community to support a reduced diversity of reproductive, feeding, and locomotion traits (Larsen *et al.*, 2011). While we have an understanding of the

potential adverse effects of deposited sediment on streams, we lack the tools necessary to monitor and assess the level of damage from deposited sediment on the ecological condition of a stream, specifically in the NGP (Benoy, 2012; Bryce *et al.*, 2008; 2010)

### **1.3 Biomonitoring using Benthic Macroinvertebrates**

To monitor the biological effects of a stressor in streams, biomonitoring programs were developed (Rosenberg & Resh, 1993). These programs sample the distribution and abundance of resident native biota, as they are ultimately the indicators of the success or failure of management efforts (Courtemanch, 1996). Freshwater biomonitoring programs rely on the sampling of aquatic macroinvertebrates, algae, mussels and/or fish (Barbour *et al.*, 1999). Invertebrates, primarily insects, are most commonly used in biomonitoring programs, as they present fewer sampling issues relative to other organisms, especially as indicators of disturbance. Algae are shorter lived, respond rapidly to small disturbances, and their response is not necessarily representative of the disturbance. For example, chlorophyll *a* decreases in response to a minor spate, however invertebrate communities will remain unchanged (Grimm & Fisher, 1989). Mussels can be used as bioindicators; however, they are naturally missing from many stream environments in the NGP. Fish are longer lived, but are highly mobile, difficult to sample, and take longer to respond to disturbances (Karr, 1981)

Benthic macroinvertebrates are excellent candidates for bioindicators as they are ubiquitous, relatively long lived, relatively immobile, and taxa vary in tolerance levels to environmental characteristics, making them good indicators of the type of habitat in which they are collected (Rosenberg & Resh, 1993). They also represent an intermediate trophic level between algae and higher vertebrates, so we can make inferences at upper and lower trophic levels. Benthic macroinvertebrate communities respond to conditions of the entire watershed,

making them indicators of integrated effects of disturbances and therefore have the potential to be very useful in monitoring disturbances to communities related to sediment (Minshall, 1984).

In biomonitoring programs, benthic macroinvertebrates are collected in a standardized sample, identified to the lowest taxonomic designation possible, and identity data are translated into metrics for data analysis. Traditional metrics include abundance, taxa richness, Shannon's diversity, % Ephemeroptera/Plecoptera/Trichoptera (EPT) or EPT/Diptera. These can be integrated into multimetric indices, such as the index of biotic integrity (Karr, 1991). These metrics do not consider the effects of a specific stressor, but treat all disturbances equally through enumeration and identification of taxa from a given site to assess overall ecosystem health.

There have been a few single-stressor metrics developed to detect the effects of organic pollution (Hilsenhoff, 1988), acidification (Davy-Bowker *et al.*, 2005), non-point stressors, metals (Lenat, 1988) and eutrophication (Rosenberg & Resh, 1993). These are established by determining indicator species and their tolerances (Davy- Bowker *et al.*, 2005), or by identifying a metric that can detect small changes in response to a stressor (Hering *et al.*, 2006). The isolation of the effects of a single stressor is the next step in bioassessment metrics (Camargo *et al.*, 2004; Clews & Ormerod, 2009) and provides the opportunity for targeted management when impairment from that stressor is determined. Few deposited sediment metrics or standards exist (Kaller & Hartman, 2004; Sutherland *et al.*, 2010; Benoy *et al.*, 2012; Chambers *et al.*, 2012), although visual estimation of deposited sediment characteristics is included in rapid bioassessment protocols (Barbour *et al.*, 1999).

#### **1.4 Development of Regional-Specific Metrics**

As habitat assessments including visualization of deposited sediment are common, a metric specific to this stressor would be widely applicable. Other metrics have been developed to

address the issue of deposited sediment, although their application in the NGP is limited due to the unique taxa and streams that are especially sensitive to sedimentation (Zweig & Rabeni, 2001; Relyea *et al.*, 2000).

In the United States, a single fine sediment biotic index was applied to the Northwest (Relyea *et al.*, 2000); however, this proved to be insufficient in depicting macroinvertebrate-substrate relationships across such a broad scale. Monitoring at an ecoregion-scale is suggested so the metric reflects underlying differences in the geologic, thermal, and hydrologic regimes and past/present human alterations (Relyea *et al.*, 2012). Depending on the size of the watershed, typical watersheds within an ecoregion would be exposed to the same conditions and would therefore have similar index scores. Any differences can then be attributed to different levels of disturbance due to land management history or current practices. Since landscapes in the NGP are dominated by agriculture and are naturally prone to erosion, it is imperative that proper ecosystem health assessment and monitoring tools developed.

Seven sediment-sensitive species, predominantly EPT (Relyea *et al.*, 2000; Carlisle *et al.*, 2009) are typically found in streams with cobble substrates and are inherently missing from many NGP rivers and streams. The average stream in SK is 60% mud bottom in sampled reaches (SKCDC, 2014) and would be considered highly impacted by sediment through other standards (Barbour *et al.*, 1999). A metric using taxa naturally occurring in NGP rivers is needed so that the metric can be ecologically relevant. Few manipulative studies exist (Schofield *et al.*, 2004; de Castro Vasconcelos & Melo, 2008); however, they are among the most valuable in determining the impacts of a given stressor (Angradi, 1999; Larsen *et al.*, 2011) and offer even more strength when examined in the context of landscape-scale distributions of invertebrates in the same region.

## 1.5 Objectives

I tested the hypothesis that sedimentation is an important driver in benthic macroinvertebrate communities using experimental and survey approaches. There were two research phases, each containing three main objectives. The objectives of the first phase were: 1) to quantify the response of BMI community metrics using an experimental approach, whereby I manipulated the amount of deposited sediment in *in-situ* cobble baskets, 2) to identify indicator species for given deposited sediment levels, and 3) to determine which community or individual metrics are sensitive to sediment addition. In the second phase, I applied the same approach to analyze data from a landscape-scale survey of benthic macroinvertebrate communities across gradients of habitat complexity and deposited sediment to: 1) determine if the patterns seen in the first phase approach apply at a broader scale, 2) identify indicator species at a broader level, and 3) determine if the metrics analyzed in the first phase match results at the landscape scale. I hypothesized that if deposited sediment determines benthic community composition, I would observe a shift to a community dominated by sediment-tolerant taxa as sediment volumes increased.

## CHAPTER 2 2.0 EXPERIMENTAL MANIPULATION OF SEDIMENT DEPTH

### 2.1 Introduction

Deposited sediment is one of the most widespread forms of pollution affecting freshwaters globally (Davies-Colley *et al.*, 2001). However, the biological implications are still poorly understood, and field experiments to single out the effects of sediment loads are limited. In agricultural watersheds, excessive sedimentation can arise from point sources, livestock, tillage, altered flow, or bank erosion (Larsen *et al.*, 2011), affecting microhabitat for animals by clogging interstitial spaces and reducing oxygen (Rosenberg & Snow, 1975). Improved quantification of the biological effects of sedimentation is necessary to develop biomonitoring protocols specific to determining impacts of a specific stressor to overall ecosystem health (Sutherland *et al.*, 2010).

Along streams there are different degrees of sedimentation. Therefore, even in streams with relatively high sedimentation rates, there are patches with lower amounts of fine sediment that are occupied by relatively sediment-sensitive taxa. Colonization of stream benthos occurs by four mechanisms: drift, upstream active migration, within habitat migration, and aerial sources such as oviposition (Williams & Hynes, 1976). Benthic macroinvertebrates (BMIs) can opportunistically choose their habitat through these mechanisms. Previous experimental studies assessing the biological impacts of deposited sediment have used the addition of artificial substratum mixtures (Angradi, 1999) or the addition of sediment to laboratory streams (Luedtke & Brusven, 1976) or entire reaches (Rosenberg & Wiens, 1978). These studies have generally shown a decrease in overall density and a shift to sediment-tolerant species through within habitat migration, and an increase in drift and upstream migration when sediment was added to the entire reach (Rosenberg & Wiens, 1978).



To isolate the effects of a specific stressor using an experimental approach, researchers manipulate variables of interest while holding other confounding environmental variables constant. Ideally, a study will use introduced substrate from the experimental area to ensure realism and test a gradient of treatments to potentially identify threshold responses (Chambers *et al.*, 2012). Sediment effects on stream invertebrates can develop within 24 hours (Culp *et al.*, 1986). Species richness often reaches equilibrium with surrounding areas within four weeks (Reice, 1985), therefore it is important to have a minimum experimental length of 4 weeks, but longer times are preferable.

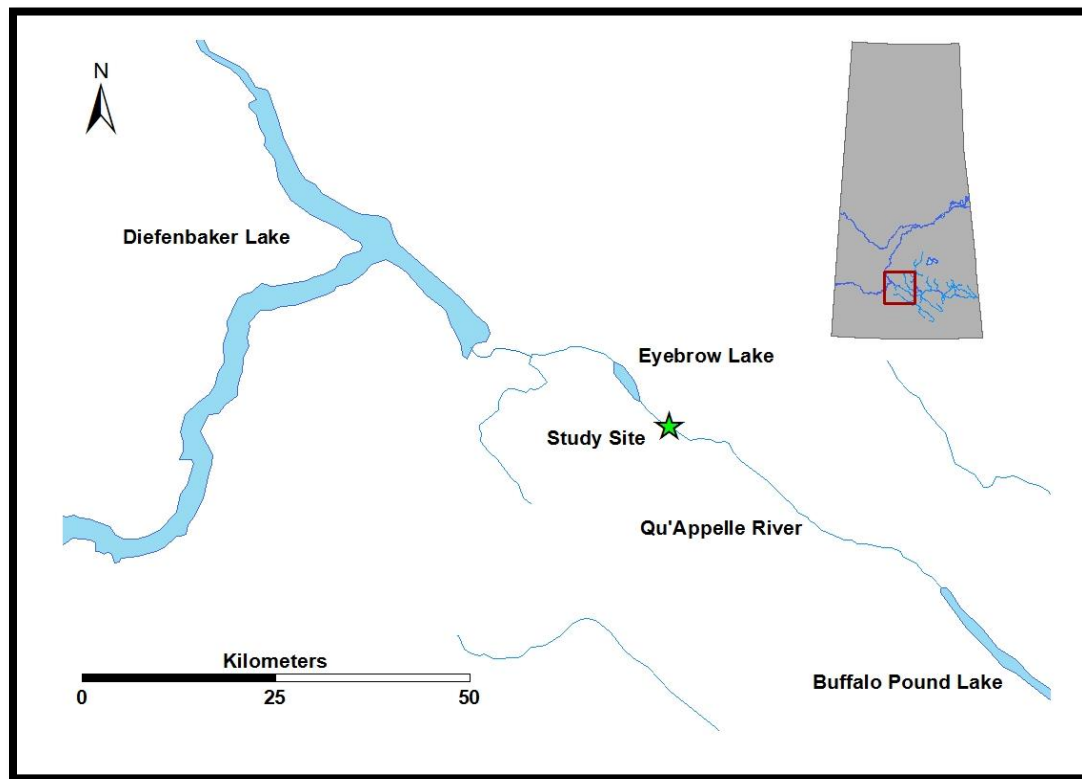
I studied the colonization of replicated cobble substratum mesocosms with differing amounts of fine sediment, and evaluated BMIs as an extension of the environmental characteristics in the ecosystem. I aimed first, to quantify the effect of sediment on BMI communities; second, to identify potential indicator species that are characteristic of a given sediment volume; and, finally, to identify community metrics that respond to deposited sediment. Based on a review of current literature, I expected metrics of diversity and evenness, percent intolerant taxa such as Ephemeroptera, Plecoptera and Trichoptera (EPT), and taxa richness to have a negative relationship with sediment addition. I also expected changes in habit and feeding groups, as they are potential indicators of deposited sediment (Jessup & Gerritsen, 2002; Rabeni *et al.*, 2005; Pollard & Yuan, 2010).

## **2.2 Methods**

### **2.2.1 Study Site**

The mesocosm experiment was conducted in a homogeneous run along the Upper Qu'Appelle River at Eyebrow Marsh [50°53'49. 4"N 106°06'09. 8"W], Saskatchewan, Canada, in the summer of 2012 (Fig. 1). This wadeable stream is in the moist mixed grasslands ecozone in the Northern Great Plains. The riparian zone is primarily dominated by cropped and grazed

land. It was historically an intermittent stream but now is augmented by inflow of the South Saskatchewan River due to the Qu'Appelle Dam upstream of the study site (Hammer, 1971). This channel supplies over a quarter of Saskatchewan's drinking water with flow demands expected to increase (Davies, 2006). The Upper Qu'Appelle River is a 5<sup>th</sup> order stream and is approximately 1.1 m deep at the center at the study location. Mud is the primary substrate with a visual estimation of embeddedness score of 1 (poor condition, very highly impaired) and few macrophytes. Water chemistry measures taken September 17, 2012 were temperature = 13.3°C, conductivity = 368.2µs, dissolved oxygen = 9.0 mg/L, turbidity = 5.53 NTU, and average stage (2012) = 528 masl. This area supports fisheries including whitefish (*Coregonus clupeaformis*), walleye (*Sander vitreus*), common carp (*Cyprinus carpio*) and pike (*Esox lucius*) as well as a species of concern, bigmouth buffalo (*Ictiobus cyprinellus*).



**Fig.1:** Study site (green star) located downstream of Eyebrow Lake in the Upper Qu'Appelle River, Saskatchewan, Canada.

### 2.2.2 Experimental Design

I constructed 25 baskets each measuring 25 cm x 25 cm x 7.5 cm (volume = 4700 mL, Fig. 2) by joining thick mesh together with zip ties. Baskets were lined with a polypropylene geotextile fabric to keep sediment and invertebrates in place and were filled with dry cobble from the area. Inorganic sediment, primarily silt, from the study site was measured with a graduated cylinder and was added to the baskets in the following treatments: A= Control= 0 mL, B= 400 mL, C= 800 mL, D= 1200 mL, E= 2400 mL with 5 replicates in each treatment. These treatments were chosen to match the US EPA guidelines for visual estimation of embeddedness on a scale from 1-20, divided into four groups (Kaufmann *et al.*, 1999). Treatment A acts as a control with no sediment, Treatment B represents embeddedness of 16 - 20 (optimal, very low impairment), Treatment C represents embeddedness of 11-15 (sub-optimal, somewhat impaired), Treatment D represents embeddedness of 6-10 (marginal, highly impaired), and Treatment E represents embeddedness of 0-5 (poor, very highly impaired).

The experiment spanned 16 weeks (June 5 to September 17, 2012). Baskets were placed in a straight line along the length of a homogenous run along the stream. Baskets were 1 m off shore and 1 m apart. The order of the treatments was randomized.



**Fig. 2:** Cobble baskets deployed June 5, 2012 at Eyebrow Marsh in the Upper Qu'Appelle River. Sediment depths range from control (left) with no sediment added to smothered (right) with 2400 mL sediment added.

### **2.2.3 Sample Processing**

#### **2.2.3.1 Field collection**

Cobble baskets were collected Sept 17, 2012. The extended colonization period allowed the insects to be fully-grown, making taxonomic identifications possible, as well as the timing to be comparable to biomonitoring surveys that typically take place in the fall (Rosenberg & Resh, 1993). The baskets were raised in a 38L container and care was taken not to lose sediment and invertebrates in the baskets. Cobble and geotextile were cleaned off by hand and visually inspected for organisms. The sample was then poured through a 500  $\mu\text{m}$  D-frame net, including the coarse sediment, into a sample container (Larsen *et al.*, 2011). The benthic sample was preserved in 95% ethanol in the field and transported to the laboratory.

### **2.2.3.2 Laboratory processing**

Post-experiment sediment depth was measured as the volume of coarse sediment in the sample container. Inorganic material was then separated from organic material by swirling the entire sample in a pail of water and decanting the suspended invertebrates and detritus into a 250 $\mu$ m mesh sieve. Samples were subsampled to 25%- 50% by weight (Sebastien *et al.*, 1988) to approximately 300 organisms (Barbour & Gerritson, 1996). Sorting was performed under 7.5X magnification and identified to lowest practical taxonomic level (e.g. genus for most insect orders) using Merritt *et al.* (2008). Chironomidae were mounted on slides and identified by Dale Parker (Aquatax.ca). All voucher specimens were also verified by Aquatax.ca (1204 Main Street, Saskatoon, Saskatchewan). Functional feeding and habit groups were assigned based on classifications determined by Merritt *et al.* (2008). Coarsely identified taxa were analyzed as pseudo-species to the lowest taxonomic level possible.

### **2.2.4 Statistical Analyses**

#### **2.2.4.1 Redundancy analysis**

To determine the effect of sediment volume on the benthic communities (species abundances) while holding other variables constant, distance-based redundancy analysis (db-RDA) was used with a Bray Curtis distance measure to carry out constrained ordination using non-Euclidean distances. Bray Curtis dissimilarity is a semi-quantitative measure that emphasizes community composition, as differences between abundant species contribute the same to the value as differences between rare species (Bloom, 1981). To avoid negative eigenvalues when using a semi-metric distance measure in RDA, a constant is added and the square root of the distance measure is taken (Cailliez, 1983). Statistical significance of the overall model and individual axes was determined using ANOVA by axis with 999 permutations at  $\alpha = 0.05$ . To visualize the RDA, a triplot with the treatments was produced with sites and

species vectors, given a significant model. Species vectors were overlaid to visualize association with each treatment (McCune, 1997). Partial RDA was used to isolate the effects of sedimentation while keeping other variables constant (Borcard *et al.*, 1992). This partial canonical ordination was performed using the package *vegan* (Oksanen *et al.*, 2012). All statistical analyses were performed in R version 2.15.2 (R Core Team, 2012).

#### **2.2.4.2 Indicator species analysis**

To identify species to potentially be used as bioindicators, indicator species values were calculated using the IndVal index in the *labdsv* package in R (Roberts, 2013) based on species abundance within a group compared to other groups (specificity) and the presence in most replicates within a treatment (fidelity) (Dufrêne & Legendre, 1997). The statistical significance of the indicator values was tested by 1000 permutations at  $\alpha = 0.05$ .

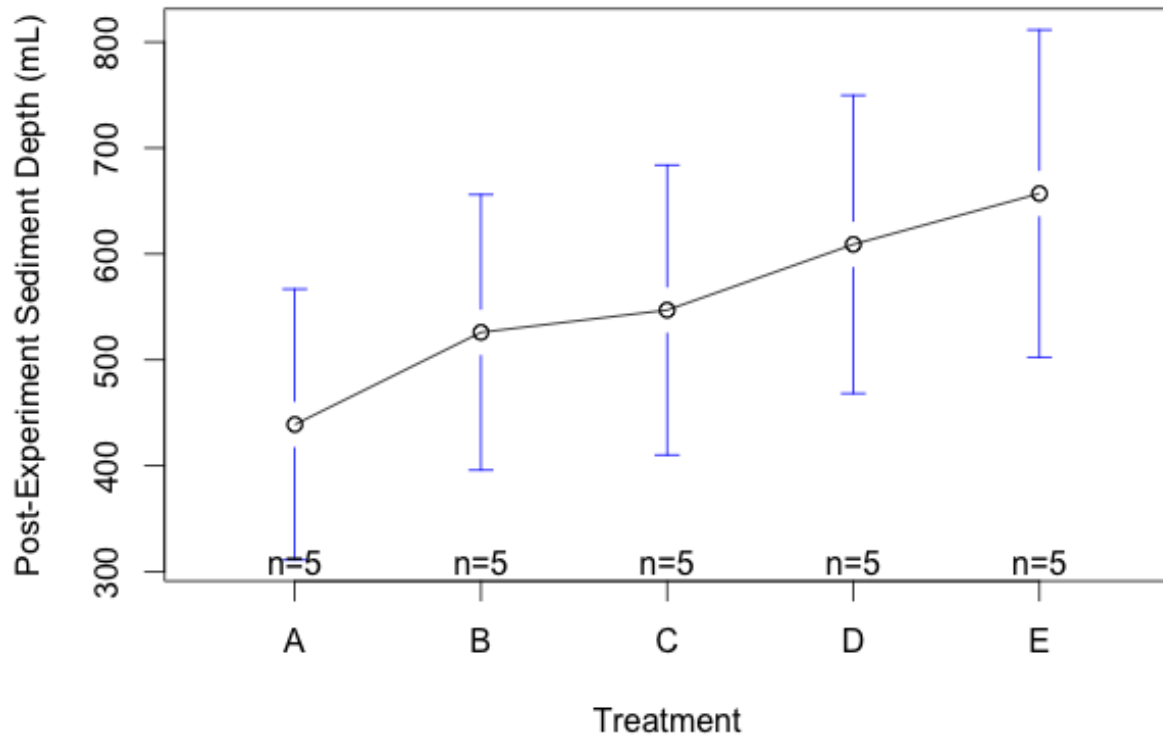
#### **2.2.4. Index Sensitivities**

Indices were calculated at the lowest taxonomic level (typically genus), except Nematoda and Oligochaeta were classified to the phylum level. Biomonitoring metrics and indices were chosen *a priori* from conventional metrics used by stream monitoring programs (Barbour *et al.*, 1999) or shown by others to be sensitive to sediment deposition (Angradi, 1999; Waters 1995; Relyea *et al.*, 2000). Metrics and their associated predictions for tolerance to sedimentation are shown in Appendix A. They include: Percent intolerant taxa (%EPT, ratio of EPT to Diptera), intolerant habit groups (clingers) and functional feeding groups (filterers, scrapers) (Jessup & Gerritsen, 2002), percent tolerant taxa (Chironomidae, Cricotopus/Chironomous, Oligochaeta), and tolerant habit (%Burrowers and %Swimmers) and functional feeding groups (collector gatherers) (Rabeni *et al.*, 2005; Pollard & Yuan, 2009). Metrics of taxa diversity, richness, and evenness typically decrease with increasing sediment (Rosenberg & Snow, 1975).

Normality of the data was tested using the Kolmogorov-Smirnov test and homogeneity of variance verified using Levene's test. For the index sensitivities assessments, the ANOVA alpha values were not corrected using a Bonferroni-type correction, as they are intended to adjust probabilities of multiple comparisons where tests lack specific *a priori* hypotheses (Moran, 2003; Garcia, 2004). Habitat and community metrics were explicitly chosen to be sensitive to sediment deposition and represent specific *a priori* hypotheses rather than randomly selected contrasts, making Bonferroni adjustments excessively conservative (Nakagawa, 2004). A post-hoc power analysis was performed to assess the likelihood of type 2 errors, to examine effects across studies, and to inform follow-up experiment sample sizes (Cohen, 1992). Calculations were performed with the pwr package in R (Champely & Champely, 2012)

### 2.3 Results

The sediment added at the beginning of the experiments maintained treatment levels (ANOVA,  $F_{(4,20)} = 2.97$ ,  $p = 0.045$ ; Fig. 3). A total of 59 taxa representing 13 orders and 26 families were identified and enumerated (Appendix B). Average density was  $15000 \pm 1000$  individuals/m<sup>2</sup> with no significant difference among Treatments (ANOVA,  $F_{(4,20)} = 0.7041$ ,  $p = 0.60$ ). Common invertebrates ( $n > 500$ ) included *Agraylea multipunctata* (Trichoptera: Hydroptilidae), *Cryptochironomus* (Diptera: Chironomidae), *Hyalella azteca* (Amphipoda: Talitridae), *Oligochaeta*, *Paratanytarsus* (Diptera: Chironomidae) and *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae).



**Fig. 3:** Mean  $\pm$  standard error of post-experiment sediment volume (mL) in each treatment, demonstrating that the treatments were effective at maintaining different sediment levels.

### 2.3.1 Distance-Based Redundancy Analysis (db-RDA)

The overall db-RDA model of benthic taxa abundance was not significant (5,5) ( $F_{(4,18)}=0.96$ ,  $p=0.63$ , with 999 permutations). In total, 15.9% of the variance was constrained by the treatment and 14.8% was conditioned, with 69.7% residual, unconstrained variation. The variable constrained was treatment and the variables held as conditions in the environmental matrix were position in the mesocosm array, as a proxy for flow, and sediment depth measured at the end of the experiment. Because the overall model was non-significant, an RDA triplot was not drawn.



### 2.3.2 Indicator Species Analysis

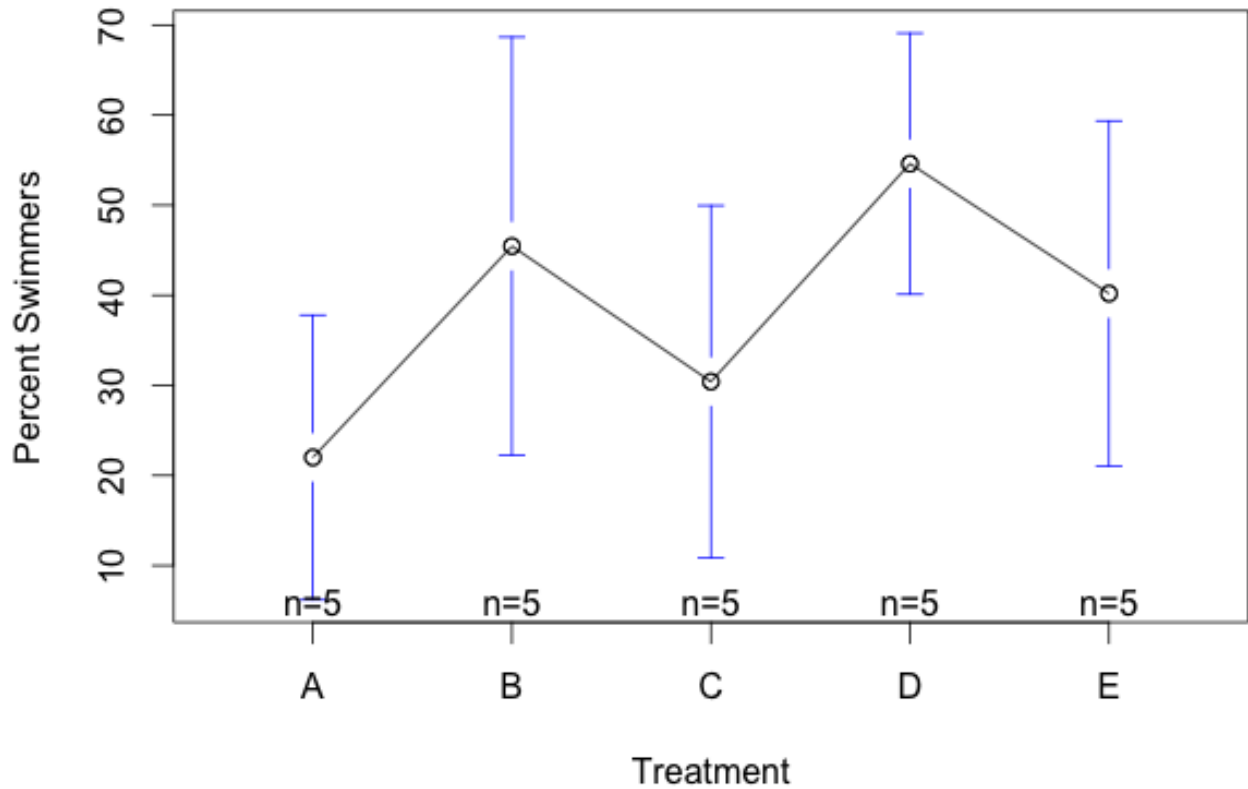
Four taxa had high specificity and fidelity to a treatment and are considered weakly indicative of a given sediment volume (Table 1). Chironomidae (Diptera) and *Caenis* (Ephemeroptera: Caenidae) were associated with shallow sediment conditions of the control group (Treatment A). *Cricotopus* (Diptera: Chironomidae) and *Enallagma* (Zygoptera: Coenagrionidae) were associated with deeper sediment conditions found in Treatment D.

**Table 1:** Indicator species and their associated treatment group from a mesocosm experiment whereby deposited sediment was manipulated. Group A= control and Group D= Moderately Impaired treatment.

Taxa	Group	Indval	p value	Specificity	Fidelity
Chironomidae	A	0.46	0.028	0.78	0.59
<i>Caenis</i>	A	0.38	0.033	0.74	0.51
<i>Cricotopus</i>	D	0.54	0.028	0.83	0.65
<i>Enallagma</i>	D	0.52	0.002	0.91	0.57

### 2.3.3 Index Sensitivities

Of the seventeen metrics tested, only one displayed a statistically significant difference among treatments, Percent Swimmers (ANOVA,  $F_{(4,20)} = 3.58$ ,  $p = 0.023$ , Fig. 4). Only Treatment D was different from Treatment A in a post-hoc Tukey test ( $F_{(4,20)} = 3.58$ ,  $p = 0.02$ ). This habit metric primarily included Amphipoda, Coleoptera, Hydrachnidia, Hemiptera, and others. The standardized effect size of each treatment was 1.2% and the post-hoc power was 0.31. At the conventional power level of 0.8 (Cohen, 1992), 18 replicates per treatment would produce the same outcome as was observed.



**Fig. 4:** The mean and standard error of Percent Swimmers and Treatment of a one-way ANOVA with Percent Swimmers increasing with higher levels of sediment from low sediment (Treatment A) to high sediment (Treatment D).

#### 2.4 Discussion

Although individual taxa and functional groups may respond to sediment as a stressor, community composition was not a sensitive indicator of sediment impairment. Experimental manipulation is a powerful tool to determine cause and effect relationships in stream community ecology (Townsend, 1989). As sediment has been viewed as a primary stressor in freshwater systems (Sutherland *et al.*, 2010), this study focused on quantifying the responses of benthic community to varying deposited sediment levels in the NGP and attempted to identify bioindicators of that stressor. Sediment was not found to influence the community composition significantly. Four indicator taxa were revealed to be weakly associated with sediment treatments

using indicator species analysis. Furthermore, the Percent Swimmers metric was sensitive to deposited sediment levels and has the potential to be used as an indicator of sediment stress.

#### **2.4.1 Community Analysis**

Other studies have shown that cobble that is free of silt and sediment attracts greater densities of organisms than the surrounding substrates (Minshall & Minshall, 1977). However, I found that there was no difference in invertebrate densities among treatments. The Upper Qu'Appelle and the NGP in general have naturally high sediment (Lemke *et al.*, 1965) when compared to coastal and high gradient streams with cobble bottoms typically studied (Angradi, 1999). Therefore, NGP benthic communities are inherently missing sensitive taxa included in sediment-sensitivity studies.

Experimental methods have the benefit of isolating the effects of a given stressor; however, one must take precautions to ensure realism (Townsend, 1989). This experiment aimed to achieve realistic circumstances by keeping many environmental variables, such as water chemistry, geologic, thermal, and hydrologic regimes and human alterations, constant by using *in-situ* mesocosms and by using sediment and cobble from the same stream. Position and sediment depth were included in the model but together, they explained less variance than the treatment factor (15%, compared to 16% by treatment alone). Furthermore, the treatments were successful in altering deposited sediment in the mesocosms (Fig. 3). This indicates the experimental design successfully isolated the effect of deposited sediment levels in the mesocosms.

Much of the variation in the benthic communities was left unexplained (69%). This indicates there were variables that were not measured that are the drivers of the benthic macroinvertebrate community in this stream. Further variance could have been explained by biological interactions that were not explored here, including predation from macroconsumers in

the area. Other studies have excluded fish from mesocosms to determine sediment-BMI relationships (Schofield *et al.*, 2004). Furthermore, algae were not prevented from colonizing the baskets, and could have introduced further variance by serving as a food source in some treatments. However, it is not ecologically relevant to consider communities without their natural biological interactions when performing biomonitoring surveys.

#### **2.4.2 Species Response to Sediment**

Four indicator taxa were identified to potentially be used as bioindicators (Table 1). With the exception of *Enallagma*, characteristics of these taxa preclude their use as ideal bioindicators. Specifically, Chironomidae is a pseudo-taxa that includes dipterans that could not be identified past family level as they were damaged, cryptic species, or too young. Chironomidae are known to increase with increasing sediment volume (Culp & Davies, 1983); however, I observed the opposite effect, with Chironomidae indicative of low sediment conditions. *Caenis* is a widespread collector-gatherer mayfly with a pollution tolerance value of 7 (Merritt *et al.*, 2008). In this study, *Caenis* was indicative of low sediment conditions but with its high tolerance, other sediment indices have included it as an indicator of high sediment (Relyea *et al.* 2012). These examples support the notion that the BMI communities in the NGP are unique. Other studies indicate that *Cricotopus* is tolerant to deposited sediment, however there is a broad range of tolerance values within the genus (Carew *et al.*, 2007). My study identified intact specimens of Chironomidae to genus, however many biomonitoring programs cannot always feasibly identify Chironomidae down to genus, and especially down to species. Future studies, however, should consider the use of *Enallagma* as a bioindicator. *Enallagma* is a predator with a pollution tolerance value of 8 (Merritt *et al.*, 2008). With its high tolerance value, *Enallagma* is an appropriate indicator of high sedimentation as it was found in high abundance in Treatment D. Because of their close association with substrate, they have been found to be indicators of cattle

grazing activity in prairie wetlands as they are found in higher abundance when there is vegetation for cattle to graze (Lee Foote & Hornung, 2005).

### **2.4.3 Index Sensitivities**

Of the common indices tested that are known to respond to sediment deposition (Appendix A), only percent swimmers was sensitive to any differences in treatments in this experiment. Logan (2004) found that percent swimmers were positively correlated with deposited sediment in agricultural streams in New Brunswick, Canada. This habit metric is based on invertebrate traits including morphological adaptations that result in a tolerance to sediment. Therefore, this metric is linked to ecological niche theory where organisms are adapted to a specific range of environmental conditions (Hirzel & Le Lay, 2008). A sound biological basis is key for biomonitoring metrics (Bonada *et al.*, 2006). It is also less affected by taxonomic resolution, because generally, taxa in the same family share the same habit or functional feeding group.

Because only one metric was sensitive to any difference in treatments, the exploration of multimetric indices that combine metrics to detect responses to stressors was not possible (Reynoldson *et al.*, 1997). Furthermore, I did not see a threshold-type response (Fig.4). Perhaps threshold levels of sediment volumes were not reached and the difference between the amounts of sediment added was not enough to achieve a threshold response (Benoy *et al.*, 2012). The highest treatment matches the embeddedness scale of the EPA, however there are many sites in the NGP with no cobble substrate underneath. Furthermore, the 25cm x 25cm cobble basket may not have been a large enough area to study to see the full effects on the community.

Generally, finer substrates tend to attract more burrowers such as oligochaetes, clams and Chironomidae (Williams & Hynes, 1976). Metrics generally accepted to be sensitive to deposited sediment including richness, diversity, Percent EPT, and Percent Burrowers were all similar

among treatments. This indicates that the communities are adapted to the range of deposited sediment introduced. The biological effects of sediment were found to be no better than a random model and *Enallagma* spp. and Percent Swimmers were identified as sensitive to sedimentation, indicating they require further investigation into their potential use in biomonitoring in the NGP.

CHAPTER 3  
3.0 LANDSCAPE-SCALE SURVEY OF BENTHIC MACROINVERTEBRATES IN  
RELATION TO DEPOSITED SEDIMENT

**3.1 Introduction**

The deposition of inert fine particles, arising from catchment and riparian modification, is one of the most widespread of all pollution problems affecting freshwaters globally (Davies-Colley & Smith, 2001). Excessive sedimentation has been identified as the most important cause of lotic ecosystem degradation in the United States in terms of stream distance impacted (USEPA, 2000; CCME, 2002). Soil erosion from agricultural practices, urban runoff, altered stream banks and flow regimes contribute to the natural erosional processes present especially in the Northern Great Plains (NGP) of North America (Sutherland, 2012). However, the tools to identify the effects of deposited sediment on ecosystem health are not well defined (Benoy *et al.*, 2012).

Indicator species can act as warning signs of a stressor and are especially useful when found in high specificity to a particular typology relative to others (Dufrière & Legendre, 1997). Taxa have unique responses to environmental variables (Whittaker, 1956) and the community composition can respond with a shift to more tolerant taxa (Angradi, 1999). They are the biological extension of stressors and reflect impacts at multiple trophic levels in the ecosystem.

Benthic macroinvertebrates (BMIs) are excellent candidates as bioindicators of sediment disturbance as substrate type can be a driver of freshwater invertebrate community composition (Minshall, 1984; Rosenberg & Resh, 1993). Deposited sediment (inert particles <2mm) can affect benthic communities directly by smothering organisms or indirectly by reducing microhabitat complexity through clogging of interstitial spaces and introducing changes to the shape of the streambed (Waters *et al.*, 1995). The changes to microhabitat can affect BMIs

through the reduction of intra-substrate current velocity, lowered dissolved oxygen and decreased visual predation (Rosenberg & Wiens, 1987). Species that are sensitive to sedimentation are biological extensions of the impact of anthropogenic disturbance through erosion and sediment deposition that will reflect the impact at an intermediate trophic level, allowing extension to both primary and tertiary levels.

To determine the impact of sediment deposition, measured as embeddedness, on BMI communities in the NGP, I used a large-scale regional BMI survey data from wadeable streams that ranged in sediment conditions. I hypothesized that sediment is a major driver of BMI community composition, and predicted that intolerant taxa would be less common in reaches of high sedimentation due to direct smothering or indirectly through reduced habitat complexity or other mechanisms. I predicted a shift from sensitive taxa such as Ephemeroptera/ Plecoptera/ Trichoptera (EPT) to a community dominated by sediment-tolerant taxa as sediment levels increase (Waters *et al.*, 1995). A lack of change in response to sediment would run counter to the paradigm that sediment is a major stressor to freshwater ecosystems, and instead suggest that BMIs in the NGP possess adaptations to the naturally high sediment loads in these rivers. My specific objectives were three fold: firstly, to quantify the impact of embeddedness on benthic macroinvertebrate communities; secondly, to identify indicator taxa within classes of sediment impairment; and, thirdly, to assess the index sensitivity to sedimentation such as %EPT that are known elsewhere to respond to sediment addition. These results will provide insight into the impact of sediment on benthic macroinvertebrates in the NGP and inform managers in developing methods to identify ecosystem health impairment due to sediment.



## **3.2 Methods**

### **3.2.1 Sampling Methods**

Benthic macroinvertebrates were sampled as part of a biological survey of 78 wadeable streams (<1.3m depth at center of transect) in the Northern Great Plains as part of the “Reference Condition Approach (RCA)” project by the Water Security Agency of Saskatchewan in September of 2009 (Fig. 5). In this survey, sites were targeted with the least amount of ambient human disturbance. Specifically, these sites were chosen based on common characteristics of <50% cropland, <5% urban land use, <50% pasture, <80% total land under human influence (combination of cropland, urban and pasture, fewer than two landfills, oilwells, bridges or road crossings upstream, and no reservoirs) within 10 km. Sites were at least 10 km apart and are considered to be independent sampling units, where the sample at one site is expected to be unrelated to the sample at another (MoE & SWA, 2012).



**Fig. 5:** Map of 78 study sites in wadeable streams across the Northern Great Plains, Saskatchewan, Canada.

At each site four BMI transects were sampled with a D-frame net using a travelling kick and sweep method in a 1 m<sup>2</sup> area according to Water Security Agency methods outlined in MoE and SWA (2012) based on biomonitoring protocols including Environment Canada's CABIN program (Reynoldson, *et al.*, 2007), the US EPA Rapid Biomonitoring Program (Barbour *et al.*, 1999), and biomonitoring protocols developed by Rosenberg & Resh (1993). Five 1 m<sup>2</sup> points perpendicular to the stream banks were sampled along each transect and were pooled to form one sample per transect, moving upstream. Samples were

placed in ethanol and processed at the lab. Samples were sorted until completion but if the samples were large they were subsampled by weight and sorted from organic matter until a maximum of 300 organisms were counted (Barbour & Gerritsen, 1996). Organisms were identified to the lowest possible taxonomic designation, typically genus (Resh & Unzicker, 1975; Bowman & Bailey, 1997). To avoid incomplete processing or damaged samples, only sites with three sampling transects were analyzed. This maximizes the breadth of taxa diversity while maximizing the number of sites included. The three transects from a site were pooled into a single sample for analysis which reduces variation by including averages. If there were four transects at a site, samples from one randomly selected transect were omitted.

Visual descriptions of reach habitat, condition, water chemistry, and riparian health were conducted at each site following the protocol developed for the EMAP program through a standardized approach (Kaufmann *et al.*, 1999). Embeddedness and sediment deposition were assessed as a univariate estimate of sediment impairment measured by visual estimation of the substrate on a scale of 0-20 (Barbour *et al.*, 1999). A score of 0-5 is considered poor habitat (very highly impaired), 6-10 is marginal (highly impaired), 11-15 is sub-optimal (somewhat impaired) and 16-20 optimal (very low impairment) for both embeddedness and sediment deposition (Kaufmann *et al.*, 1999). Embeddedness refers to the extent to which rocks (gravel, cobble, and boulders) and snags are covered or sunken into the silt, sand, or mud of the stream bottom. Generally, as rocks become embedded, the surface area available to macroinvertebrates is decreased. Sediment deposition refers to the amount that sediment has accumulated and altered the streambed (Barbour *et al.*, 1999). The database is made available through the Saskatchewan Data Conservation Centre by the Water Security Agency of Saskatchewan.

## **3.2.2 Statistical Analyses**

### **3.2.2.1 Data standardizations**

Lone taxa that occurred only once in the dataset were eliminated and coarse taxonomic identifications were considered pseudo-species at the lowest taxonomic designation possible. Species and environmental data were first transformed prior to indicator species analysis and community level analysis. Species abundance data were log+1 transformed as sites were sampled over a wide environmental gradient. Total invertebrate abundances also varied over an order of magnitude among sites. Species data were then Hellinger standardized by sites (Legendre & Legendre, 2012). This has two benefits: firstly, it alleviates the problem of analyzing double zeros in Euclidean analyses (Legendre & Gallagher, 2001). Secondly, it up weights the species in sparse plots and down weights species in plots with high abundances placing the emphasis on species proportions and community composition. Hellinger standardization combined with the Euclidean distance is a metric distance measure as it preserves the Euclidean distances among sites and performs best when RDA or PCA will be used for species data across a wide range of environmental variables (Prentice 1980; Rao, 1995). Environmental data were dimensionally heterogeneous and were therefore standardized to Z-scores. To ensure variable independence, Pearson's  $r$  correlation coefficients were calculated; variables correlated with others above a threshold of 0.6 were omitted from the models. Environmental variables were chosen by forward selection in the RDA.

### **3.2.2.2 Redundancy analysis (RDA)**

To assess the impact of sedimentation on the community composition, both transformation- based Redundancy Analysis (tb-RDA) and partial RDA were used. Tb-RDA is a constrained ordination analysis that preserves the Euclidean distance among Hellinger transformed species variables (Legendre & Gallagher, 2001). It was used to determine the

community level response to sediment including all environmental variables. Forward selection reduces interdependences and selects a parsimonious model from all environmental variables (Blanchet *et al.*, 2008). Forward selection was performed using the package packfor (Dray *et al.*, 2011) with 999 permutations. Variance inflation factors were also calculated and were kept below 5 to complement forward selection in finding parsimonious variables and to reduce model overfitting (Borcard *et al.*, 2011). To visualize the RDA, a triplot with all parsimonious environmental variables was produced with sites and species vectors. The species associated with embeddedness are considered sensitive to sediment. Species vectors were overlaid to visualize association with environmental variables (McCune, 1997).

Partial RDA is a tool that isolates the unique effects of a stressor on the transformed species data while keeping other environmental covariables constant (Borcard *et al.*, 1992). Specifically, this was used to produce a single canonical axis that represents the partial effect of embeddedness on the species data. The corresponding canonical eigenvalue divided by the total variance of the response matrix quantifies the partial fraction of the variation of Y that is accounted for by embeddedness (Legendre & Legendre, 2012). This partial canonical ordination was performed on Hellinger transformed species data to determine the unique effects of sedimentation on community composition as the parsimonious environmental variables identified in the full RDA are held constant using the package vegan (Oksanen *et al.*, 2012). All statistical analyses were performed in R version 2.15.2 (R Core Team, 2012).

### **3.2.2.3 Indicator species analysis**

To assess the impact of sediment on species, sites were grouped based on embeddedness scores into four distinct groups to match the classification guidelines by the US EPA (Kaufmann *et al.*, 1999). Indicator species values are calculated using the IndVal index, which combines species mean abundance and its frequency of occurrence in groups. Both the R packages labsdv

(Roberts, 2012) and *indicspecies* (De Cáceres & Legendre, 2009) in R calculate the same value *IndVal*, although *indicspecies* calculates the square root of *IndVal*, which was then squared to match *labsdv*'s *IndVal*. Benefits of using both packages are that they provide complementary results; *labsdv* provides the overall frequency and *indicspecies* separates the contribution of species abundance within a group compared to other groups (specificity) and the presence in most sites of that group (fidelity) (Dufrene & Legendre, 1997). When *indicspecies* did not identify the same species, specificity and fidelity were listed as not available. The statistical significance of the indicator values was tested by 1000 permutations at  $\alpha= 0.05$ . Indicator species for each class were chosen based on high specificity and fidelity (Dufrene & Legendre, 1997). The range for specificity is 0 (not specific, present in all groups) to 1 (highly specific, present in only one group) and fidelity is 0 (not loyal, not present in all replicates of a group) to 1 (highly loyal, present in all replicates of a group).

#### **3.2.2.4 Index Sensitivities**

Index sensitivity assessments were used to determine the response of benthic community metrics to embeddedness using a series of one-way ANOVAs with  $\alpha= 0.05$ . Metrics evaluated in this study were identical to those assessed in Chapter 2 (Appendix A).

### **3.3 Results**

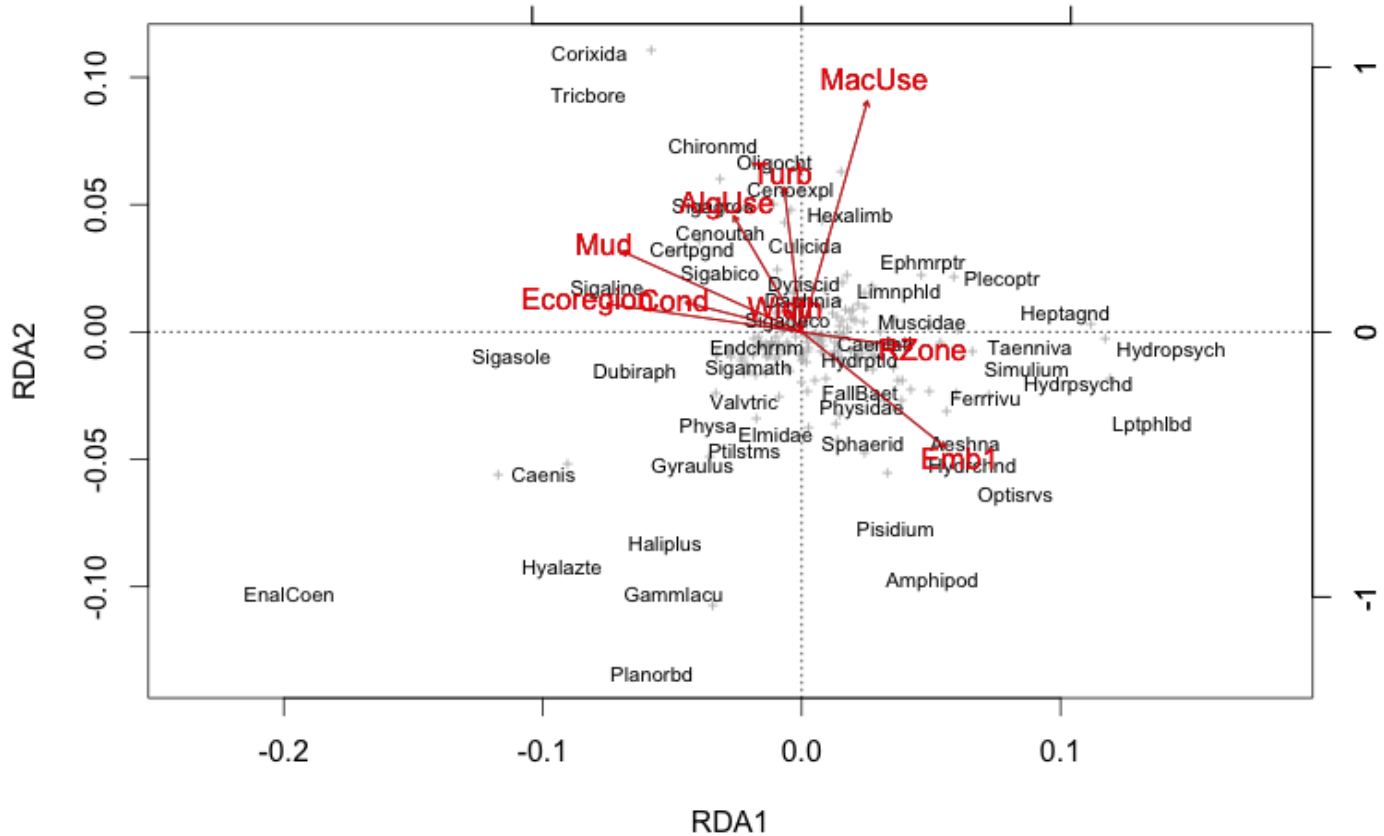
A total of 78 sites were analyzed and 218 taxa were identified (Appendix C). Twenty environmental variables were measured during the habitat assessment and ten were eliminated with correlation coefficients greater than 0.6, including the substrate parameter “sediment deposition”, leaving embeddedness as a proxy for sediment impairment.

#### **3.3.1 Community Analyses**

The *tb*-RDA model retained ten environmental variables in forward selection. The variables included (and their associated ranges): ecozone, channel depth (0.14-1.5 m), channel

width (0.3-25 m), conductivity (1.41-2725  $\mu\text{S}$ ), turbidity (0.14-1813 NTUs), land use (agricultural use or forest), vegetation on banks (0-100%), algae presence/absence, macrophyte presence/absence, % mud (0-100%), and embeddedness (0-20) ( $p < 0.05$ ). The overall canonical relationship of the species response data and environmental dataset were significant (Fig. 6;  $F_{(10,67)} = 1.82$ ;  $p < 0.001$ ) after 1000 permutations. Four canonical axes were significant ( $p < 0.05$ ) and explained 3.1%, 1.8%, 1.3% and 1.0% of the variance of the response data, respectively, for a total  $R^2_{\text{adj}}$  of 0.096. Mud was negatively aligned with embeddedness and *Sigara lineata* was close to the % mud vector (Fig. 6). To validate the environmental drivers in the RDA model, variables were tested for their correlation to the RDA axes. Embeddedness was only weakly correlated to RDA1 and RDA2 ( $r = 0.40$  and  $-0.34$ , respectively). Ecoregion was positively correlated to RDA1 ( $r = 0.61$ ), and macrophytes were positively correlated to RDA2 ( $r = 0.67$ ). Sites located near the origin could mean that the species is at its optimum in the mid-range of the ecological gradients represented by the axes or that it is present everywhere along the gradient.

The partial RDA model to isolate the effect of embeddedness on the BMI community was non-significant ( $F_{(1,67)} = 1.17$ ;  $p = 0.26$  with 999 permutations). The  $R^2_{\text{adj}}$  value for the partial RDA was 0.0026, meaning the proportion of variance in the response matrix explained uniquely by embeddedness was very low (only 0.2%). The covariates explained 9.8% of the total variance and their shared explained variance was 1.9%. The residual variance left unexplained by the model was 88.0%. The unconstrained variance identified by tb-RDA was 80%. When partitioning the variance using partial RDA to isolate the effects of sediment on community composition, the residuals form 89% of the variance. This is because the tb-RDA values are the unadjusted values with inflation due to shared variance between the two datasets (Peres-Neto *et al.*, 2006).



**Fig. 6:** Redundancy analysis triplot (Scaling 2) on log Hellinger transformed benthic macroinvertebrate species data from wadeable streams in the Northern Great Plains. The first four axes are significant (All  $P < 0.05$ ), however only the first two are potted. Distances among sites (grey dots) are the Hellinger distances in multidimensional space. Black text shows the locations of weighted average species scores and vectors showing the relation between ordination axes and standardized parsimonious environmental variables are in red.

### 3.3.2 Indicator Species Analysis

The sites were grouped according to the embeddedness scores into 4 sediment impairment classes: poor (1;  $n=32$ ), marginal (2;  $n=11$ ), sub-optimal (3;  $n=15$ ) and optimal (4;  $n=20$ ). Indicator species were found for each of the four classes (Table 2). For very highly embedded sites (Poor; Class 1) *Sigara lineata* (Hemiptera: Corixidae) was identified as an indicator species. For highly embedded sites (Marginal; Class 2) *Caenis* (Ephemeroptera:



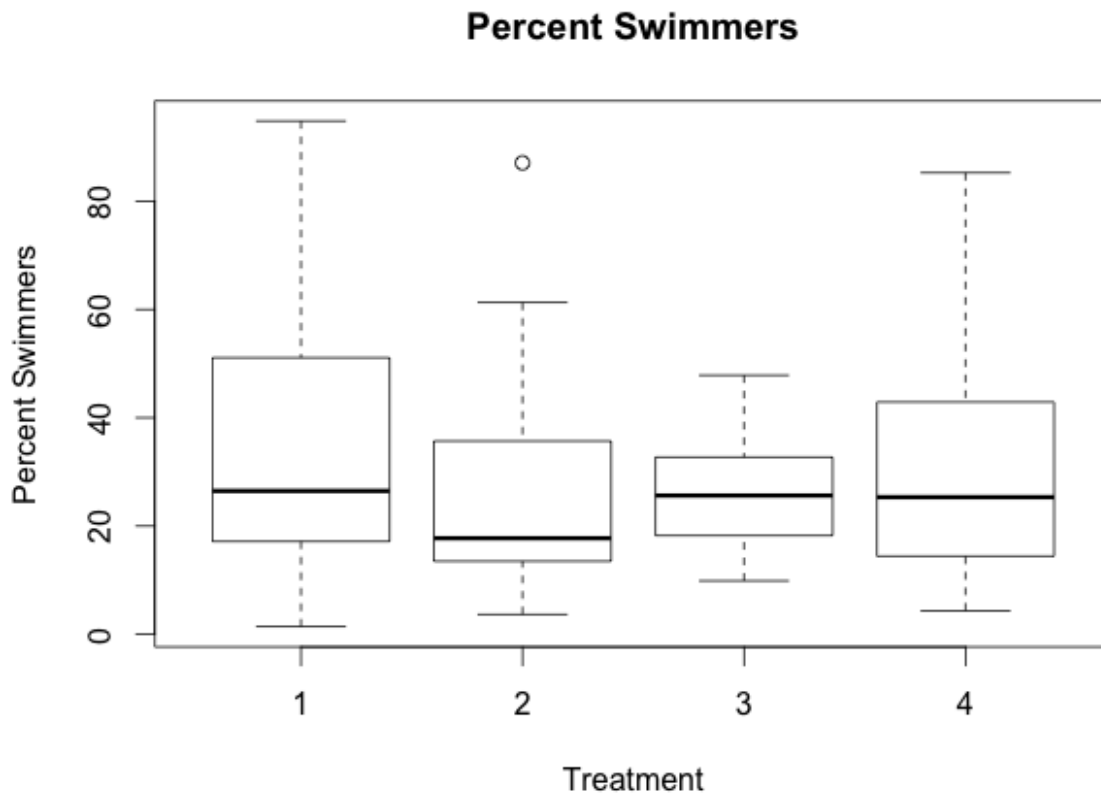
Caenidae), *Haliphus* (Coleoptera: Haliplidae), *Oecetis* (Trichoptera: Leptoceridae) *Aeshna* (Odonata: Aeshnidae) and *Stagnicola exilis* (Gastropoda: Lymnaeidae) were indicator taxa. For somewhat embedded sites (sub-optimal; Class 3) *Dicranota* (Diptera: Pediciidae) was an indicator taxon. For low embeddedness (Optimal; Class 4) Ephemeroptera, Plecoptera, *Tipula* (Diptera: Tipulidae), *Hydropsyche* (Trichoptera: Hydropsychidae), *Acroneuria* (Plecoptera: Perlidae) and Capniidae (Plecoptera) were indicator taxa. Both IndVal and indicpecies identified the same indicator taxa. Overall, the specificity values for the indicator taxa were high (specificity = 0.65-0.96), whereas the fidelity values were quite low (fidelity = 0.19-0.6).

**Table 2:** Indicator species identified from landscape-scale survey of benthic macroinvertebrates in the Northern Great Plains and their associated indicator value, significance, specificity and fidelity to a group. Sediment Class 1 is considered impaired and sediment class 4 is optimal sediment levels.

Indicator Taxa	Sediment				
	Class	IndVal	p-value	Specificity	Fidelity
<i>Sigara lineata</i>	1	0.26	0.03	0.83	0.34
<i>Caenis</i> spp.	2	0.46	0.03	0.89	0.52
<i>Haliphus</i> spp.	2	0.38	0.01	0.65	0.60
<i>Oecetis</i> spp.	2	0.24	0.03	0.81	0.3
<i>Aeshna</i> spp.	2	0.31	0.01	0.66	0.47
<i>Stagnicola exilis</i>	2	0.19	0.01	0.93	0.20
<i>Dicranota</i> spp.	3	0.28	0.01	0.98	0.29
Ephemeroptera	4	0.5	0.03	0.93	0.53
Plecoptera	4	0.28	<0.01	0.89	0.31
<i>Tipula</i> spp.	4	0.24	0.02	0.97	0.25
<i>Hydropsyche</i> spp.	4	0.38	0.02	0.9	0.43
<i>Acroneuria</i> spp.	4	0.19	0.02	0.96	0.19
Capniidae	4	0.18	0.03	0.98	0.19

### 3.3.3 Index Sensitivity

Commonly used benthic community metrics assessed in the Index Sensitivities (Appendix A) yielded non-significant responses ( $p > 0.05$  for all metrics). Noteworthy, however, is the habit group Percent Swimmers that was also non-significant (ANOVA,  $F_{(3,32)}=0.6907$ ;  $p=0.56$ , Fig. 7).



**Fig. 7:** The minimum, 25<sup>th</sup> and 75<sup>th</sup> percentiles, median, and maximum are used to construct a boxplot of percent swimmers in groups 1 (high sediment) through 4 (low sediment) with no significant difference among groups in a one-way ANOVA test ( $p=0.56$ ).

### 3.4 Discussion

Benthic macroinvertebrates are often used as bioindicators of stressors in freshwater systems (Rosenberg & Resh, 1993). As sedimentation has been viewed as a primary stressor in these systems (Sutherland *et al.*, 2010), this study focused on determining the responses of

species to sediment volume and was expanded to include the response of the entire benthic community in the NGP. A survey of BMIs with associated environmental and habitat data revealed indicator taxa associated with low and high sedimentation. However, at the community level, sediment volume had little influence, accounting for only 0.2% of the variation in the species data. None of the metrics were sensitive to deposited sediment despite being identified elsewhere as indicative of sediment impairment. This suggests that although taxa may respond to sedimentation as a stressor, community composition cannot be used as an indicator of sediment impairment in NGP rivers.

### **3.4.1 Community Responses to Sediment**

The overall tb-RDA using all parsimonious environmental variables captured 20% of the variation in the species data with four significant canonical axes. The RDA triplot (Fig. 6) is very information rich and displays complex community interactions. There is valuable information regarding both the species and the environmental variables. The species scores along the first two RDA axes will be located along the vectors of the environmental variables with which they are most associated (Borcard *et al.*, 2011). Along the substrate vector, *Aeshna* was closely associated with embeddedness. This supports the identification of *Aeshna* as an indicator species in the indicator species analysis. *Sigara lineata* is closely associated with the percent mud vector on the opposite end of embeddedness, supporting its inclusion as an indicator of high levels of sediment. Because the data were log Hellinger transformed, the RDA will pull out even minor shifts in community composition based on proportions emphasizing rare individuals (Rao, 1995).

Macrophytes and ecoregion contribute the most to the overall model, as their vectors are longest, with mud and embeddedness long as well (Fig. 6). The presence of macrophytes can increase invertebrate abundance by an order of magnitude (Downing, 1979). This was the reason the data were log transformed; however, macrophytes contributed greatly to the second RDA

axis indicating that species found at a given site are highly associated with macrophytes (Hammer *et al.*, 1990). Ecozone can be a major driver as it reflects flow regimes, plant communities, climate, and relative geographic position (Relyea *et al.*, 2000). Embeddedness was aligned in opposition to percent mud. This indicates that embeddedness is an accurate measure of sediment, as percent mud is expected to decrease as the class of embeddedness gets closer to optimal, a score of 20. However, embeddedness was only weakly correlated to the first two canonical axes, indicating sediment depth is not a major driver of BMI communities.

The proportion of variation explained uniquely by sediment depth was only 0.2% in the partial RDA model. This is a very small proportion of the overall variance in the response data, of which 88% was unexplained. Further work is therefore needed to explain this variation. Ecozone was identified as important indicating that spatial processes are potentially occurring. Including parameters such as hydrology, surficial geology, and seasonal variations in suspended sediments in the model has the potential to further explain some of the unconstrained variance. Due to the large unexplained variance in community data, neutral processes of community composition may better predict community organization rather than local niche partitioning (Rosindell *et al.*, 2012), and stochastic events that cause episodic sedimentation may influence community composition greater than environmental variables in the NGP.

### **3.4.2 Species Response to Sediment**

Sites were placed into groups to match classes of sediment impairment outlined by the US EPA classification system (Barbour *et al.*, 1999) and to identify indicator species associated with different classes. Indicator species should be both necessary and sufficient, meaning that if you find that species it should be in a given class of sites, and if you are in that class of sites you should find that species (Roberts *et al.*, 2012). Fourteen taxa were identified as indicator taxa of a given sediment class (Table 2). The specificity values overall were high and fidelity values

low. This indicates the taxa were found in high frequency within a treatment, however the taxa were also often found in other treatments as well.

#### **3.4.2.1 Class 1 (Poor; very highly embedded)**

*Sigara lineata* (Corixidae) is highly tolerant to human disturbance (Brooks & Kelton, 1967). Corixidae are highly mobile and difficult to identify to species and therefore generally not included in biomonitoring programs (Reynoldson *et al.*, 2007), although in biomonitoring surveys, Corixidae can make up close to 90% of a sample at some sites. Its identification as an indicator of sediment disturbance supports their inclusion in biomonitoring programs and are easily distinguishable from other cryptic Corixidae (Brooks & Kelton, 1967).

#### **3.4.2.2 Class 2 (Marginal; highly embedded)**

*Caenis* (Caenidae) is a widespread collector gatherer mayfly with a pollution tolerance value of 7 (Merritt *et al.*, 2008) and has been used as an indicator of high sedimentation (Relyea *et al.*, 2012). However, in an experimental sediment manipulation in the Upper Qu'Appelle River, *Caenis* was found to be an indicator of low sedimentation (Table 1: Chapter 2). *Haliphus* spp. (Elmidae) has a high tolerance to human disturbance of 7 (Merritt *et al.*, 2008) and has been used as an indicator of disturbance in other studies (Smith & Galladay, 2011). *Oecetis* spp. (Leptoceridae) is a case-making caddisfly and a predator that has a tolerance to human disturbance of 8 (Merritt *et al.*, 2008). *Aeshna* spp. (Aeshnidae) is a dragonfly with widespread distribution in lentic and lotic environments. They are climbers and predators by engulfing with a tolerance value of 3 (Merritt *et al.*, 2008). *Stagnicola exilis* (Lymnaeidae) is a typically known as a habitat generalist with widespread distribution (Clifford, 1991).

#### **3.4.2.3 Class 3 (Suboptimal; somewhat embedded)**

*Dicranota* spp. (Pediidae) or crane flies are sprawlers and predators with a tolerance value of 3 (Merritt *et al.*, 2008).

#### **3.4.2.4 Class 4 (Optimal; low embeddedness)**

*Tipula* spp. (Tipulidae) is a burrower and shredder with a tolerance value of 4 (Merritt *et al.*, 2008). *Hydropsyche* spp. (Hydropsychidae) has a tolerance of 4 (Merritt *et al.*, 2008). They are clingers (net spinners) and therefore need a holdfast in lotic environments. *Acroneuria* spp. (Perlidae) and Capniidae are both members of Plecoptera that are common indicators of stressors. Ephemeroptera are also a common indicator of stressors. In this case, Ephemeroptera and Plecoptera are coarsely identified and pseudo-species and represent the organisms that are fragile and immature making them difficult to distinguish taxonomically. Members of Ephemeroptera are not always sensitive to sedimentation, as burrowing mayflies such as *Hexagenia limbata* are easier to identify and are frequent in the dataset. This species, however, was not identified as an indicator.

#### **3.4.3 Index Sensitivity**

No metrics that typically respond to habitat were different among embeddedness classes. Embeddedness is therefore not a driver of BMIs in the study region or is potentially not an accurate visual assessment method. An alternative quantitative method of assessing sedimentation, Percent Fines, was most closely associated with BMI communities in agricultural streams (Culp & Davies, 1983; Sutherland *et al.*, 2012). Perhaps this method would be more appropriate as it is a standardized, objective estimation that has the potential to be more accurate than visual estimation methods, although this method has the disadvantage of being more time-intensive (Culp *et al.*, 2009).

Overall, response to sedimentation was only observed at the species level. Sediment volume did not impact the benthic community composition. In multimetric studies, response to sedimentation at the community level typically involve shifts in functional feeding groups from shredders to filterers and gatherers, and shifts from intolerant taxa including Ephemeroptera,

Plecoptera and Trichoptera and (EPT) to burrowing organisms including Oligochaeta and Chironomidae with increasing sediment levels (Rabeni *et al.*, 2005). In other multivariate studies in cobble-bottom streams, benthic community assemblage structure was altered with *Baetis* most negatively correlated and Chironomidae most positively associated with the first PCA axes representing increasing embeddedness (Angradi, 1999).

There was no shift in community structure with sediment levels in this study, and shifts at the species level were inconsistent. Though sensitive EPT, particularly net-spinning Trichoptera (*Hydropsyche* spp.) and immature Ephemeroptera and Plecoptera were associated with lower sediment levels, the case- building Trichoptera *Oecetis* spp. was found to be an indicator of high sediment depth. Furthermore, tolerant taxa such as Oligochaeta and Chironomidae were not identified as indicators of high levels of embeddedness. These taxa are both ubiquitous and abundant in the dataset, found in all sites with  $n > 23$  individuals and 16, respectively. This implies that sites in the NGP have benthic communities that would be considered “sediment impaired” relative to those in clear, cobble streams of the Missouri River (Zweig & Rabeni, 2001) or the Appalachian mountains (Angradi, 1999), where sediment has a low residence time. However, in their regional context, they are clearly not impaired, as they share all the characteristics of potential reference sites in the NGP biomonitoring program (MoE & SWA, 2012). Therefore, this warrants a classification system specific to the NGP

## CHAPTER 4 4.0 SYNTHESIS

### 4.1 Discussion

There was no significant response of BMI communities to sedimentation through experimental manipulation of deposited sediment and sediment is quantified as explaining 0.2% of community variation in the analysis of a landscape-scale survey of the NGP. The indicator invertebrate common to both datasets was *Caenis* spp. (Ephemeroptera: Caenidae). In the experimental approach, *Caenis* spp. was indicative of the control group, with no deposited sediment added. The survey approach identified *Caenis* spp. as indicative of moderately impaired sediment class (Class 2). This contradictory finding does not support its use as a bioindicator of deposited sediment. Percent Swimmers was the only metric that showed sensitivity to deposited sediment levels (Fig. 2) but this was not matched at the broader, landscape level.

Experimental methods allow for a better isolation of the effects of a stressor by reducing the variability associated with other factors. For example, the variance explained by sediment volume in the experimental approach was much greater than that explained in the survey, although still not statistically significant in relation to community composition. In the experiment, ecozone was held constant and macrophytes were held constant, which were variables that explained the most variance in the survey data set. Relying solely on large-scale surveys can be misleading, as they are often correlative and do not provide a clear indication of cause and effect (Manel *et al.*, 2000). However, when used in conjunction with an experiment, the results can be used to corroborate larger-scale patterns (Angradi, 1999; Kreutzweiser, 2000), or to determine direct relationships in the response of impacted organisms (Rabeni *et al.*, 2005). In this case, they can also be used to formulate further hypotheses.



One hypothesis worth testing in future work is that Percent Swimmers is a valid metric for deposited sediment, but only in the Upper Qu'Appelle system where the experiment was conducted. Though it did not differ amongst sediment classes in the survey, it is possible that the NGP is too broad of a scale over which to apply only one metric. Regional metrics are based on smaller land areas than the ecoregion scale (Relyea *et al.*, 2000) and should contain a smaller subset of the total community assemblage. The variance of environmental variables is so great in the NGP, that potentially isolating biological groupings such as plant ecozones, and identifying metrics specific to those regions, will create more applicable metrics.

Comparing the results of percent swimmers in both the experiment and the survey, there was an average of 20-25% swimmers in the survey (Fig. 6), which is similar to the control in the experiment (Fig. 4). The only difference seen in percent swimmers is in treatments B, C, D, and E in the experiment, where they have 30-50% swimmers (Fig. 4). Perhaps swimmers are quickest to colonize in the experiment, accounting for the unusually high percentages in higher sediment treatments.

Further hypotheses also include the possibility that sediment is not a driver of BMI communities and that neutral models of community organization may apply in this region that experiences regular disturbance in the form of floods and droughts. Hydrological modifications from hydroelectric dams, and water diversion for agriculture, and human use in these streams may exacerbate the influence of stochastic events on community organization, further driving communities towards neutral models of organization (Rosindall *et al.*, 2012). The environmental variables measured in both the experiment and survey were only able to explain 31% and 20% of the variation, respectively, indicating that unmeasured variables are accounting for the majority of the variance in species abundances. As an example, algae was not kept constant in the

experiment and a dominant species in the data set, *Agraylea* spp. (Trichoptera: Hydroptilidae) is highly associated with filamentous algae (Merritt *et al.*, 2008). Perhaps quantifying the amount of filamentous algae in samples would increase the amount of variance explained.

Furthermore, benthic communities in streams with different substrate types could respond differently to sediment depth manipulation. As the study site here was already a mud-dominated bottom, other cobble-bottom communities may respond differently to sediment addition. Sites in the survey were chosen that had little human disturbance. Benthic communities in naturally mud-dominated rivers may behave very differently than rivers that were once cobble but have been subject to erosion in the period of human settlement. Further studies can include experiments in multiple streams of different orders and substrate types. In addition, the use of indicator species analysis to identify the tolerance of BMIs warrants further attention. Indicator species will be easier to identify when there are more than subtle differences in responses to sedimentation among treatments.

That only subtle effects on invertebrates were detected indicates that BMI communities in the NGP can exhibit some degree of resilience to fine sediments. Stream substrates are composed of mud prior the sediment manipulation and the insect communities may have already been naturally adapted to these fine substrates. Additional experiments at a larger scale, in other watersheds would be useful to determine the degree of tolerance BMI communities have in this region. Because there was very little difference from the cobble community composition, there is little evidence to support cobble introduction as a management solution to enhance benthic richness, at this scale.

## 4.2 Conclusion

I observed some evidence for responses to sediment at the species level but not at the community level. This goes against the paradigm that sedimentation is a major driver of BMI communities, suggesting benthic communities in the NGP are well adapted to high sedimentation levels. What is considered sediment impairment, or poor sediment conditions in other regions, are not applicable to these communities. However, specific taxa are associated with particular sediment volume. These indicator taxa have the potential to be used as a metric of sediment impairment in the NGP. In future work, I recommend that when developing metrics or indicator taxa for use in BMI monitoring, ecoregions be further broken down into ecozones to identify endpoints that are sensitive to sediment in each of these smaller landscape sub-units. Furthermore, I recommend that the habit metrics such as Percent Swimmers for the Moist Mixed Grassland ecozone be validated with an independent dataset to determine if it should be incorporated into the biomonitoring program for the NGP.

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## APPENDIX A

Benthic macroinvertebrate metrics and the direction of the predicted response to deposited sediment addition with associated references.

<b>Metric</b>	<b>Predicted Response to Sediment</b>	<b>Reference</b>
Percent EPT	-	Waters, 1995
EPT Richness	-	Angradi, 1999
Ratio EPT/Diptera	-	Rosenberg & Resh, 1993
Percent Clingers	-	Rabeni <i>et al.</i> , 2005
Sprawler density	-	Rabeni <i>et al.</i> , 2005
Percent Filterers	-	Jessup & Gerritsen, 2002
Percent Scrapers	-	Jessup & Gerritsen, 2003
Evenness	-	Waters, 1995
Shannon Diversity	-	Lenat <i>et al.</i> , 1981
Richness	-	Zweig & Rabeni, 2001
Chironomidae density	+	Angradi, 1999
Orthocladiinae density	+	Lenat <i>et al.</i> 1981
Percent Oligochaeta	+	Wood & Armitage, 1997
Percent Burrowers	+	Rabeni <i>et al.</i> , 2005
Percent Climbers	+	Rabeni <i>et al.</i> , 2005
Percent Swimmers	+	Logan, 2004
Percent Collector Gatherers	+	Pollard & Yuan, 2009

## APPENDIX B

Taxa list from experimental manipulation of sediment depth in cobble baskets in the Upper Qu'Appelle River of the Northern Great Plains, Saskatchewan, Canada. Total column represents total individuals identified. Non-integers are approximations from subsampling.

<b>Taxa</b>	<b>Total</b>
<i>Ablabesymia</i>	99.5
<i>Agabus</i>	66.5
<i>Agraylea multipunctata</i>	5068.4
<i>Caenis</i>	422.0
<i>Ceratopogonidae</i>	7.3
<i>Chironomidae</i>	1565.4
<i>Chironomini</i>	36.6
<i>Chironomus</i>	4.5
<i>Chrysops</i>	55.5
<i>Cricotopus</i>	99.0
<i>Cryptochironomus</i>	1854.4
<i>Dicrotendipes</i>	211.1
<i>Dubiraphia</i>	382.4
<i>Enallagma/Coenagrion</i>	127.6
<i>Epoicocladius</i>	166.8
<i>Gammarus lacustris</i>	24.1
<i>Glossophoniidae</i>	56.7
<i>Glossiphonia complanata</i>	5.8
<i>Glyptotendipes</i>	13.2
<i>Haliplus</i>	209.8
<i>Helobdella stagnalis</i>	19.5
<i>Heptageniidae</i>	468.5
<i>Hexagenia limbata</i>	190.1
<i>Hirudinea</i>	464.6
<i>Hyalabella azteca</i>	7828.4
<i>Hydroporinae</i>	12.9
<i>Hydropsyche</i>	62.9
<i>Hydroptila</i>	30.1
<i>Micropsectra</i>	23.6
<i>Microtendipes</i>	2.3
<i>Nematoda</i>	14.6
<i>Nephropsis obscura</i>	293.4
<i>Neuroclepsis</i>	462.0
<i>Noctuidae</i>	45.0
<i>Oecetis</i>	19.7
<i>Oligochaeta</i>	1107.5
<i>Placobdella ornata</i>	14.0
<i>Orthoclaadiinae</i>	104.4
<i>Orthocladius1</i>	489.8
<i>Orthocladius2</i>	19.0
<i>Ostracoda</i>	36.3
<i>Paratanytarsus</i>	815.0
<i>Phaenopsectra</i>	135.9
<i>Phyrganea cinera</i>	9.1
<i>Physa</i>	9.4
<i>Placobdella montifera</i>	28.8
<i>Polypedilum</i>	3.5
<i>Potthastia</i>	4.0
<i>Procladius</i>	16.6
<i>Psectrocladius</i>	75.1
<i>Sphaerium</i>	130.0
<i>Lymnaea stagnalis</i>	317.9
<i>Tanypodinae</i>	28.6
<i>Tanytarsini</i>	310.7
<i>Tanytarsus</i>	7.9
<i>Thienemannimyia complex</i>	12.3
<i>Promenetus umbilicatellus</i>	22.2
<i>Orconectes virilus</i>	51.6
<i>Wormaldia gabriella</i>	6.4

## APPENDIX C

Taxa list from landscape-scale survey of benthic macroinvertebrates from wadeable streams in the Northern Great Plains, Saskatchewan, Canada. Total column represents total individuals identified. Non-integers are approximations from subsampling.

<b>Taxa</b>	<b>Total</b>		
<i>Ablabesmyia</i>	57.1	<i>Chironomini</i>	158.6
<i>Acricotopus</i>	14.3	<i>Chrysops</i>	35.8
<i>Acroneuria</i>	26.5	<i>Cladopelma</i>	14.3
<i>Acroneuria lycorias</i>	5.3	<i>Cloeon/Procloeon</i>	8.7
<i>Aeshna</i>	145.0	<i>Coleoptera</i>	53.4
<i>Agabus</i>	44.6	<i>Colymbetes exaratus</i>	13.0
<i>Agraylea</i>	15.9	<i>Coptotomus</i>	53.8
<i>Agrypnia</i>	33.2	<i>Corixidae</i>	13712.2
<i>Amphipoda</i>	2795.6	<i>Corynoneura</i>	157.1
<i>Armiger crista</i>	23.2	<i>Crenitis</i>	1.5
<i>Baetidae</i>	3971.6	<i>Culicidae</i>	14.6
<i>Baetis sensu Webb</i>	63.8	<i>Culiseta</i>	13.0
<i>Baetisca</i>	45.0	<i>Cymatia americana</i>	18.0
<i>Baetisca laurentina</i>	4.0	<i>Daphnia</i>	1543.5
<i>Berosus</i>	63.2	<i>Dicranota</i>	49.2
<i>Brachycentrus occidentalis</i>	22.8	<i>Dicrotendipes</i>	500.0
<i>Brachycercus edmundsi</i>	2.0	<i>Diptera</i>	90.8
<i>Caenis</i>	27445.2	<i>Dixidae</i>	17.6
<i>Caenis latipennis</i>	928.5	<i>Dolichopodidae</i>	21.3
<i>Callibaetis</i>	330.5	<i>Dryopidae</i>	8.4
<i>Callicorixa audeni</i>	444.2	<i>Dubiraphia</i>	12545.0
<i>Capniidae</i>	123.5	<i>Dytiscidae</i>	28.2
<i>Cenocorixa</i>	74.5	<i>Elmidae</i>	627.1
<i>Cenocorixa bifida</i>	66.5	<i>Empididae</i>	68.1
<i>Cenocorixa dakotensis</i>	188.1	<i>Enallagma/Coenagrion</i>	4470.9
<i>Cenocorixa expleta</i>	12.0	<i>Endochironomus</i>	571.4
<i>Cenocorixa utahensis</i>	32.9	<i>Ephemera simulans</i>	382.4
<i>Centroptilum</i>	22.9	<i>Ephemerellidae</i>	34.6
<i>Ceraclea</i>	21.0	<i>Ephemeridae</i>	7.5
<i>Ceratopogonidae</i>	5340.8	<i>Ephemeridae/Polymatricydae</i>	792.0
<i>Chaoborus</i>	36.6	<i>Ephemeroptera</i>	9451.3
<i>Chelifera</i>	54.7	<i>Ephoron album</i>	7.0
<i>Cheumatopsyche</i>	267.5	<i>Erpobdella punctata</i>	18.1
<i>Chironomidae</i>	144290.6	<i>Fallceon/Baetis</i>	394.4
		<i>Ferrissia rivularis</i>	510.7

<i>Fossaria/Stagnicola</i>	36.9	<i>Laccophilus biguttatus</i>	153.1
<i>Gammarus lacustris</i>	7871.2	<i>Leptoceridae</i>	390.1
<i>Gastropoda</i>	99.9	<i>Leptophlebia</i>	170.2
<i>Glossiphonia complanata</i>	138.9	<i>Leptophlebiidae</i>	2459.1
<i>Glossiphoniidae</i>	29.6	<i>Libellulidae</i>	13.0
<i>Glyptotendipes</i>	15.6	<i>Limnephilidae</i>	103.2
<i>Gomphidae</i>	53.1	<i>Limnephilus</i>	40.8
<i>Gyraulus</i>	5426.7	<i>Liodessus</i>	126.2
<i>Gyrinus</i>	11.3	<i>Lymnaeidae</i>	275.8
<i>Haliplus</i>	707.7	<i>Maccaffertium</i>	13.0
<i>Haliplus apicalis</i>	64.8	<i>Maccaffertium terminatum</i>	14.0
<i>Haliplus borealis</i>	34.5	<i>Maccaffertium vicarium</i>	126.8
<i>Haliplus immaculicollis</i>	530.4	<i>Maccaffertium/Stenonema</i>	70.6
<i>Harpacticoidia</i>	12.9	<i>Microsema</i>	2.0
<i>Helicopsyche borealis</i>	75.0	<i>Muscidae</i>	113.0
<i>Helisoma</i>	629.2	<i>Mystacides</i>	140.1
<i>Helisoma anceps</i>	4.4	<i>Nebrioporus macronychus</i>	25.0
<i>Helobdella</i>	9.7	<i>Nectopsyche</i>	33.2
<i>Helobdella stagnalis</i>	168.1	<i>Nematoda</i>	606.4
<i>Helophorus</i>	11.4	<i>Nemotelus</i>	50.4
<i>Hemerodromia</i>	200.1	<i>Neoporus superiorus</i>	149.3
<i>Heptagenia</i>	10.1	<i>Nephelopsis obscura</i>	164.5
<i>Heptageniidae</i>	1408.6	<i>Notonecta</i>	16.9
<i>Hesperocorixa</i>	193.8	<i>Notonecta kirbyi</i>	30.0
<i>Hesperocorixa atopodonta</i>	10.7	<i>Oecetis</i>	46.8
<i>Hesperocorixa laevigata</i>	33.4	<i>Ohiogomphus</i>	6.6
<i>Hesperocorixa vulgaris</i>	35.7	<i>Oligochaeta</i>	22359.4
<i>Hexagenia limbata</i>	1529.9	<i>Ophiogomphus</i>	10.7
<i>Hexatoma</i>	8.3	<i>Optioservus</i>	657.4
<i>Hyalella azteca</i>	48775.7	<i>Orconectes virilis</i>	436.8
<i>Hydra</i>	291.1	<i>Orthoclaadiinae</i>	14.3
<i>Hydrachnidia</i>	6038.5	<i>Ostracoda</i>	1661.8
<i>Hydraenidae</i>	9.6	<i>Oxyethira</i>	836.5
<i>Hydrobiidae</i>	8.5	<i>Palmacorixa</i>	5.5
<i>Hydrophilidae</i>	24.2	<i>Palmacorixa buenoi</i>	14.0
<i>Hydroporinae</i>	29.0	<i>Palmacorixa gillettei</i>	6.2
<i>Hydropsyche</i>	1147.5	<i>Paraleptophlebia</i>	125.3
<i>Hydropsychidae</i>	602.6	<i>Paratanytarsus</i>	134.3
<i>Hydroptila</i>	53.6	<i>Pericoma/Telmatoscopus</i>	55.0
<i>Hydroptilidae</i>	95.4	<i>Perlodidae</i>	94.8
<i>Hygrotus sayii</i>	4.8	<i>Phryganea cinerea</i>	46.6
<i>Isoperla</i>	23.0	<i>Phryganeidae</i>	66.0
<i>Labrundinia</i>	14.3	<i>Physa</i>	595.7



<i>Physa gyrina</i>	471.6	<i>Sigara solensis</i>	586.2
<i>Physa skinneri</i>	19.1	<i>Sigara trilineata</i>	51.1
<i>Physidae</i>	437.4	<i>Simuliidae</i>	21.0
<i>Pilaria</i>	3.5	<i>Simulium</i>	4392.0
<i>Piscicolidae</i>	19.3	<i>Simulium</i>	
<i>Pisidium</i>	3163.7	<i>venustum/verecundum</i>	32.0
<i>Placobdella ornata</i>	9.6	<i>Simulium vittatum</i>	942.6
<i>Planorbidae</i>	2668.2	<i>Sisyra fuscata</i>	12.2
<i>Plecoptera</i>	115.6	<i>Somatochlora</i>	6.1
<i>Polycentropodidae</i>	302.8	<i>Sphaeriidae</i>	3560.0
<i>Polycentropus</i>	60.5	<i>Sphaerium</i>	245.9
<i>Procladius</i>	74.3	<i>Stagnicola</i>	59.6
<i>Proclleon</i>	27.9	<i>Stagnicola elodes</i>	32.2
<i>Promenetus exacuous</i>	23.1	<i>Stagnicola exilis</i>	24.1
<i>Psychodidae</i>	71.3	<i>Stenacron</i>	131.1
<i>Psychomyia</i>	38.9	<i>Stenacron interpunctatum</i>	246.3
<i>Psychomyiidae</i>	14.2	<i>Tabanidae</i>	211.4
<i>Ptilostomis</i>	148.7	<i>Tabanus</i>	18.2
<i>Ranatra fusca</i>	23.1	<i>Taeniopteryx</i>	16.0
<i>Rhantus sericans</i>	11.3	<i>Taeniopteryx nivalis</i>	585.1
<i>Sialis velata</i>	92.3	<i>Tanytarsini</i>	708.6
<i>Sigara alternata</i>	111.0	<i>Tipula</i>	43.8
<i>Sigara bicoloripennis</i>	187.0	<i>Tipulidae</i>	121.9
<i>Sigara conocephela</i>	5.5	<i>Triaenodes</i>	21.6
<i>Sigara decorata</i>	30.2	<i>Trichocorixa borealis</i>	2150.2
<i>Sigara decoratella</i>	110.7	<i>Trichocorixa sexcincta</i>	1643.2
<i>Sigara fallenoidea</i>	3.0	<i>Trichocorixa verticalis</i>	202.9
<i>Sigara grossolineata</i>	935.9	<i>Trichoptera</i>	448.4
<i>Sigara lineata</i>	1383.2	<i>Tricorythodes minutus</i>	664.7
<i>Sigara mathesoni</i>	1084.3	<i>Valvata tricarinata</i>	1936.6
<i>Sigara mullettensis</i>	49.2	<i>Zygoptera</i>	26.9
<i>Sigara peniensis</i>	4.0		