AN ABSTRACT OF THE THESIS OF

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Title: The Impacts of Multiple Stressors on Aquatic Invertebrate Communities in the Umatilla River

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Rivers impacted by human activities often have multiple stressors present. The effects of multiple stressors on biological communities can often be difficult to predict, due to the potential for complex interactions between stressors and communities. This thesis explores the impacts of two stressors often associated with agricultural land use, increased sediment and reduced discharge, on aquatic invertebrate communities and taxa behavior. The study design of these experiments allowed for the analysis of both independent and interactive effects of these two stressors on aquatic invertebrates.

To test the impact of multiple stressors on aquatic invertebrate communities, in-stream techniques were used in the Umatilla River in the summer of 2014. The impacts of these stressors were analyzed by focusing on community metrics and total community structure. There was evidence for both independent and interactive effects of sediment and discharge on the aquatic invertebrate communities. Increased sediment and reduced discharge both had negative impacts on Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa, and both changed functional group proportions and taxa abundances in the

communities. The presence of complex stressor interactions in this study highlighted the need to continue studying multiple stressors in rivers in order to better understand these complex relationships.

To test the impact of multiple stressors on invertebrate behavior, laboratory techniques were used. Movement behavior (i.e. crawling and drift behavior) was assessed for stonefly nymphs (family: Perlodidae, genus *Isoperla*) to determine how increased sediment and reduced discharge impacted habitat selection. Perlodidae nymphs found in experimental channels in the Umatilla River were also assessed to test how these two stressors may impact their abundances in rivers faced with increased sediment and reduced discharge. There was evidence that increased sediment influenced *Isoperla* movement behavior, with more nymphs moving off of habitat subjected to added sediment compared to habitat without sediment. There was also evidence that increased sediment had a significant negative influence on Perlodidae absolute and percent abundance in the Umatilla River. Discharge was found to have a marginally negative influence on Perlodidae absolute abundance.

Both of these studies show the important consequences of increased sediment and decreased discharge for individual taxa and functioning aquatic invertebrate communities. Continued study of multiple stressors in rivers will help us to better understand how to manage stressors to reduce the number of negative effects in river communities. In addition, continued use of laboratory studies may allow us to better understand the impacts on individual taxa within communities and may help us understand the mechanisms influencing taxa abundances found in nature.

The Impacts of Multiple Stressors on Aquatic Invertebrate Communities in the Umatilla River

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Hannah Clark, Author

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CONTRIBUTION OF AUTHORS

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CHAPTER 1 - General Introduction

Human land use activities, including agriculture, urbanization, and forestry have negatively impacted river systems worldwide, threatening freshwater biodiversity and water resources (Strayer & Dudgeon 2010; Vorosmarty et al. 2010). Agricultural land use is often of particular interest to stream ecologists, since agriculture uses 85% of human consumed water worldwide (Gleick 2003) and negatively impacts aquatic systems through increasing nonpoint-source inputs of pollutants, changing riparian and stream channel habitat, and altering flows (Allan 2004). About 40% of the Earth's land has been converted to agriculture (Foley et al. 2005). The high demand for fresh water in both agriculture and other land uses has created a great need for providing enough fresh water for human use, while at the same time minimizing the harmful environmental consequences, such as degradation of ecosystems, loss of biodiversity, and loss of ecosystem services (Allan & Castillo 2007).

Two common negative impacts to river systems are fine sediment input and surface water withdrawal for irrigation. Water is regularly removed from rivers for agricultural irrigation and agricultural practices often result in sediment inputs through field and bank erosion (Oki and Kanae 2006; Waters 1995). Both increased sediment and decreased water discharge can alter river habitats, which is considered the most significant threat to biodiversity and ecosystem function in most rivers impacted by humans (Allan & Castillo 2007).

Sediment addition is known to negatively impact aquatic organisms, including benthic invertebrates (Waters 1995). As human disturbance increases in watersheds,

increased levels of fine sediments are delivered to streams from bank erosion or from re-suspended bed sediments (Sutherland et al. 2002). Changes caused by increased sediment levels in rivers include increased turbidity, and increased scouring and abrasion of benthic substrate, which result in decreased periphyton and biofilms on benthic substrate, decreased primary production and bottom-up effects on food webs (Allan 2004). Increased sediment also results in sediment filling interstitial spaces between rocks, which eliminates habitat for some taxa of macroinvertebrates and gravel spawning areas for fish (Allan 2004). Suspended sediments can impair respiration in benthic invertebrates with gills, due to both damage by abrasion and buildup of sediment in the gills (Jones et al. 2011; Waters 1995).

Periods of low discharge are natural for many stream systems, but human activities that artificially create or extend periods of low discharge may cause negative consequences for aquatic communities (Dewson et al. 2007). Low flows in rivers can cause significant changes in habitat conditions and a loss in habitat diversity (Dewson et al. 2007). Changes in aquatic invertebrate communities can result from reduced water discharge interacting with indirect effects of irrigation water withdrawal, such as increased water temperature and conductivity (Miller et al. 2007). Stressor effects can often be unpredictable based only on the knowledge of single effects, and managers should take into account the possible complex interactions of multiple stressors (Townsend et al. 2008). Since flow reduction is typically associated with increased sediment cover in rivers (Dewson et al. 2007), it is important to study these two stressors together in order to try to understand the effects on communities that are caused by these possible complex interactions.

Biomonitoring is the systematic use of living organisms or their responses to evaluate the quality or health of an aquatic environment (Rosenberg et al. 2008). Depending on the study area and the objectives of the study, different groups of organisms have been chosen for aquatic biomonitoring, including aquatic invertebrates, algae, fish, and zooplankton (Resh 2008). Aquatic invertebrates have been used to evaluate water quality and to study the food base for aquatic vertebrates and decomposition (Cummins et al. 2008), and they are used more often than any other group to study the health of streams and rivers through biomonitoring (Friberg et al. 2010). Advantages for using benthic macroinvertebrates for biomonitoring studies include: 1) they are found in many different aquatic habitats, 2) there are a large number of benthic macroinvertebrate species, which have a broad spectrum of responses to environmental variables, 3) they are generally sedentary, and 4) they have life cycles long enough to show changes in the habitat quality over time (Rosenburg and Resh 1993). Aquatic invertebrates are also generally easily collected and observed and they are more closely associated with local habitats compared to fish and other more mobile animals (Cummins et al. 2008). Studies have shown that aquatic invertebrate communities can be strongly related to land use (Allan 2004; Moore & Palmer 2005).

Researchers have called for further study of land use linked stressors on river and stream communities because the effects can be complex and difficult to predict (Wagenhoff et al. 2011). More information is needed on multiple stressors linked to agriculture to better inform land managers of possible environmental risks associated with land use. Few studies have explored the effects of multiple, interacting stressors on river ecosystems and those that have used out-of-stream experimental channels or

mesocosms (Matthaei et al. 2010, Piggott et al. 2012, Waggenhoff et al. 2012; Magbanua et al. 2013; Elbrecht et al. 2016). Matthaei et al. (2010) showed experimental evidence that increased fine sediment and reduced water flow often had interactive effects. This suggested that abstracting water from rivers with already high levels of fine sediment can have stronger negative impacts on aquatic invertebrate communities compared to abstracting water from rivers that have lower levels of fine sediment.

The following chapters cover two experiments investigating the impacts of sediment addition and reduced discharge an aquatic invertebrate communities. Through both in-stream community analysis and a laboratory behavior study, the individual and combined effects of sediment and discharge on aquatic invertebrates were determined. Both experiments allowed for investigation of stressor interactions, in order to attempt to better understand possible complex relationships between sediment and discharge and the aquatic invertebrate communities.

CHAPTER 2 - The Impacts of Multiple Stressors on Aquatic Invertebrate Communities in the Umatilla River

ABSTRACT

Human land use has caused negative impacts to rivers worldwide, which threatens biodiversity, ecosystem function, and important water resources. Agricultural land use is of particular interest in stream ecology, due to its negative impacts to rivers such as added pollution, sediment addition, altering flows, and changing riparian and stream habitat. Rivers impacted by human activities often have multiple stressors present. The effects of multiple stressors on biological communities can often be difficult to predict, due to the potential for complex interactions between stressors and communities.

To test the impact of multiple stressors on aquatic invertebrate communities, in-stream techniques were used in the Umatilla River in the summer of 2014. Community response variables and total community structure were analyzed. There was evidence for both independent and interactive effects of sediment and discharge on the aquatic invertebrate communities. Increased sediment and reduced discharge both had negative impacts on Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa, and both changed functional group proportions and taxa abundances in the communities. Increased sediment had a negative impact on macroinvertebrates with gills. Reduced discharge had a negative impact on taxa diversity, as well as a positive impact on collector-gatherers and a negative impact on collector-filterers. Each stressor resulted in negative impacts for some aquatic invertebrate taxa abundances, while also having positive impacts for other aquatic invertebrate taxa abundances. This shows that each stressor is independently important in these macroinvertebrate communities, but that there is a

greater impact on the community when both of these stressors are present. The presence of complex stressor interactions in this study highlights the need to continue studying multiple stressors in rivers in order to better understand these complex relationships.

INTRODUCTION

Human land use activities, including agriculture, urbanization, and forestry have negatively impacted river systems worldwide, threatening freshwater biodiversity and water resources (Strayer & Dudgeon 2010; Vorosmarty et al. 2010). Agricultural land use is often of particular interest to stream ecologists, since agriculture uses 85% of human consumed water worldwide (Gleick 2003) and negatively impacts aquatic systems through increasing nonpoint-source inputs of pollutants, changing riparian and stream channel habitat, and altering flows (Allan 2004).

A stressor can be defined as a variable that exceeds its normal range of variation, as a result of human activity, and negatively affects individual taxa or community composition (Townsend et al. 2008). Stressor effects can often be unpredictable based only on the knowledge of single effects, and managers should take into account the possible complex interactions of multiple stressors (Townsend et al. 2008). However, few studies have explored the effects of multiple, interacting stressors on river ecosystems and those that have used out-of-stream experimental channels or mesocosms (Matthaei et al. 2010, Piggott et al. 2012, Waggenhoff et al. 2012; Magbanua et al. 2013; Elbrecht et al. 2016). Aquatic macroinvertebrates are often used to study stressor effects on aquatic ecosystems due to the wide range of sensitivities to environmental variables (Rosenburg and Resh 1993) and their strong links to local habitats (Cummins et al. 2008).

Functional traits of aquatic macroinvertebrate taxa have also been used to study how stressors impact river communities (Lange et al. 2014; Wagenhoff et al. 2012), and may give information about the mechanisms that drive stressor impacts on aquatic invertebrate communities (Statzner & Beche 2010).

Two of the common agricultural impacts to river systems are fine sediment input and surface water withdrawal for irrigation. Water is regularly removed from rivers and streams for agricultural irrigation and agricultural practices often result in sediment inputs through field and bank erosion (Oki and Kanae 2006; Waters 1995). Sediment addition is known to negatively impact aquatic organisms, including benthic invertebrates (Waters 1995). Fine sediment is a natural component in many streams with no human disturbance, and organisms have evolved and adapted to these settings. Changes caused by increased sediment levels in rivers include increased turbidity, and increased scouring and abrasion of benthic substrate, which result in decreased periphyton and biofilms on benthic substrate, decreased primary production and bottom-up effects on food webs (Allan 2004). Deposited sediment can fill interstitial spaces between rocks, which eliminates habitat for some taxa of macroinvertebrates (Allan 2004). Suspended sediments can impair respiration in benthic invertebrates with gills, due to both damage by abrasion and buildup of sediment in the gills (Jones et al. 2011; Waters 1995).

Periods of low discharge are natural for many stream systems, but human activities that artificially create or extend periods of low discharge may have negative consequences for aquatic communities (Dewson et al. 2007). Changes in aquatic invertebrate communities can result from reduced water discharge interacting with indirect effects of irrigation water withdrawal, such as increased water temperature and

conductivity (Miller et al. 2007). Low flows in rivers can cause significant changes in habitat conditions and a loss in habitat diversity (Dewson et al. 2007).

Researchers have called for further study of multiple, land use linked stressors on river and stream communities because the effects can be complex and difficult to predict (Wagenhoff et al. 2011). Matthaei et al. (2010) showed experimental evidence that increased fine sediment and reduced water flow often had interactive effects in aquatic macroinvertebrate communities. More information is needed on multiple stressors, including increased sediment and reduced discharge, to better inform land managers of possible environmental risks associated with land use.

This study focused on how increased sediment levels and reduced discharge would impact aquatic invertebrate communities in the Umatilla River in eastern Oregon. The main objective of this study was to investigate the effects of both single and multiple agriculturally-linked stressors (fine sediment addition and decreased water discharge) on aquatic invertebrate communities using in-stream techniques. It was predicted that sediment addition and reduced water discharge would result in negative impacts and change functional traits in aquatic macroinvertebrate communities. It was also predicted that the presence of multiple and interacting stressors would have a greater effect on the invertebrate community compared to single stressors.

METHODS

Study site description

To study how multiple stressors would impact aquatic invertebrate communities in the Umatilla River, sediment and discharge levels were experimentally manipulated in

in-stream channels. Experimental channels were constructed and maintained in the lower mainstem Umatilla River near the town of Echo, Oregon at river mile ~29 (45º43'29.9" N, 119º11'14.74" W) from August 1 through October 1, 2014. The Umatilla River and its tributaries originate in the Blue Mountains and drain an area of ~2,290 square miles, and the mainstem of the Umatilla River is 89 miles long (Northwest Power and Conservation Council 2004). The land uses of the Umatilla River Basin include rangeland and range-forest transition areas (42%), cropland (39%, of which 73% is dryland and 27% is irrigated), forest cover (14%), and urban and developed areas (1%) (Northwest Power and Conservation Council 2004).

A total of 24 channels were constructed with six replicates of four experimental treatments. Experimental treatments involved the manipulation of water discharge and sediment in a factorial design (Figure 2.1). The four treatments consisted of a control treatment, a reduced discharge treatment, an increased sediment treatment, and a treatment with both reduced discharge and increased sediment. Three study blocks were established in the study site, each containing eight channels and two replicates of each of the treatments. The first study block was constructed ~ 1.3 miles downstream from the last two study blocks. Study blocks were constructed within one to two weeks of each other. Information on study blocks can be found in the Appendix.

Experimental channel description

Each channel was two meters long and one meter wide, with a permeable enclosure located at the downstream end (dimensions: 0.61 m x 0.61 m). Channels were constructed out of black plastic sheets anchored to rebar secured into the riverbed parallel to the stream flow. Enclosure frames were constructed out of 1.27 cm diameter PVC.

The enclosure frames were covered with 2.54 cm diameter mesh garden fence and layered with window screen material on the bottom to keep in sediment. Each enclosure contained river rocks scrubbed free of invertebrates from the area that the channel was constructed from. Within each study block, channels were approximately three meters away from each other and were offset such that the closest upstream and downstream channels were not directly in front of each other, which was an adequate distance to ensure minimal impact of treatments from upstream channels, while staying within the study area.

Treatments were added two weeks after channels were constructed. To achieve reduced water discharge, rock dams were constructed at the upstream ends of the channels to divert water, while still allowing some water and invertebrates to flow through. The average reduction in discharge among these treatments was 74%. To create sediment addition treatments (< 2 mm grain size, Zweig & Rabeni 2001), sand was added to enclosures to cover approximately 50% of the substrate (Figure 2.1). Visual estimates were used in percent coverage, and sediment weights were recorded from sample substrates after invertebrates were removed to assess treatment effectiveness. Along with maintaining treatments throughout the study, channels were monitored 2-3 times weekly and cleared of any accumulated debris.

Each enclosure contained three rock packs and three leaf packs, which were mesh bags containing substrate for invertebrate communities to colonize. Leaf packs were composed of leaf litter from a black cottonwood tree (*Populus trichocarpa*) near the study site. Leaf litter was collected, brought back to the lab, and dried for 24 hours at 60ºC. After the leaf litter was dried, 5 g of dried weight leaf litter was placed into 32 cm

x 12 cm mesh bags with openings 1 cm wide. Rock packs were composed of rocks collected from each channel site and placed in 20 cm x 25 cm plastic mesh bags with 2.54 cm diameter openings. All rocks used in rock packs were scrubbed free of invertebrates and periphyton in order to allow for equal colonization opportunities throughout the study site. The average volume of rocks used in rock packs was 48 mL for each pack, and rocks were on average 47.6 mm (±1.7 SE) in median width. There were no significant differences in volume ($F = 0.354$, $p = 0.787$, one-way ANOVA) or median width ($F = 0.531$, $p = 0.666$, one-way ANOVA) of rocks between treatment groups.

Stressors as continuous variables

Though stressor treatments were maintained throughout the study in order to achieve an average reduction in discharge of 74% and a 50% sediment substrate coverage, the natural variability within the Umatilla River made this difficult to do successfully. Within each treatment group, there was a range in both discharge and sediment treatments (Table 2.1). Due to the range of discharges and sediment levels achieved in this experiment, the two stressors were treated as continuous variables for all analyses rather than discrete variables with two levels.

Sampling protocol

Temperature, flow velocity, and sediment coverage were recorded ~2 times weekly throughout the duration of the study. During sampling one rock pack and one leaf pack were sampled at random from the channels. The first samples were collected two weeks after the channels were constructed and before treatments were implemented, representing the aquatic invertebrate community before treatments were implemented.

The post-treatment samples were collected four weeks after treatments were implemented, six weeks into the study.

After picking out all invertebrates, sediment attached to leaf and rock packs (>250µm) was dried at 60ºC for 24 hours, and weighed. This level of sediment was used as the continuous variable in all analyses. Analyses of temperature, leaf decomposition, and study blocks can be found in the appendix.

Aquatic invertebrate samples were preserved in 95% ethanol and identified in the laboratory with a dissection microscope. Insect taxa were identified to family or genus, when possible, while non-insect invertebrate taxa were identified at a coarser level. Identification guides provided by Merritt et al. (2008) were used for all insect identification and Thorp & Covich (2010) were used for non-insect invertebrates. Functional trait characteristics were assigned to all insect taxa. Habit and functional feeding groups were assigned from Merritt et al. (2008), while attachment and respiration were assigned from Poff et al. (2006). Tolerance values were assigned to insect taxa from Whittier & Van Sickle (2010).

Data analysis

Aquatic invertebrate community functional traits:

Functional traits that were analyzed for rock pack and leaf pack communities included respiration (gills), habit (burrowers, climbers, and clingers), and functional feeding group (collector-gatherers, collector-filterers, scrapers, and predators). For diverse invertebrate taxa that were reported to have multiple states of a trait, the first/main state was chosen and secondary traits were not considered (Table 2.2).

Aquatic Invertebrate Community metrics:

Community metrics analyzed in rock pack and leaf pack communities included assemblage tolerance index (ATI), total abundance, Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa abundance, % EPT taxa, total richness, EPT taxa richness, and diversity (Shannon's Diversity Index) (Table 2.3). ATI was calculated for each sample as the sum of the products of each taxon's tolerance value (TV_i) and its relative abundance ($Abund_i$), according to Whittier & Van Sickle (2010):

$$ATI = \Sigma(TV_i \times Abund_i)$$

Diversity was calculated using Shannon's Diversity Index:

$$H' = -\Sigma p_i \ln(p_i)$$

where p_i is the proportion of individuals from the ith taxa

Multiple linear regression:

Multiple linear regression models were used to assess how community metrics and functional traits were impacted by water discharge and sediment level as independent and interacting stressors. The following "full" model was used initially:

Response variable ~ Discharge + Sediment + Discharge*Sediment + ε

However, if the interaction term was not significant it was removed from the model and the data were analyzed with a "reduced" model:

Response variable ~ Discharge + Sediment + ε

The sediment explanatory variable was the log transformed sediment weight (g) collected from community samples. The discharge explanatory variable was the average discharge for each channel taken over the four weeks after treatments were implemented. R statistical software, version 0.99.486 was used to run all models (R Core Team 2013).

Response variable data sets were transformed when necessary to fit assumptions of multiple linear regression models.

The α level for all models was set at 0.05. There were a total of 15 multiple linear regression models produced for both leaf pack and rock pack communities. To correct for multiple comparisons, the Benjamini-Hochberg procedure was used (Benjamini & Hochberg 1995, Waite & Campbell 2006, McDonald 2014). A false discovery rate of 0.20 was used which assumes a 20% chance of Type I error (false positive). Calculations for the Benjamini-Hochberg procedure were completed using a spreadsheet from McDonald (2014). Though there was some evidence that study block may have been an important predictor to include in the regression models, it was left out due to concern of over-fitting the models (Harrell 2015). A summary of any differences in study blocks can be found in the appendix.

Ordination:

To study the impact of treatments (control, increased sediment only, reduced discharge only, and increased sediment & reduced discharge together), as well as average treatment discharge and sample sediment weights (log-transformed) on the aquatic invertebrate communities, non-metric multidimensional scaling (NMS) ordination was used. NMS ordinations were performed on post-treatment communities using Sorenson's distance on log transformed abundance data using the software PC-ORD v. 6.0 (McCune & Mefford 2011). A maximum of six axes were examined, 500 iterations were conducted, 250 runs with real data and a Monte Carlo randomization test with 250 runs were made to examine stress in relation to dimensionality. Forty eight samples were included in this analysis, with a total of 50 taxa/life stage combinations. Life stages

included larvae, pupae, and adult invertebrates. Log transformed sediment weights and average post-treatment discharge were also included as continuous variables in this analysis, and analyzed with Pearson correlations with ordination axes.

Multi-Response Permutation Procedure (MRPP) was used to examine whether invertebrate communities differed by treatment group and substrate. MRPP is a nonparametric procedure for testing for whether samples from two or more pre-determined groups are significantly more similar to each other than expected by chance (McCune and Grace 2002).

An Indicator Species Analysis (ISA) was conducted in order to determine if there were any taxa indicative of particular treatments or sample substrates. ISA calculates an indicator value for each taxa by taking into account the number of sites (in this case, treatments or substrates) the taxa are found in and also the relative abundance of each taxa (Dufrene & Legendre 1997). Indicator taxa are taxa that are used as proxy measures of ecosystem conditions (Hitley & Merenlender 2000).

RESULTS

There were a total of 16,409 individuals that were collected and identified from both leaf pack and rock pack communities, with 50 different taxa/life stage combinations identified. There were many similarities in the dominant taxa found in each treatment group in leaf packs and rock packs (Figures 2.2 & 2.3). Dominant taxa were considered the top five most abundant taxa for each treatment group. For all treatment groups, Chironomidae were the most abundant taxa. There were other similarities in dominant

taxa among treatment groups, including Leptohyphidae, Gastropoda, Elmidae, and Annelida.

Multiple Linear Regressions

Leaf pack community metrics:

In leaf packs, both % EPT taxa and diversity were significantly and positively related to discharge (Table 2.4). Sediment alone and the interaction between discharge and sediment did not have any significant effects on invertebrate community metrics in leaf packs.

Leaf pack community functional traits:

Two significant models for leaf pack community functional traits were found. Proportions of collector-gatherers had a significant negative relationship with discharge while collector-filterers had a significant positive relationship with discharge (Table 2.4). Sediment alone and the interaction between discharge and sediment did not have any significant effects on invertebrate community functional traits in leaf packs.

Rock pack community metrics:

In rock packs the % EPT taxa was significantly and negatively related to sediment level (Table 2.5). Discharge alone and the interaction between discharge and sediment did not have any significant effects on rock pack community metrics.

Rock pack community functional traits:

There were four significant models for rock pack community functional traits. Proportions of invertebrates with gills had a significant negative relationship with sediment (Table 2.5). Proportions of collector-gatherers had a significant negative relationship with discharge, while collector-filterers and scrapers had significant positive

relationships with discharge (Table 2.5). Collector-filterers and scrapers also had a significant interaction between sediment and discharge (Table 2.5; Figure 2.4 and 2.5, respectively).

Assemblage composition

NMS ordination returned a 3-dimensional solution that explained 84.0% of variation in community composition. The ordination solution had a final stress level of 14.84 and final instability of <0.0001, indicating a stable solution for the final ordination. Average post-treatment discharge was correlated with axis 2 ($r = -0.605$, $p < 0.05$), and sample sediment weight (log transformed) was correlated with axis 3 ($r = 0.334$, $p < 0.05$) (Table 5). There were several taxa that were also correlated with these two axes (Tables 2.7 & 2.8).

Differences between treatment groups

Based on Multi-Response Permutation Procedures (MRPP) the invertebrate communities differed significantly by treatment group ($A = 0.020$, $p = 0.022$) (Figure 2.6). Pairwise comparisons showed significant differences between control and reduced discharge only treatments ($p = 0.01$), control and increased sediment & reduced discharge treatments ($p = 0.029$), and increased sediment only and reduced discharge only treatments ($p = 0.017$). A marginally significant difference was also found between increased sediment only treatments and increased sediment & reduced discharge ($p = 0.089$). Multi-Response Permutation Procedures (MRPP) also showed that invertebrate communities differed significantly by substrate ($A = 0.026$, $p = 0.0003$) (Figure 2.7).

Indicator taxa analysis

Indicator taxa analysis of treatment groups showed that there were indicator taxa for two of the treatment groups, increased sediment only and increased sediment with reduced discharge (Table 6). Indicator taxa of the increased sediment treatment were Hydroptilidae and an unidentified Trichoptera family (Table 2.8). The indicator taxa for increased sediment with reduced discharge treatments was Elmidae. There were no indicator taxa found in either control or reduced discharge only treatments. For substrate types, two significant indicator taxa were found for leaf packs and five for rock packs (Table 2.9).

DISCUSSION

The main objective of this experiment was to study the effects of both single and multiple stressors (fine sediment addition and reduced water discharge) on aquatic invertebrate communities using experimental, in-stream techniques. This study showed evidence that discharge and sediment, as both independent and interacting stressors, have important impacts on aquatic invertebrate communities. One of the most concerning findings from this study is that both sediment and discharge had negative consequences for EPT taxa, which includes sensitive bioindicator species, signaling that both of these stressors may reduce stream health.

Impacts of sediment

As predicted, increased sediment levels had significant impacts on the macroinvertebrate communities. In rock packs, percent EPT taxa was found to have a significant negative relationship with sediment such that channels with high sediment had

on average 16.8% EPT taxa and as low as only 3.3% while channels with low sediment had an average 20.9% EPT taxa and up to 39.9%. Many taxa within the EPT group inhabit surfaces or interstitial spaces between and beneath river stones (Waters 1995). Deposited sediment in the rock packs may have covered enough of the surface and interstitial spaces to change or reduce important habitat for EPT taxa. It is possible for communities to recover after a short period of sediment deposition, but continuous high levels of sediment may completely alter the natural community structure (Wood & Armitage 1997).

Negative relationships between sediment and percent EPT taxa have been found in other studies as well, including field studies on agricultural land-use gradients (Niyogi et al. 2007). Deposited sediment negatively influences other measures of EPT taxa as well, such as EPT density (Zweig & Rabeni 2001) and EPT richness (Angradi 1999; Zweig & Rabeni 2001). Manipulative stressor experiments have also shown negative relationships between added sediment and EPT richness (Matthaei et al. 2006; Matthaei et al. 2010) and EPT abundance (Elbrecht et al. 2016).

In rock packs, proportions of invertebrates with gills had a negative relationship with sediment. Suspended sediments can impair respiration in benthic invertebrates with gills, due to both damage by abrasion and buildup of sediment in the gills (Jones et al. 2011; Waters 1995). Damage to gills and impaired respiration may have played an important role in shaping the communities that were subjected to elevated levels of sediment.

There were no significant relationships found between sediment and any response variables in leaf pack communities. This could be due to a greater resiliency in leaf pack

communities to sediment compared to rock pack communities. Communities in leaf packs may not have relied on interstitial spaces as much as those in rock pack communities, or perhaps the interstitial spaces between leaves were not as severely impacted as those in rock packs.

However, there was evidence that sediment played an important role in shaping the overall community structure based on taxa and life stage abundances for both leaf packs and rock packs. There were a number of taxa that were found to be correlated with the same ordination axis as sediment, indicating that these particular taxa could have been significantly influenced by sediment (Table 2.7). Coleoptera larvae (family Elmidae), Diptera larvae (family Chironomidae), and Ephemeroptera larve (family Leptohyphidae) were all positively correlated with sediment. Meanwhile, two families of Ephemeroptera larvae (family Heptageniidae and Leptophlebiidae) were negatively correlated with sediment.

An increase in fine sediment often creates favorable conditions for certain taxa of macroinvertebrates at the expense of others (Wood & Armitage 1997). Chironomidae were very important members of the macroinvertebrate communities in this study, since this taxa comprised a majority of the macroinvertebrate community for several of the treatment groups in leaf packs and rock packs (Figures 2.2 & 2.3). Chironomidae are generally considered burrowers, and since Chironomidae are a very diverse family, they are found in all types of aquatic habitats (Merritt et al. 2008). These characteristics likely allowed the Chironomidae larvae to flourish in areas with high sediment. Some genera of Leptohyphidae live in habitats with deposited sediment (Merritt et al. 2008), which may explain the positive relationship between Leptohyphidae larvae and sediment. There are

also some genera of Elmidae that live in depositional habitats, but this is a very diverse family, with over 99 species within it (Merritt et al. 2008), so it is unclear if this could explain the positive relationship between Elmidae larvae and sediment.

The negative relationship between two families of Ephemeroptera larvae (Heptageniidae and Leptophlebiidae) and sediment is not surprising. Ephemeroptera, as well as other members of the EPT taxa, have been documented in many studies to have negative relationships with sediment (see above: Niyogi et al. 2007; Angradi 1999; Zweig & Rabeni 2001; Matthaei et al. 2006; Matthaei et al. 2010; Elbrecht et al. 2016).

Impacts of discharge

As predicted, reduced discharge had significant impacts on the macroinvertebrate communities. In leaf packs, there were negative impacts for percent EPT taxa and overall diversity with decreases in discharge. Periods of low discharge are natural for many stream systems, but human activities that artificially create or extend periods of low discharge may have negative consequences for aquatic communities (Dewson et al. 2007; Webb et al. 2013).

The negative relationship found between discharge and EPT taxa is supported in other studies, though the experimental variables differ somewhat. A mesocosm study by Elbrecht et al. (2016) found that reduced water velocity was associated with reduced EPT abundance and richness. Matthaei et al. (2010) found that stream flow impacted EPT richness by an interaction with sediment, where at normal flow, EPT richness increased with increased sediment, until intermediate levels of sediment, and then decreased at high levels. At reduced flow, Matthaei et al. (2010) found a different pattern, where EPT richness decreased steadily with increasing sediment levels. A larger scale study of the

influence of diversion dams in the lower Umatilla River found that EPT taxa richness was lower in areas subject to severe water withdrawals during one year of a two year study (Miller et al. 2007).

Reduced discharge led to a decrease in diversity in leaf pack communities. Low flows in rivers can cause significant changes in habitat conditions and a loss in habitat diversity (Dewson et al. 2007). A review by Webb et al. (2013) found that decreased flows consistently resulted in decreased macroinvertebrate diversity. It is probable that changes in habitat associated with reduced flow/discharge may have led to reduced taxa diversity in this study.

Decreased discharge led to a negative impact on proportions of collector-filterers in leaf pack communities across all sediment levels. However, decreased discharge had a positive impact on collector-gatherers in both leaf pack and rock pack communities across all sediment levels. Collector-filterers consume fine particulate organic matter (FPOM) that is transported within the water column. Baker et al. (2011) studied fine sediment dynamics above and below diversions and found that areas with slower flows accumulated greater amounts of fine sediment compared to areas with faster flows. It is likely that FPOM dynamics would behave in the same way, with amounts of available FPOM in the water column declining with reduced discharge, as it settles onto the benthic substrate. This is likely to have an effect on collector-filterer feeding rates. For example, Georgian & Thorp (1992) found that Hydropsychidae caddisfly larvae are found in areas with higher water velocity, where their filter-feeding rates were higher than in areas with lower water velocity. This effect on feeding rates is likely to translate into an effect on abundance. In contrast, collector-gatherers consume FPOM that settles

onto the benthic substrate. Decreased discharge increases the amount of FPOM settling (Baker et al. 2010), thus increasing the amount of available food for collector-gatherers. Food availability is likely the mechanism responsible for the changes in functional feeding group proportions documented in this study.

Like sediment, not only did water discharge influence many community metrics, but it also played an important role in shaping the overall community structure in leaf packs and rock packs based on taxa and life stage abundances. There were many taxa that were found to be correlated with the same ordination axis as discharge, showing evidence for both positive and negative relationships between discharge and the abundance of certain taxa (Table 2.6). There were many more taxa that had a positive correlation to discharge (ten taxa) compared to taxa that had a negative correlation to discharge (four taxa).

Out of the four taxa with a negative correlation with discharge, two were Ephemeroptera larvae (families Ephemeridae and Leptophlebiidae), and the other two were Plecoptera larvae (family Perlodidae) and Odonata larvae (family Coenagrionidae). Ephemeridae are known to be burrowers (Merritt et al. 2008), and it was expected in this study that burrowers would increase in areas with more sediment. Even though Ephemeridae were not significantly correlated with sediment, the negative correlation with discharge may be explained by the fact that areas of low discharge may be more likely to have sediment settle on the substrate, which provides habitat for Ephemeridae. Both Coenagrionidae larvae and Perlodidae larvae have been found in both lotic and lentic habitats (Merritt et al. 2008), indicating they can live in the lower discharge areas.

However, Leptophlebiidae are generally only found in lotic habitats (Merritt et al. 2008), so the reasons for the negative relationship with discharge are unclear.

Interactions of multiple stressors

In rock packs, interactions between sediment and discharge significantly impacted proportions of both collector-filterers and scrapers. At low sediment levels, proportions of collector-filterers increased steadily with increasing discharge. This relationship fits well with what was previously discussed with the relationship with collector-filterers and discharge, with food sources in fine particles (FPOM) more readily available in the water column at high discharges and less available in low discharges due to settling onto benthic substrate. It was assumed that this food availability was linked to abundances. At mid and high levels of sediment, proportions of collector-filterers appeared unchanged by increasing discharge. Similar to damage to gills discussed previously, filter feeding structures can also get damaged by abrasion and clogged with sediment, which may reduce feeding efficiency for these animals (Jones et al. 2011). This may explain the influence of discharge on proportions of collector-filterers was changed at higher levels of sediment.

At low sediment levels, there was an increase in the proportion of scrapers with increased discharge, while at high sediment levels, proportions of scrapers decreased with increasing discharge. At mid sediment levels, proportions of scrapers were relatively unchanged by increasing discharge. Scrapers consume periphyton, which is a biofilm that grows on rock surfaces and is composed of benthic algae and heterotrophic microbes (Allan & Castillo 2007). Periphyton biomass is thought to be significantly influenced by current velocity (Biggs & Close 1989). In addition, water velocity can impact the feeding

efficiency of grazers, including scrapers (however, this effect varies by species; Hart 1992; Poff et al. 2003). Therefore, water discharge may have influenced the proportion of scrapers within the communities through differences in periphyton biomass and/or through differences in feeding efficiencies. However, the impact of discharge on scraper communities differed depending on the level of sediment. Sediment could impact scraper's food sources by smothering periphyton or damaging it through increased abrasion.

These results reveal complex interactions between discharge and sediment on proportions of two functional groups. Discharge had an important influence on proportions of collector-filterers, but only at low levels of sediment. Discharge also had important impacts on proportions of scrapers, and this relationship also depended on sediment levels, but was a bit more complex. At low proportions of sediment, scrapers increased with increasing discharge, while at high sediment, proportions of scrapers decreased with increasing discharge.

Management implications and future directions for research

While both increased sediment and decreased discharge had similar consequences for EPT taxa, these stressors also impacted additional and different parts of the macroinvertebrate communities. Increased sediment had a negative impact on macroinvertebrates with gills. Reduced discharge had a negative impact on taxa diversity, as well as a positive impact on collector-gatherers and a negative impact on collector-filterers. Both stressors also had negative impacts on abundances for certain macroinvertebrate taxa, and positive impacts for other taxa, that did not appear to be significantly influenced by the other stressor. This shows that each stressor is

independently important in these macroinvertebrate communities, but that there is a greater impact on the community when both of these stressors are present.

The presence of complex stressor interactions in this study highlighted the need to continue studying multiple stressors in rivers. For both collector-filterers and scrapers, discharge was an important influence at low sediment levels. However, with increased sediment, the relationship between discharge and these two functional groups changed – either having little pattern or reversing the pattern that was observed at low sediment levels. These complex relationships are important to keep in mind from a management standpoint. Restoring natural flows to a river may help restore some balance to some functional groups of the community, but for others groups, recovery will depend on how much sediment is in the river.

Functional groups, such as scrapers and collector-filterers, serve important roles in stream ecosystems in processing organic matter and transferring energy through food webs. Changes in functional feeding groups due to stressors are a signal that human activities are impacting the ways that stream ecosystems function. Future studies should consider including functional feeding group analysis in future studies as a way to gauge how stream communities are functioning in relation to stressors.

It has been shown elsewhere that abstracting water from a river with high sediment levels may have more severe consequences for the aquatic invertebrate communities compared to abstracting water from rivers with relatively low sediment levels (Matthaei et al 2010), and managers should keep this in mind if they are able to choose from different river sources for irrigation water. Though there is natural variability in discharge and sediment in river systems, there are important ecological

consequences when human land use alters these variables from their natural conditions. Efforts to limit the amount of water abstracted from rivers, especially during low flows, could help to preserve the health of aquatic invertebrate communities. Taking steps to prevent unnatural amounts of sediment from entering streams and restoring degraded sites (see Waters 1995) can prevent further negative consequences for aquatic invertebrate communities. Restoration planning should focus on restoring natural flows and managing sediment levels in streams to more closely match natural conditions in order to support a healthy functioning ecosystem. Increasing our understanding of the impacts of single and interacting stressors can allow for more informed management decisions in the future to provide enough fresh water for human use, while reducing negative impacts to river ecosystems.

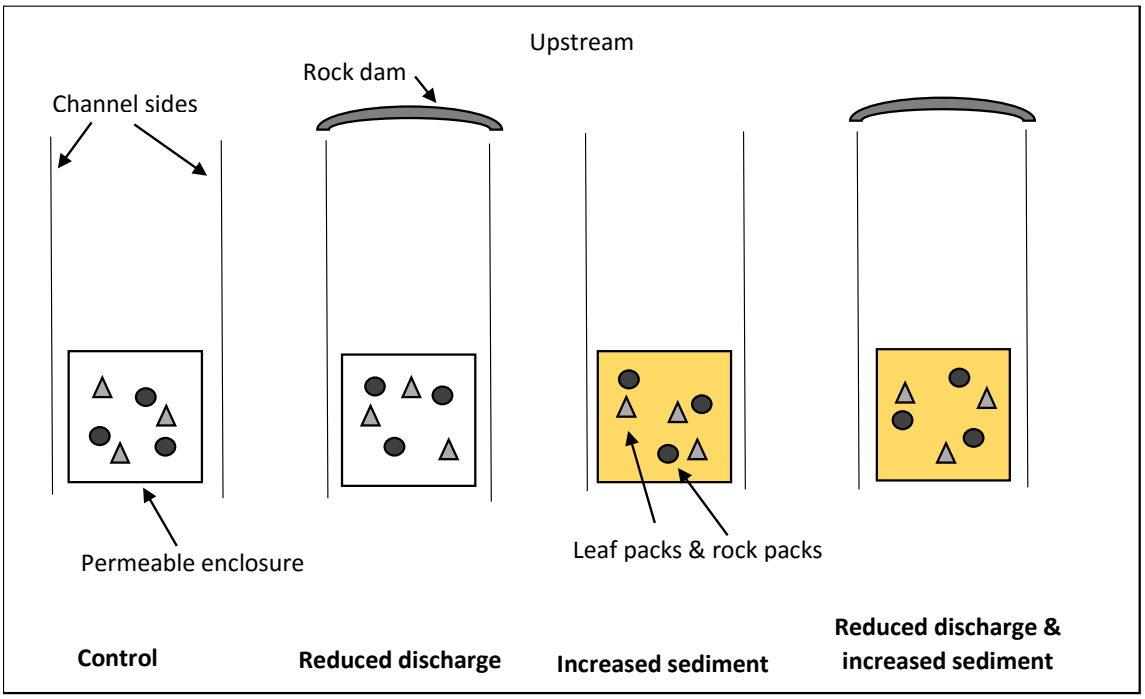

Figure 2.1: In-stream channel factorial, treatment design. Tan shaded experimental enclosures represents a sediment addition treatment.

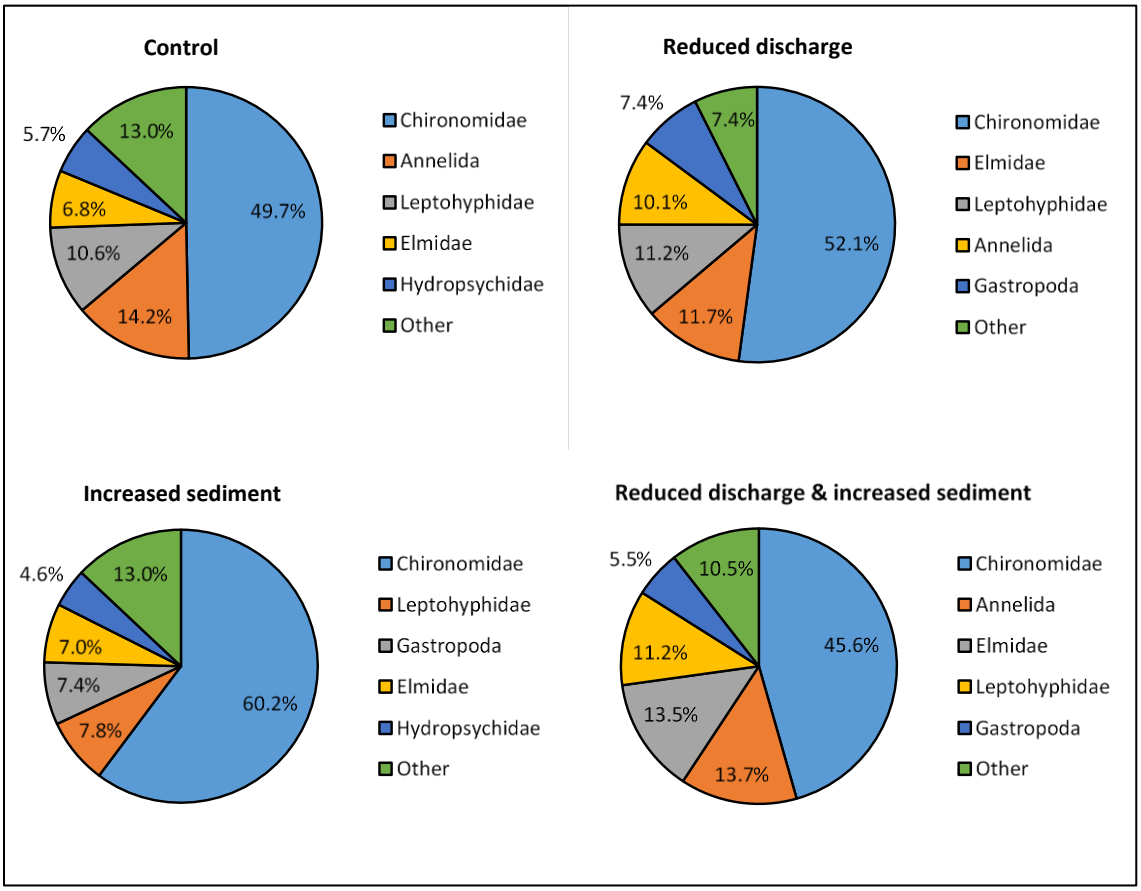

Figure 2.2: Leaf pack community taxa percent abundances by treatment group. Top left: control, top right: reduced discharge, bottom left: increased sediment, bottom right: reduced discharge & increased sediment. “Other” taxa include any taxa that were not included in the top five abundances in the leaf pack communities.

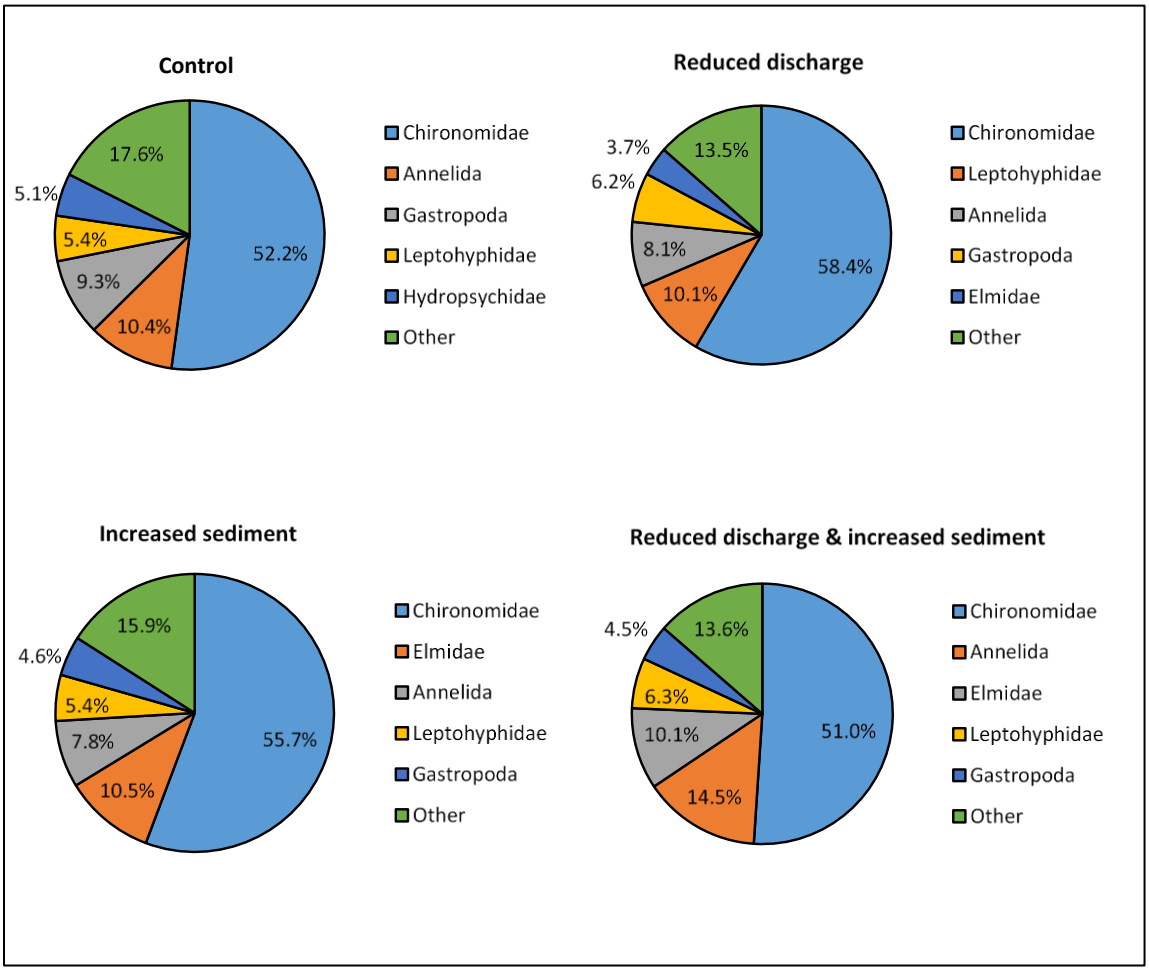

Figure 2.3: Rock pack community taxa percent abundances by treatment group. Top left: control, top right: reduced discharge, bottom left: increased sediment, bottom right: reduced discharge & increased sediment. “Other” taxa include any taxa that were not included in the top five abundances in the rock pack communities.

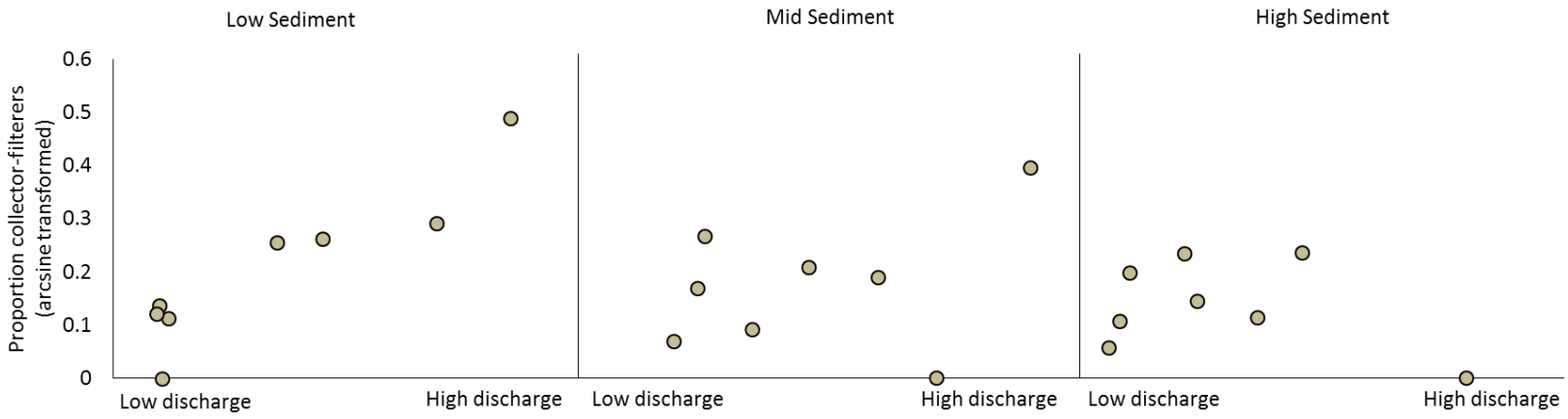

Figure 2.4: Scatterplots of the influence of discharge and sediment on the proportion of collector-filterers in rock pack communities.

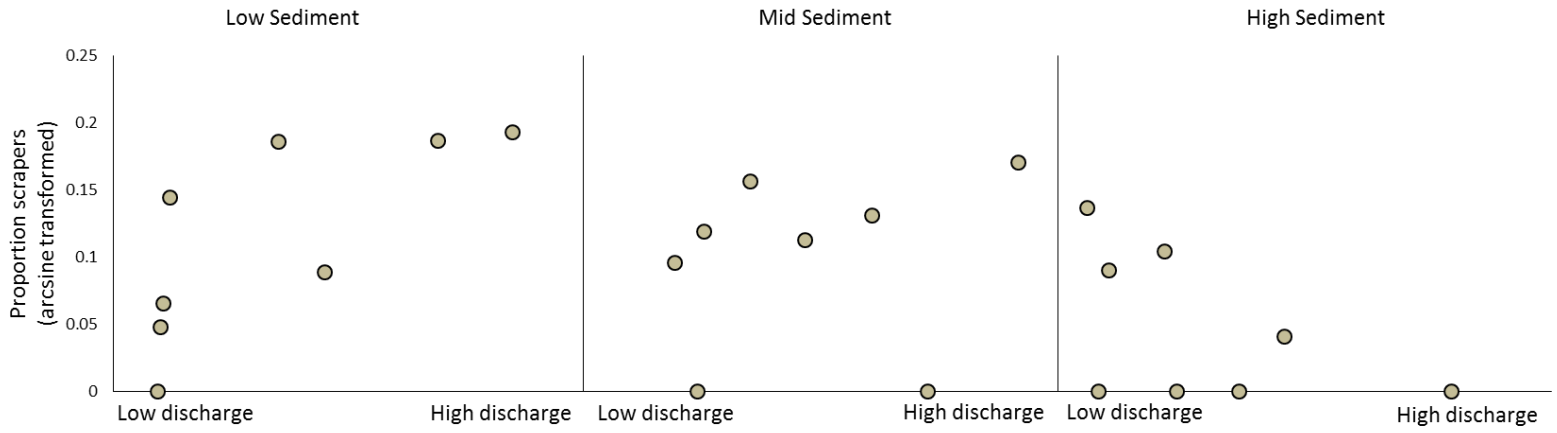

Figure 2.5: Scatterplots of the influence of discharge and sediment on the proportion of scrapers in rock pack communities.

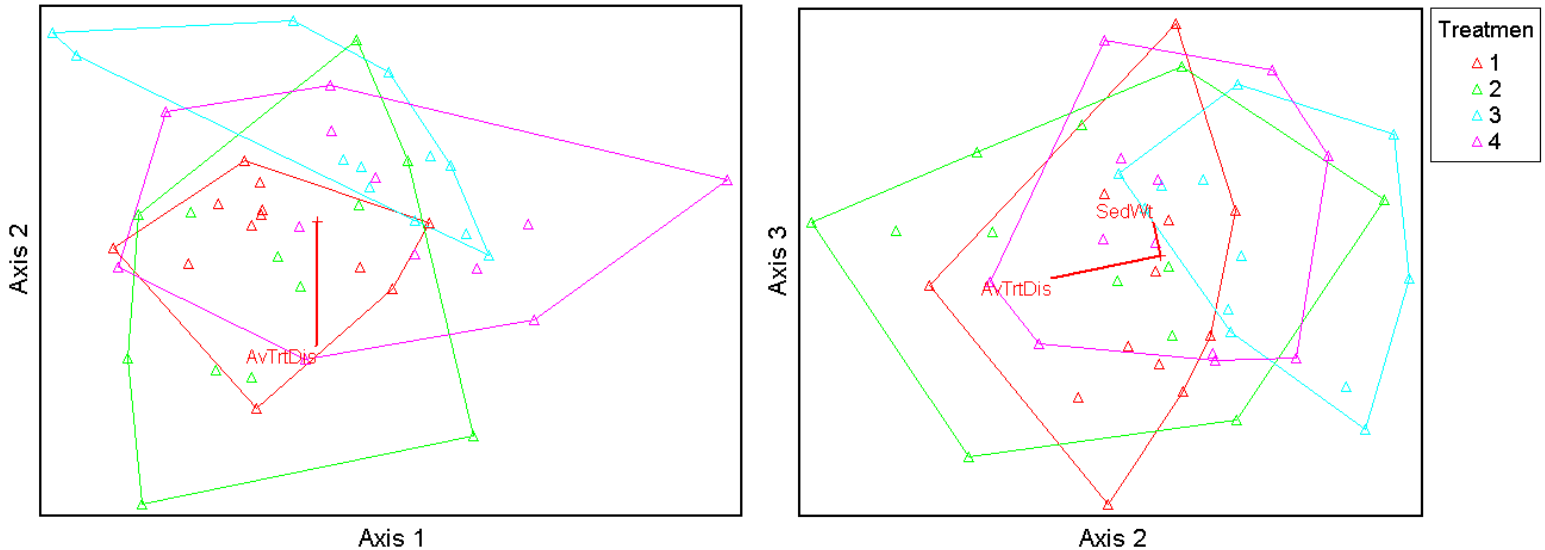

Figure 2.6: NMS ordination graph of communities based on treatment group. Treatment 1 = control, treatment 2 = increased sediment, treatment 3 = reduced discharge, treatment 4 = reduced discharge & increased sediment.

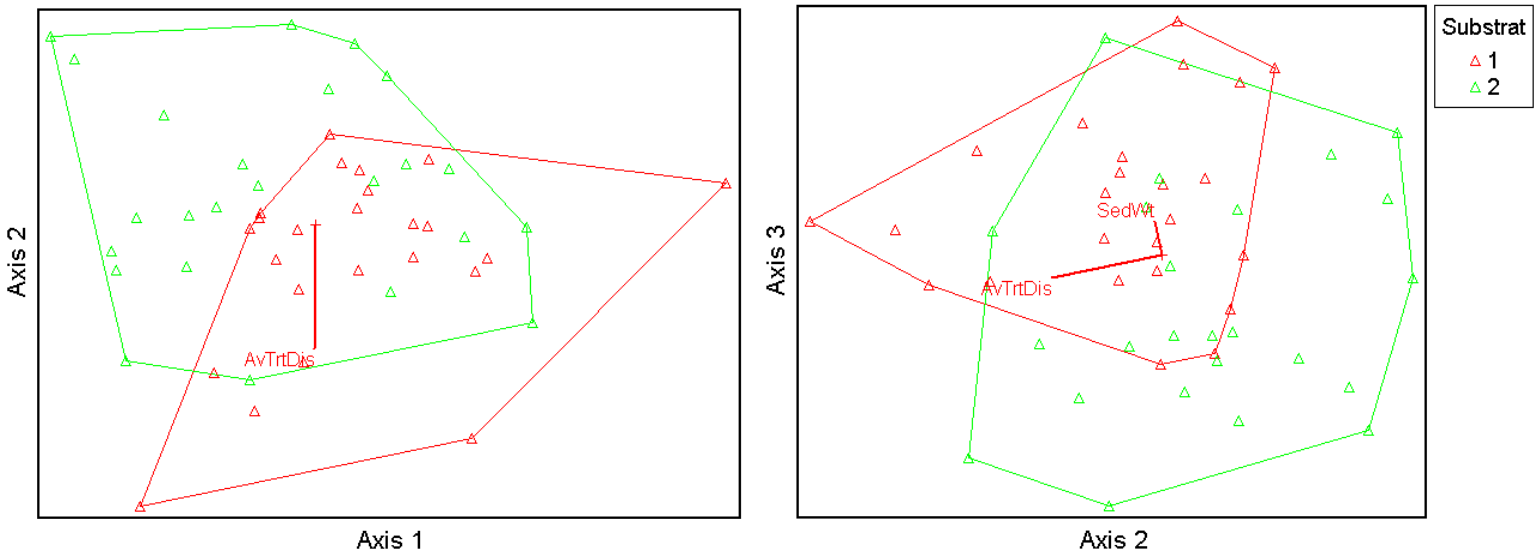

Figure 2.7: NMS ordination graph of communities based on substrate. Substrate 1 = leaf pack, treatment 2 = rock pack.

Table 2.1: Highest and lowest values of average post-treatment discharge and sample sediment weights for treatment groups. Ranges based on raw values.

Treatment	**Lowest discharge (m^3/s)**	**Highest discharge (m^3/s)**	**Lowest sediment weight (g)**	**Highest sediment weight (g)**
Control	0.008	0.059	0.07	0.34
Reduced discharge	0.006	0.050	0	0.77
Increased sediment	0.004	0.063	1.77	105.21
Reduced discharge & increased sediment	0.006	0.056	3.40	203.44

Table 2.2: Descriptions of community functional traits used in multiple linear regression analysis (from Merritt et al. 2008).

Community functional trait	**Description**
Gills	Includes aquatic invertebrates that rely on gills for respiration.
Burrower	Includes aquatic invertebrates that have adaptations for living in fine sediment.
Climber	Includes aquatic invertebrates that have adaptations for living on aquatic plants or detritus and are able to move vertically on stem surfaces.
Clinger	Includes aquatic invertebrates that have adaptations for attaching to surfaces in riffle habitat.
Collector-gatherer	Includes aquatic invertebrates that decompose fine particulate organic matter (FPOM) by feeding on deposited material and surface films.
Collector-filterer	Includes aquatic invertebrates that decompose fine particulate organic matter (FPOM) through filter feeding.
Scraper	Includes aquatic invertebrates that consume periphyton by scraping it off of substrate surfaces.
Predator	Includes aquatic invertebrates that consume living animal tissue through engulfing whole animals or piercing prey.

Table 2.3. Community metrics calculated for rock and leaf packs.

Community Metric	**Description**
Assemblage Tolerance Index (ATI)	An index based on the sum of taxa tolerance values weighted by taxon abundance values (Wittier & Van Sickle 2010)
Total abundance	The sum of the total number of individuals from every taxa of aquatic invertebrates.
EPT taxa abundance	The sum of the total number of individuals from EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa.
% EPT taxa	The percentage of the sum of the individuals from EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa out of the total abundance.
Total richness	The number of different taxa of aquatic invertebrates.
EPT taxa richness	The number of different EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa.
Diversity (Shannon's Diversity Index)	A diversity index based on the proportional abundances of taxa (Magurran 1988)

Table 2.4: Multiple linear regression results of the influence of discharge and sediment on leaf pack invertebrate communities. Response variables were transformed when appropriate. The Benjamini-Hochberg procedure was applied with a false discovery rate of 0.20.

Response variable	Explanatory variables	Coefficient estimate	Std Err	t value	P-value	Multiple R^2 value	P-value
Community Metrics							
ATI	Discharge	0.037	0.256	0.145	0.886	0.023	0.786
	Sediment	-0.005	0.007	-0.666	0.513		
Total Abundance	Discharge	-931.910	1630.190	-0.572	0.574	0.060	0.521
	Sediment	43.630	46.020	0.948	0.354		
EPT Taxa Abundance	Discharge	48.809	25.100	1.945	0.065	0.153	0.174
	Sediment	0.242	0.709	0.342	0.736		
% EPT Taxa	Discharge	260.650	92.536	2.817	**0.010 ***	0.279	**0.032***
	Sediment	-0.418	2.612	-0.160	0.874		
Total Taxa Richness	Discharge	26.218	25.598	1.024	0.317	0.049	0.589
	Sediment	0.213	0.723	0.294	0.771		
EPT Taxa Richness	Discharge	27.947	20.086	1.391	0.179	0.086	0.391
	Sediment	0.176	0.567	0.310	0.760		
Diversity	Discharge	9.258	3.467	2.671	**0.014 ***	0.258	**0.043***
	Sediment	-0.016	0.098	-0.160	0.874		
Functional Traits							
Gills	Discharge	-1.099	0.795	-1.383	0.181	0.162	0.156
	Sediment	-0.036	0.022	-1.596	0.125		
Burrowers	Discharge	-3.123	1.665	-1.875	0.075	0.144	0.197
	Sediment	-0.010	0.047	-0.220	0.828		
Climbers	Discharge	2.330	1.172	1.988	0.060	0.163	0.155
	Sediment	-0.005	0.033	-0.153	0.880		
Clingers	Discharge	2.771	1.330	2.083	**0.050 ***	0.173	0.137
	Sediment	0.016	0.038	0.414	0.683		
Collector-gatherers	Discharge	-4.690	1.552	-3.023	**0.007 ****	0.309	**0.021***
	Sediment	0.010	0.044	0.218	0.830		
Collector-filterers	Discharge	4.672	1.424	3.281	**0.004 ****	0.348	**0.011***
	Sediment	-0.014	0.040	-0.347	0.732		
Scrapers	Discharge	-0.049	0.034	-1.415	0.172	0.089	0.376
	Sediment	0.000	0.001	0.083	0.934		
Predators	Discharge	0.537	1.098	0.489	0.630	0.017	0.838
	Sediment	-0.009	0.031	-0.293	0.773		

Table 2.5: Multiple linear regression results of the influence of discharge and sediment on rock pack invertebrate communities. Response variables were transformed when appropriate. The Benjamini-Hochberg procedure was applied with a false discovery rate of 0.20.

Response variable	Explanatory variables	Coefficient estimate	Std Err	t value	P-value	Multiple R^2 value	P-value
Community Metrics							
ATI	Discharge Sediment	0.189 0.000	0.259 0.006	0.731 0.049	0.473 0.962	0.025	0.766
Total Abundance	Discharge Sediment	-2.014 0.052	2.166 0.050	-0.930 1.027	0.363 0.316	0.097	0.342
EPT Taxa Abundance	Discharge Sediment	32.890 -0.777	298.865 6.963	0.110 -0.112	0.913 0.912	0.001	0.986
% EPT Taxa	Discharge Sediment	125.790 -3.575	79.405 1.850	1.584 -1.933	0.128 **0.067 .**	0.259	**0.043***
Total Taxa Richness	Discharge Sediment	0.741 -0.994	36.309 0.846	0.020 -1.175	0.984 0.253	0.063	0.503
EPT Taxa Richness	Discharge Sediment	-2.579 -1.097	24.503 0.571	-0.105 -1.921	0.917 0.068	0.151	0.180
Diversity	Discharge Sediment	6.270 -0.088	3.503 0.082	1.790 -1.076	0.088 0.294	0.194	0.104
Functional Traits							
Gills	Discharge Sediment	-0.444 -0.065	1.005 0.023	-0.442 -2.763	0.663 **0.012 ***	0.267	**0.039***
Burrowers	Discharge Sediment	-1.714 -0.006	1.240 0.029	-1.382 -0.205	0.181 0.839	0.083	0.401
Climbers	Discharge Sediment	-0.025 -0.006	0.374 0.009	-0.067 -0.631	0.948 0.535	0.019	0.821
Clingers	Discharge Sediment	1.797 0.012	1.125 0.026	1.597 0.465	0.125 0.647	0.110	0.293
Collector-gatherers	Discharge Sediment	-1.663 0.027	0.734 0.017	-2.266 1.577	**0.034 *** 0.130	0.298	**0.024***
Collector-filterers	Discharge Sediment Discharge *Sediment	5.073 0.059 -3.571	1.494 0.043 1.495	3.395 1.365 -2.389	**0.003 **** 0.187 **0.027 ***	0.396	**0.016***
Scrapers	Discharge Sediment Discharge *Sediment	2.057 0.012 -1.943	0.887 0.026 0.888	2.318 0.481 -2.189	**0.031 *** 0.636 **0.041 ***	0.360	**0.028***
Predators	Discharge Sediment	-0.394 -0.033	0.761 0.018	-0.518 -1.872	0.610 0.075	0.145	0.193

Table 2.6: Pearson correlations with ordination axis 2, $p < 0.05$.

Phylum	Class	Order	Family	Lifestage	Axis 2 r-value
Annelida				Adult	-0.398
Arthropoda	Arachnida: Acari			Adult	-0.379
Arthropoda	Insecta	Coleoptera	Elmidae	Larvae	-0.321
Arthropoda	Insecta	Ephemeroptera	Baetidae	Larvae	-0.443
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	Larvae	0.398
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Larvae	0.478
Arthropoda	Insecta	Ephemeroptera		Larvae	-0.475
Arthropoda	Insecta	Megaloptera	Corydalidae	Larvae	-0.402
Arthropoda	Insecta	Odonata	Coenagrionidae	Larvae	0.358
Arthropoda	Insecta	Plecoptera	Perlodidae	Larvae	0.536
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Larvae	-0.427
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Larvae	-0.468
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Pupae	-0.3
Arthropoda	Insecta	Trichoptera		Larvae	-0.607

Table 2.7: Pearson correlations with ordination axis 3, $p < 0.05$.

Phylum	Class	Order	Family	Lifestage	Axis 3 r-value
Arthropoda	Insecta	Coleoptera	Elmidae	Larvae	0.442
Arthropoda	Insecta	Diptera	Chironomidae	Larvae	0.545
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Larvae	-0.499
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	Larvae	0.689
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Larvae	-0.626
Arthropoda	Insecta			Pupae	0.397

Table 2.8: Post-treatment indicator taxa in treatment groups

Treatment	**Taxa**	**Life stage**	**Indicator value**	**P-value**
Control	*NA*			
Increased sediment	Hydroptilidae	Larvae	31.2	0.090
	Unknown trichoptera	Larvae	33.0	0.042
Reduced discharge	*NA*			
Increased sediment & reduced discharge	Elmidae	Larvae	28.4	0.090

Table 2.9: Post-treatment indicator taxa in leaf and rock packs

Type	**Taxon**	**Life stage**	**Indicator value**	**P-value**
Leaf pack	Elmidae	Larvae	55.2	0.014
	Leptohyphidae	Larvae	54	0.002
Rock pack	Crambidae	Larvae	55.8	0.008
	Heptageniidae	Larvae	47.8	0.001
	Leptophlebiidae	Larvae	68.2	<0.001
	Perlodidae	Larvae	44.8	0.016
	Unknown trichoptera	Larvae	25	0.021

CHAPTER 3 - The Impact of Multiple Stressors on the Behavior and Abundance of Stonefly Nymphs (Family: Perlodidae) in the Umatilla River

ABSTRACT

Rivers impacted by human activities can have multiple stressors present. The effects of multiple stressors on aquatic communities can often be difficult to predict, due to the potential for complex interactions between stressors. This study explored the impacts of two stressors often associated with agricultural land use, increased sediment and reduced discharge, and their impacts on the abundance and behavior of a taxa of stonefly nymph (family: Perlodidae, genus *Isoperla*). This study allowed for the analysis of both independent and interactive effects of these two stressors on stonefly nymph behavior and abundance.

Movement (i.e. crawling and drift behavior) was assessed for stonefly nymphs (family: Perlodidae, genus *Isoperla*) to determine how increased sediment and reduced discharge impacted habitat selection in a laboratory experiment. Further, abundances of Perlodidae nymphs found in experimental channels in the Umatilla River were also assessed to test how these two stressors may impact their distribution in a natural setting. Increased sediment influenced *Isoperla* movement, with more nymphs moving off of habitat subjected to added sediment compared to habitat without sediment in the laboratory. Increased sediment had a significant negative influence on Perlodidae absolute and percent abundance in the Umatilla River. Reduced discharge was found to have a positive influence on Perlodidae absolute abundance. This experiment was

designed in a way to relate multiple stressors to behavior of *Isoperla* nymphs, in order to understand a possible mechanism behind nymph abundances in the Umatilla River.

INTRODUCTION

Human land use activities, including agriculture, urbanization, and forestry have negatively impacted river systems worldwide, threatening freshwater biodiversity and water resources (Strayer & Dudgeon 2010; Vorosmarty et al. 2010). Two common agricultural impacts to river systems are fine sediment input and surface water withdrawal for irrigation (Allan 2004). Agriculture irrigation using water abstracted from rivers can decrease discharge, and field and bank erosion can result in increased sediment levels (Oki and Kanae 2006; Waters 1995). River systems can be impacted by multiple stressors at one time. Studies have shown experimental evidence that increased fine sediment and reduced water flow often have interactive effects (Matthaei et al. 2010). More information is needed on multiple stressors linked to agriculture to better inform land managers of possible environmental risks associated with land use

Sediment addition is known to negatively impact aquatic organisms, including benthic invertebrates (Waters 1995). Changes caused by increased sediment levels in rivers include increased turbidity, and increased scouring and abrasion of benthic substrate, which result in decreased periphyton and biofilms on benthic substrate, decreased primary production and bottom-up effects on food webs (Allan 2004). Increased sediment can fill interstitial spaces between rocks, which eliminates habitat for some taxa of macroinvertebrates (Allan 2004). Suspended sediments can impair

respiration in benthic invertebrates with gills, due to both damage by abrasion and buildup of sediment in the gills (Jones et al. 2011; Waters 1995).

Periods of low discharge are natural for many stream systems, but human activities that artificially create or extend periods of low discharge may have negative consequences for aquatic communities (Dewson et al. 2007). River habitats can be altered by both increased sediment and decreased water discharge, and changes to river habitat are considered the most significant threat to biodiversity and ecosystem function in rivers impacted by human activities (Allan & Castillo 2007).

Aquatic invertebrates are used more often than any other group to study the health of streams and rivers through biomonitoring (Friberg et al 2010), due to the wide range of sensitivities to environmental variables (Rosenburg and Resh 1993) and their strong links to local habitats (Cummins et al 2008). Many studies have investigated community responses to stressors, including the impact of stressors on abundances of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa. Studies have shown negative outcomes for measures of EPT taxa in communities subjected to elevated levels of sediment in both field studies (Niyogi et al. 2007; Zweig & Rabeni 2001; Angradi 1999) and experimental sediment manipulations (Matthaei et al. 2006; Matthaei et al. 2010; Elbrecht et al. 2016).

Studies have also investigated the impacts of stressors on individual taxa of aquatic invertebrates. For example, Scherr et al. (2010) found that a taxa of mayfly (*Epeorus albertae*) was significantly influenced in both growth and drift behavior by elevated temperatures. Others have investigated individual taxa of stoneflies in laboratory experiments, and their results showed that nymphs select substrate in areas with faster water velocity and with larger or heterogeneous substrates both in field and

laboratory assessments (Feltmate et al. 1986). The use of laboratory studies allows for better understanding of the impacts of stressors on individual taxa within communities, which may help us better understand the mechanisms influencing taxa abundances found in nature.

This study explored how two stressors, increased sediment and reduced discharge, impacted stonefly nymphs (family Perlodidae, genus *Isoperla*) in both behavior and in abundance within river communities. A laboratory experiment was designed to determine if *Isoperla* stonefly nymphs would exhibit differences in movement behavior (i.e., crawling and/or drifting) when exposed to reduced discharge and increased sediment coverage, as independent and interacting stressors. This experiment was designed in a way to relate these stressors to behavior, rather than community responses, in order to get a closer look at the mechanisms of one of the sensitive taxa found in the Umatilla River. The findings of the laboratory experiment were compared to the findings of an experiment conducted in the Umatilla River in which reduced discharge and increased sediment were manipulated to study their impacts on macroinvertebrate communities, including abundance of Perlodidae nymphs. It was predicted that the two stressors (sediment addition and reduced water discharge), as both independent and interacting stressors, would result in increased movement of *Isoperla* nymphs in search of suitable habitat. It was also predicted that these two independent and interacting stressors would result in negative impacts on Perlodidae abundance in the Umatilla River.

METHODS

Stonefly movement behavior laboratory experiment

Isoperla (family: Perlodidae) stonefly nymphs were collected from the lower mainstem Umatilla River in early December 2014. Stoneflies were transported back to the lab in coolers with river water and kept in a cooled room (approximately 7-10ºC) and kept in containers (dimensions 38.1 x 21.6 x 13.3 cm) for 4-5 days until the experiment began. Tank bottoms were covered with river stones and gravel and equipped with air pumps and air stones to maintain water circulation and oxygen levels in the water. Stoneflies were given other aquatic invertebrates (such as caddisflies and mayflies) as available prey, along with algae disks and aquatic plant debris as a supplemental food source.

Experimental drift tanks were constructed to simulate river conditions with different treatments of discharge and sediment (Figure 3.1). Drift tanks were constructed out of 37.8 liter aquariums with a horizontal Plexiglas platform 12.7 cm from the bottom. A vertical Plexiglas platform was fixed to the upstream side of the horizontal platform. A barrier of screen material was fixed to the downstream edge of the horizontal platform and the bottom and sides of the aquarium. The horizontal platform was covered in river stones and gravel. Water pumps connected to rubber hose and PVC pipes allowed for circulating water flow within the tank.

To test the impacts of sediment and discharge on stonefly nymph behavior, four different treatment tanks were created (Table 3.1). The four treatments consisted of a control treatment, an increased sediment treatment, a reduced discharge treatment, and a treatment with both increased sediment and reduced discharge. These tanks were created

as an extension to the factorial design of the field experiment in the Umatilla River (see Chapter 2). Sediment added treatments consisted of approximately 80% of the river stones and gravel substrate covered with sediment that had been sieved to remove particles < 500µm to prevent water cloudiness from suspended fine sediments, in order to more accurately observe movement behavior. Discharge treatments were achieved by using two different kinds of pumps. For the low flow treatment, a 227.1 liters/hour pump was used. For the high flow treatment, a 1059.9 liters/hour pump was used. Pumps were connected to 1.3 cm diameter PVC with holes drilled into a horizontal arm stretched across the width of the aquarium, to achieve a uniform current across the width of the tanks. The water depths above the platform of all tanks were kept equal (2.4 cm), but the water velocity was different between treatments to alter the experimental discharge. The water velocity of the low flow pumps were tested and was approximately 0.123 m/s, with a discharge of 0.0007 m/s. The water velocity of the high flow pumps was approximately 0.291 m/s, with a discharge of 0.0018 m/s. This resulted in a velocity and discharge reduction from high flow to low flow pumps of 57.8%.

Ten individuals were used together for each experimental replicate. Individuals were only used once in the experiment, with a total of 160 individuals used in four treatments groups, with four replicates each. Each group of 10 stonefly nymphs were introduced to a barricaded section of the experimental tanks for 10 minutes to acclimate to the environment. After 10 minutes of acclimation, the barriers were lifted and the water pumps were immediately turned on. Experimental trials ran for 10 minutes, and stonefly nymphs either stayed on the experimental platform with rocks and gravel or left the platform to an area of the tank with no substrate (only empty tank environment with

screen barrier). At the end of each trial, the stoneflies were collected and a description of where they were found in the tank was recorded (either on platform or off platform). A distinction was not made between stonefly nymphs that had crawled off the platform or drifted off the platform due to the inability to accurately see all movement behavior during the experiment. Stoneflies from the same trial were preserved together in 70% ethanol.

Stoneflies used in the trials were selected based on similar body size. Visual assessments were used to determine if there were any outliers in body size before the experiment began. Outliers were not used in trials. Handling time was minimized and no tools were used to aid in this body size assessment in order to avoid damaging the nymphs and to minimize influence on behavior. Stonefly body lengths were measured to the nearest millimeter after specimens were preserved at the conclusion of the experiment. Body lengths were measured from the distal end of the head to the distal end of the abdomen.

Data analysis of stonefly movement behavior

Two-way analysis of variance (ANOVA) was used to determine the influence of sediment, discharge, and their interaction on *Isoperla* nymph movement behavior. The two-way ANOVA model included two levels of each stressor (Table 3.1). One-way ANOVA was used to determine if there were any significant differences in the average body size of *Isoperla* nymphs used between treatments. Since this experiment ran over the course of two days, a one-way ANOVA was used to check if there were any significant differences in off-platform movement between time blocks.

Perlodidae abundance and percent composition in the Umatilla River

To study how multiple stressors would impact Perlodidae abundances in aquatic invertebrate communities in the Umatilla River, sediment and discharge levels were experimentally manipulated in in-stream channels. Experimental channels were constructed and maintained in the lower mainstem Umatilla River near the town of Echo, Oregon at river mile ~29 (45º43'29.9" N, 119º11'14.74" W) from August 1 through October 1, 2014. Perlodidae nymphs were identified and counted from aquatic invertebrate communities in both leaf packs and rock packs. A total of 24 channels were constructed with six replicates of four experimental treatments. Experimental treatments involved the manipulation of water discharge and sediment in a factorial design (Figure 3.2).

Experimental channel description:

Each channel was two meters long and one meter wide, with a permeable enclosure located at the downstream end (dimensions: 0.61 m x 0.61 m). Channels were constructed out of black plastic sheets anchored to rebar secured into the riverbed parallel to the stream flow. Enclosure frames were constructed out of 1.27 cm diameter PVC. The enclosure frames were covered with 2.54 cm diameter mesh garden fence and layered with window screen material on the bottom to keep in sediment. Each enclosure contained river rocks scrubbed free of invertebrates from the area that the channel was constructed from. Within each study block, channels were approximately three meters away from each other and were offset such that the closest upstream and downstream channels were not directly in front of each other, which was a distance tested to ensure

minimal impact of treatments from upstream channels, while staying within the study area.

Treatments were added two weeks after channels were constructed (Figure 3.2). To achieve reduced water discharge, rock dams were constructed at the upstream ends of the channels to divert water, while still allowing some water and invertebrates to flow through. The average reduction in discharge among these treatments was 74%. To create sediment addition treatments (< 2 mm grain size, Zweig & Rabeni 2001), sand was added to enclosures to cover approximately 50% of the substrate (Figure 3.2). Visual estimates were used in percent coverage, and sediment weights were recorded from sample substrates after invertebrates were removed to assess treatment effectiveness. Along with maintaining treatments throughout the study, channels were monitored 2-3 times weekly and cleared of any accumulated debris.

Each enclosure contained three rock packs and three leaf packs, which were mesh bags containing substrate for invertebrate communities to colonize. Leaf packs were composed of leaf litter from a black cottonwood tree (*Populus trichocarpa*) near the study site. Leaf litter was collected, brought back to the lab, and dried for 24 hours at 60ºC. After the leaf litter was dried, 5 g of dried weight leaf litter was placed into 32 cm x 12 cm mesh bags with openings 1cm wide. Rock packs were composed of rocks collected from each channel site and placed in 20 cm x 25 cm plastic mesh bags with 2.54 cm diameter openings. All rocks used in rock packs were scrubbed free of invertebrates and periphyton in order to allow for equal colonization opportunities throughout the study site. For post treatment community samples, the average volume of rocks used in rock packs was 48 mL for each pack, and rocks were on average 47.6 mm (±1.7 SE) in median

width. There were no significant differences in volume ($F = 0.354$, $p = 0.787$, one-way ANOVA) or median width ($F = 0.531$, $p = 0.666$, one-way ANOVA) of rocks between treatment groups.

Stressors as continuous variables:

Though stressor treatments were maintained throughout the study in order to achieve an average reduction in discharge of 74% and a 50% sediment substrate coverage, the natural variability within the Umatilla River made this difficult to do successfully. Within each treatment group, there was a range in both discharge and sediment treatments (Table 3.2). Due to the range of discharges and sediment levels achieved in this experiment, the two stressors were treated as continuous variables for all analyses rather than discrete variables with two levels.

Sampling protocol:

Four weeks after treatments were implemented in the experimental channels, rock pack and leaf pack invertebrate communities were sampled. After separating all invertebrates from their sample substrate, aquatic invertebrate samples were preserved in 95% ethanol and identified in the laboratory with a dissection microscope. Perlodidae nymphs were identified using Merritt et al. (2008) and abundances for each community were recorded.

Data analysis of Perlodidae abundance in the Umatilla River

Multiple linear regression models were used to assess how absolute abundances and percent abundances of Perlodidae stoneflies in invertebrate communities were impacted by water discharge and sediment level as independent and interacting stressors. Percent perlodidae and perlodidae absolute abundance datasets showed signs of

heteroscedasticty, so robust standard errors were calculated using the Car package in R (Fox & Weisberg 2011).

The following "full" model was used initially:

Response variable ~ Discharge + Sediment + Discharge*Sediment + ε

However, if the interaction term was not significant it was removed from the model and the data were analyzed with a "reduced" model:

Response variable ~ Discharge + Sediment + ε

The sediment explanatory variable was the log transformed sediment weight (g) collected from community samples. The discharge explanatory variable was the average discharge for each channel taken over the four weeks after treatments were implemented. R statistical software version 0.99.486 was used to run all models (R Core Team 2013). Response variable data sets were transformed when necessary in order to fit assumptions of multiple linear regression models. The α level for all models was set at 0.10.

RESULTS

There were no significant differences in the average body length of *Isoperla* nymphs among treatments ($p = 0.205$, one-way ANOVA). There was also no evidence of significant differences in off-platform movement behavior among the experimental replicates ($p = 0.172$, one-way ANOVA), indicating that there was no evidence of a change in movement behavior over time.

Isoperla nymph movement behavior

Sediment was a significant factor for *Isoperla* nymph movement behavior (Table 3.3). Sediment added treatments had significantly more off-platform movement

compared to trials with no sediment added (Figure 3.3). There were no significant impacts of discharge alone or interactions between discharge and sediment detected in this study. The treatment of increased sediment and reduced discharge had the most off-platform movement compared to the other treatments, with increased sediment having the second highest upstream movement, followed by reduced discharge and control (Figure 3.4).

Perlodidae abundances in field channels

In rock packs, both absolute and percent Perlodidae abundance were significantly and negatively related to sediment, as well as a negative influence of discharge on absolute Perlodidae abundance (Table 3.4). There were no significant models found for percent or abundance Perlodidae in leaf packs (Table 3.4).

DISCUSSION

The main objective of this experiment was to study the impact of reduced discharge and increased sediment as both independent and multiple stressors on behavior of *Isoperla* stonefly nymphs (Plecoptera: Perlodidae). Specifically, this study was designed to determine if stonefly nymphs would remain within an experimental habitat faced with these stressors or leave in search of more suitable habitat conditions. A secondary objective was to compare the findings of this laboratory experiment to an experiment conducted in the Umatilla River, in which Perlodidae abundances were analyzed according to reduced discharge and increased sediment manipulation. Sediment influenced *Isoperla* nymph movement, while both sediment and discharge influenced Perlodidae nymph abundance in the Umatilla River.

Influence of sediment

As predicted, sediment was found to be a significant factor in stonefly movement. In treatments with added sediment, a greater number of nymphs crawled or drifted off of the experimental habitat, compared with treatments with no sediment added. This increased movement off of the platform with added sediment could be interpreted as a greater number of nymphs leaving unsuitable habitat in search of better habitat elsewhere.

In the Umatilla River rock pack communities, percentage and abundance of Perlodidae had a significant negative relationship with sediment, with greater abundances in areas with low sediment and lower abundances in areas with high sediment. This finding complements the outcome of the movement behavior study. Based on the findings from both of these studies, it seems that habitat selection could be an important mechanism that influences stonefly abundance in nature.

Perlodidae stonefly nymphs are considered to be clingers, a "habit" category in which invertebrates have adaptations for attaching to surfaces in riffle habitat (Merritt et al 2008). Many taxa within the EPT group (Ephemeroptera, Plecoptera, and Trichoptera) inhabit surfaces or interstitial spaces between and beneath river stones (Waters 1995). Increased sediment can decrease the amount of available habitat for them to occupy, which is most likely the primary reason that led stoneflies to leaving the experimental habitat.

In the laboratory experiment, sediment particles smaller than 500µm were removed from the sand mixture used for added sediment treatments. This was done to reduce the cloudiness of the water in order to more accurately observe stonefly nymph

behavior. Suspended sediments can impair respiration in benthic invertebrates with gills, due to both damage by abrasion and buildup of sediment in the gills (Jones et al. 2011; Waters 1995). Perhaps if the finest grains of sediment (smaller than 500µm) were included in the experimental tanks, there may have been an even more dramatic difference in nymph movement between treatments with and without sediment added, due to detrimental effects of suspended sediment on respiration.

Many other studies have analyzed responses of EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) to sediment conditions in streams, rather than focusing specifically on responses of Plecoptera. Several of these studies have shown negative outcomes for measures of EPT taxa in communities subjected to elevated levels of sediment in both field studies (Niyogi et al. 2007; Zweig & Rabeni 2001; Angradi 1999) and experimental sediment manipulations (Matthaei et al. 2006; Matthaei et al. 2010; Elbrecht et al. 2016). Feltmate et al. (1986) found that the distribution of the stonefly nymph *Paragnetina media* (Plecoptera: Perlidae) was significantly influenced by single and interactive effects of the benthic substrate composition and water velocity in laboratory experiments, which was also consistent with what was observed in the field. Specifically, they found that a greater number of stonefly nymphs selected substrates in faster water velocity compared to slower water velocity, with the greatest differences observed in habitats with larger substrates and heterogeneous substrates.

Influence of discharge

Though there wasn't evidence found that discharge was influential for stonefly behavior in the laboratory experiment, discharge was found to be influential for Perlodidae abundance in the Umatilla River experiment in rock pack communities. In the

Umatilla River experiment, areas with lowest discharge were found to have more Perlodidae nymphs compared to areas with high discharge. This result is a bit unexpected, as it was predicted that reduced discharge would have a negative impact on stonefly abundances, since Perlodidae is considered to be a sensitive taxa (Whittier & Van Sickle 2010). The Umatilla River experimental manipulation of sediment and discharge resulted in a range of conditions for both stressors, and this may have been more helpful in revealing any patterns in distribution influenced by discharge. In comparison, the laboratory behavior experiment design only allowed for two levels of sediment and discharge treatment, and this limited range in stressor conditions may have also limited the range of behavioral impacts.

There are results from other studies that suggest that discharge is an important influence on Perlodidae abundance. For example, in the stressor manipulation study in the Umatilla River (Chapter 2), Perlodidae abundance was found to be negatively associated with discharge. Other studies have shown that reduced water velocity was associated with negative responses of EPT taxa (Miller et al. 2007; Elbrecht et al. 2016).

Stressor interactions

This study did not show any evidence that interactions between sediment and discharge had significant impacts on stonefly behavior in the laboratory or abundance in the field experiment. However, other studies have shown that there may be important impacts from multiple interacting stressors on EPT taxa in general. For example, Matthaei et al. (2010) found that stream flow and sediment interacted to influence EPT richness. They found that at normal flow, EPT richness increased with increased sediment, leveled out at intermediate sediment levels, and declined at high levels of

sediment. At reduced flow, they found that EPT richness declined steadily with increasing sediment.

Comparison of leaf pack and rock pack communities

There were several significant relationships found between stressors and response variables related to Perlodidae abundance in rock packs in the Umatilla River channel stressor experiment. However, there were no significant relationships found between sediment and/or discharge or any significant interactions between these stressors and Perlodidae abundance in leaf packs. There were more Perlodidae nymphs found in rock packs (1.71 ± 0.38) compared to leaf packs (0.67 ± 0.21). The populations of Perlodidae nymphs occupying leaf packs may have been so small that any pressures from stressors were inconsequential for Perlodidae abundance. As discussed previously, many stonefly nymphs rely on interstitial spaces between rocks for habitat. The interstitial spaces between leaves in the leaf packs may not have been adequate habitat for nymphs, leading to lower abundances.

Conclusion

This laboratory experiment tested how two common stressors found in streams (reduced discharge and increased sediment) impacted the behavior of a stonefly nymph, and found that sediment has an important impact on movement, which may be important for habitat selection. The same family of stonefly (family Perlodidae) was also found to be sensitive to both sediment and discharge in manipulative stressor experiments in the Umatilla River. The main implication of this study is that increased levels of sediment in rivers can negatively influence sensitive taxa, as seen in Perlodidae stonefly nymph movement and abundances. While this study highlights the influence of increased

sediment as a stressor for one particular taxa, it is important to keep in mind that in nature, there are often multiple stressors that influence entire communities. Continued study of multiple stressors in rivers will help us to better understand how to manage stressors to reduce the number of negative effects in river communities. In addition, continued use of laboratory studies may allow us to better understand the impacts on individual taxa within communities and may help us understand the mechanisms driving taxa abundances found in nature.

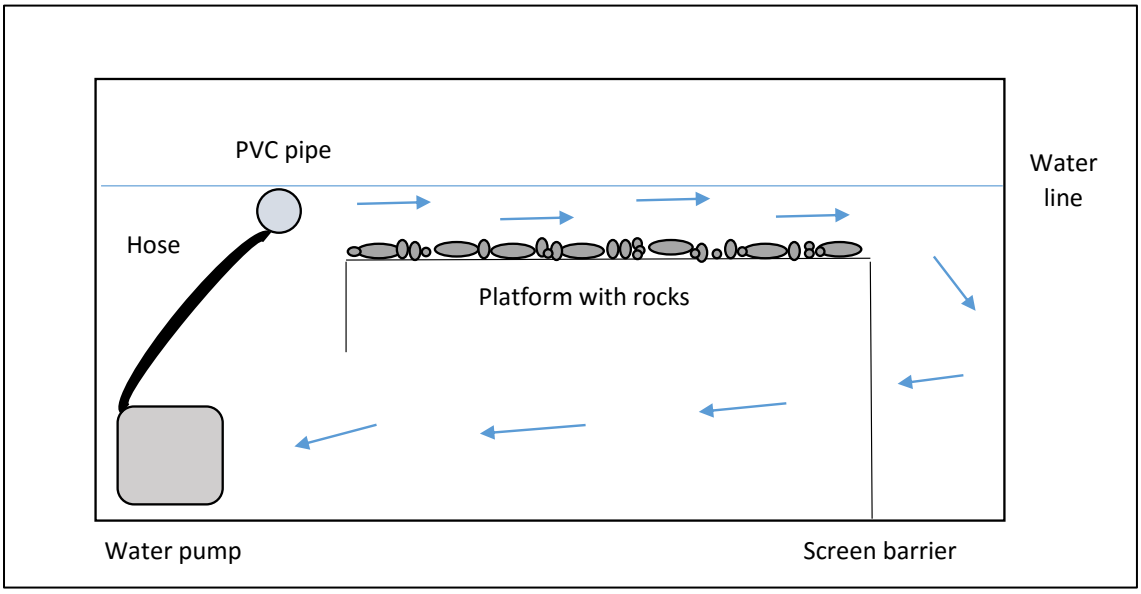

Figure 3.1: Diagram of tank design for *Isoperla* nymph movement behavior experiment. Arrows refer to direction of water flow.

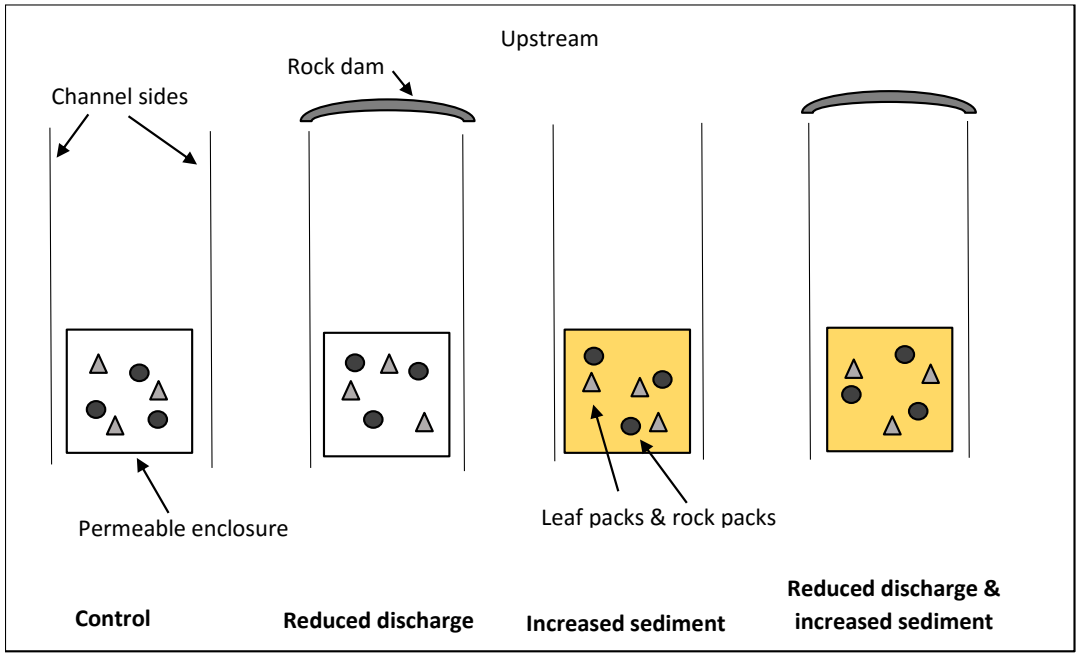

Figure 3.2: In-stream channel treatment design. Tan shaded experimental enclosures represents a sediment addition treatment.

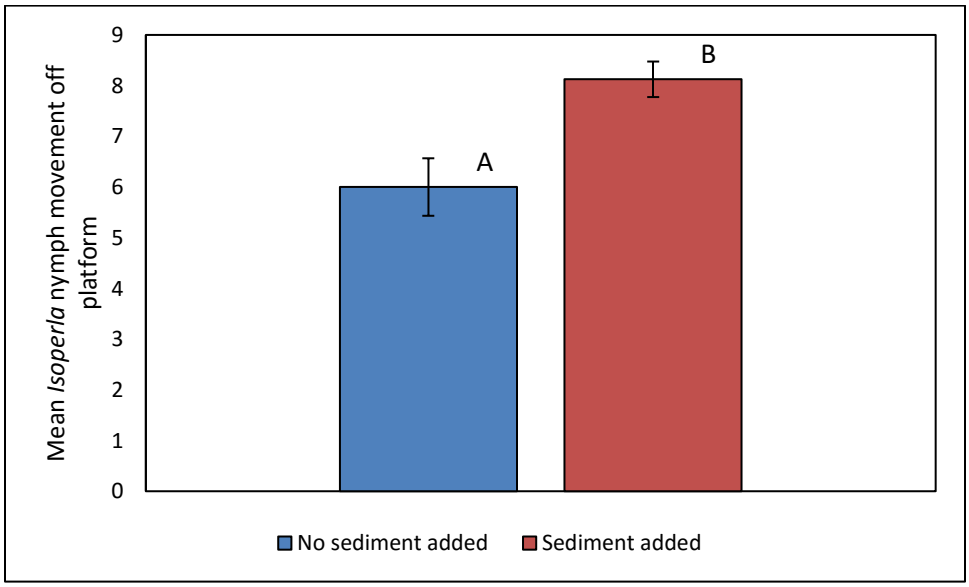

Figure 3.3: Bar chart of all off-platform movement of *Isoperla* nymphs in treatments with no sediment added and treatments with sediment added. Error bars are standard error. Different letters indicate significant differences in nymph movement (two-way ANOVA).

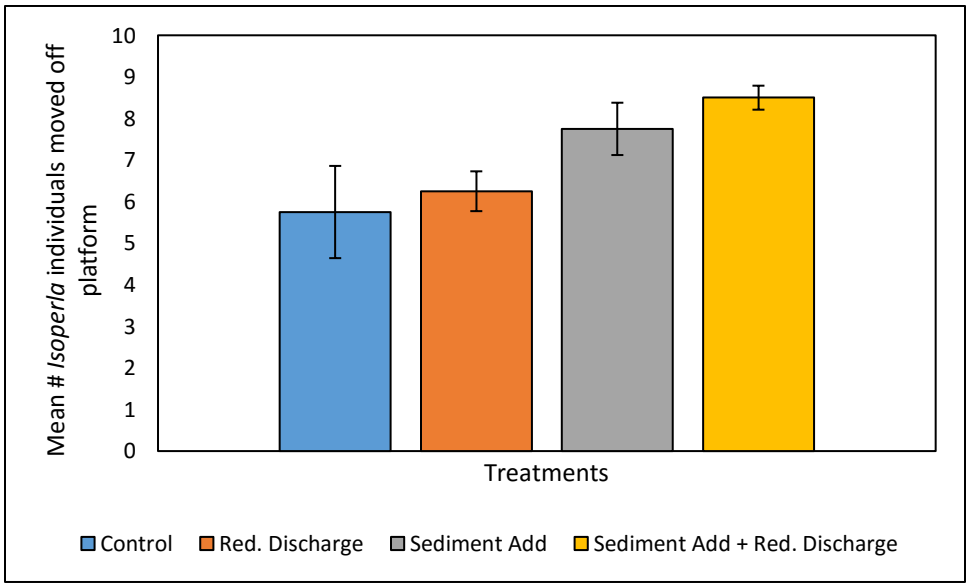

Figure 3.4: Bar graph of off-platform movement of *Isoperla* stonefly nymphs in all four treatments (control, reduced discharge, sediment added, and sediment added & reduced discharge). Error bars are standard error.

Table 3.1: Experimental treatments testing influence of sediment and discharge on *Isoperla* nymph movement behavior

Treatment	Sediment coverage	Water discharge
Control	None	0.0018 m^3/s
Sediment added	80% substrate coverage	0.0018 m^3/s
Reduced discharge	None	0.0007 m^3/s
Sediment added & reduced discharge	80% substrate coverage	0.0007 m^3/s

Table 3.2: Highest and lowest values of average post-treatment discharge and sample sediment weights for treatment groups. Ranges based on raw values.

Treatment	Lowest discharge (m^3/s)	Highest discharge (m^3/s)	Lowest sediment weight (g)	Highest sediment weight (g)
Control	0.008	0.059	0.07	0.34
Reduced discharge	0.006	0.050	0	0.77
Increased sediment	0.004	0.063	1.77	105.21
Reduced discharge & increased sediment	0.006	0.056	3.40	203.44

Table 3.3: Two-way ANOVA table of *Isoperla* off-platform movement by discharge, sediment, and interaction between discharge and sediment.

Factor	DF	Sum Sq	Mean Sq	F-value	Pr(>F)
Discharge	1	1.562	1.562	0.806	0.387
Sediment	1	18.062	18.062	9.323	**0.010***
Discharge*Sediment	1	0.062	0.062	0.032	0.860
Residuals	12	23.250	1.937		

Table 3.4: Multiple linear regression results of the influence of discharge and sediment on Perlodidae stonefly percentage and abundance in rock pack and leaf pack communities. Response variable datasets were transformed when appropriate.

Response variable	Explanatory variables	Coefficient estimate	Std Err	t value	P-value	Multiple R^2 value	P-value
Rock pack communities							
% Perlodidae	Discharge	-5.698	3.712	-1.535	0.140	0.254	**0.0456***
	Sediment	-0.194	0.085	-2.288	**0.033 ***		
Perlodidae abundance	Discharge	-11.590	6.019	-1.812	**0.084 .**	0.247	**0.051 .**
	Sediment	-0.317	0.131	-2.276	**0.033 ***		
Leaf pack communities							
% Perlodidae	Discharge	2.046	2.889	0.708	0.457	0.041	0.643
	Sediment	-0.046	0.082	-5.562	0.580		
Perlodidae abundance	Discharge	4.810	12.164	0.395	0.697	0.034	0.694
	Sediment	-0.249	0.343	-0.724	0.477		

CHAPTER 4 – General Conclusions

There are often multiple stressors that impact a river ecosystem at once, and these multiple stressors sometimes have complex interactions with aquatic communities, and the effects of interacting stressors can be difficult to predict (Townsend et al. 2008). The studies presented in Chapter two and Chapter three show different methods of investigating how two stressors (increased sediment and reduced discharge) impact aquatic invertebrate communities. The study design and analyses of these experiments allowed for testing reduced discharge and increased sediment as both independent and interacting stressors, in order to better understand these complex relationships.

CHAPTER TWO

The stressor manipulation experiment in the Umatilla River showed evidence that discharge and sediment, as both independent and interacting stressors, have important impacts on aquatic invertebrate communities. While both increased sediment and decreased discharge had similar consequences for some community response variables, these two stressors caused additional and different impacts to other community response variables. This study gave evidence that each stressor is independently important in these macroinvertebrate communities, and there is a greater impact on the community when both of these stressors are present.

Both increased sediment and reduced discharge had negative consequences for EPT taxa, which includes sensitive bioindicator species, signaling that both of these stressors may reduce stream health. These two stressors also changed functional feeding group proportions within the aquatic invertebrate communities. This indicates that

increased sediment and reduced discharge could change the ways that stream communities function.

The presence of complex stressor interactions in this study highlighted the need to continue studying multiple stressors in rivers. In this study, both scrapers and collector-filterers were significantly impacted by interactions between sediment and discharge. These complex relationships are important to keep in mind from a management standpoint. Restoring natural flows to a river may help restore some balance to some functional groups of the community, but for others this depends on how much sediment is in the river.

CHAPTER THREE

This laboratory experiment tested how reduced discharge and increased sediment impacted the behavior of a stonefly nymph, and found that sediment has an important impact on movement behavior, which may be important for habitat selection. The same family of stonefly (family Perlodidae) was also found to be influenced by sediment and discharge levels in manipulative stressor experiments in the Umatilla River. This paired behavior laboratory experiment and field manipulation study revealed a possibly important mechanisms of how Perlodidae stonefly nymphs are impacted by these two stressors.

The main implication of this study is that increased levels of sediment in rivers can reduce abundances of sensitive taxa, as seen in Perlodidae stonefly nymphs. While this study highlights the influence of increased sediment as a stressor for one particular taxa, it is important to keep in mind that in nature, there are often multiple stressors that

influence entire communities. Continued study of multiple stressors in rivers will help us to better understand how to manage stressors to reduce the number of negative effects in river communities. In addition, continued use of laboratory studies may allow us to better understand the impacts on individual taxa within communities and may help us understand the mechanisms driving taxa abundances found in nature.

MANAGEMENT IMPLICATIONS AND FUTURE STUDY

Other researchers have warned land managers that removing water from a river with high sediment levels may have more severe consequences for the aquatic invertebrate communities compared to abstracting water from rivers with relatively low sediment levels (Matthaei et al 2010). Managers should keep this in mind if they are able to choose from different river sources for irrigation water. The results of the studies covered in chapter two and chapter three show evidence that these stressors have important influences independently and through complex interactions on many aspects of aquatic invertebrate communities in the Umatilla River.

Though there is natural variability in discharge and sediment in river systems, there are important ecological consequences when human land use alters these variables from their natural conditions. Restoration planning should focus on restoring natural flows and managing sediment levels in streams to more closely match natural conditions in order to support a healthy ecosystem. Increasing our understanding of the impacts of single and interacting stressors can allow for more informed management decisions in the future.

LITERATURE CITED

Allan, J.D., M.M. Castillo. 2007. Stream ecology: structure and function of running waters. Second Edition. Springer Science & Business Media.

Allan J.D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics 35: 257-284.

Angrati, T.R. 1999. Fine sediment and macroinvertebrate assemblages in Appalachian streams: a field experiment with biomonitoring applications. Journal of North American Benthological Society 18(1): 49-66.

Baker, D.W., B.P. Bledsoe, C.M. Albano, N.L. Poff. 2011. Downstream effects of diversion dams on sediment and hydraulic conditions of Rocky Mountain streams. River Research and Applications 27: 388-401.

Benjamini Y. & Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society B, 57: 289-300.

Biggs, B.J.F., M.E. Close. 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshwater Biology 22: 209-231.

Bond, N.R., B.J. Downes. 2003. The independent and interactive effects of fine sediment and flow on benthic invertebrate communities characteristic of small upland streams. Freshwater Biology 48: 455-465.

Cummins K.W., R.W. Merritt, M.B. Berg. 2008. Ecology and distribution of aquatic insects. Pp 105-122. In Merritt R.W, K.W. Cummins, M.B. Berg (eds). An introduction to the aquatic insects of North America. Kendall Hunt Publishing Company. 1158 pp.

Dewson, Z.S., A.B.W. James, R.D. Death. 2007. A review of the consequences of decreased flow for instream habitat and macroinvertebrates. Journal of American Benthological Society 26(3): 401-415.

Dufrene, M., P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67(3): 345-366.

Elbrecht, V., A.J. Beerman, G. Goessler, J. Neumann, R. Tollrian, R. Wagner, A. Wlecklik, J.J. Piggott, C.D. Matthaei, F. Leese. 2016. Multiple-stressor effects on stream invertebrates: a mesocosm experiment manipulating nutrients, fine sediment and flow velocity. Freshwater Biology 61: 362-375.

Feltmate, B.W., R.L. Baker, P.J. Pointing. 1986. Distribution of the stonefly nymph Paragnetina media (Plecoptera: Perlidae): influence of prey, predators, current speed, and

substrate compostition. Canadian Journal of Fisheries and Aquatic Sciences 43: 1582-1587.

Fox, J. and S. Weisberg (2011). An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Friberg N., J. Skriver, S.E. Larsen, M.L. Pedersen, A. Buffagni. 2010. Stream macroinvertebrate occurrence along gradients in organic pollution and eutrophication. Freshwater Biology 55: 1405-1419.

Georgian, T., J.T. Thorp. 1992. Effects of microhabitat selection on feeding rates of net-spinning caddisfly larvae. Ecology 73(1): 229-240.

Gleick P.H. 2003. Water Use. Annual Review of Environmental Resources 28: 275-314.

Harrell, F.E. 2015. Regression modeling strategies: with applications to linear models, logistic and ordinal regression, and survival analysis, 2nd ed. Springer International Publishing, Switzerland.

Hart, D.D. 1992. Community organization in streams: the importance of species interactions, physical factors and chance. Oecologia 91: 220-228.

Hitley, J. & A. Merenlender. 2000. Faunal indicator taxa selection for monitoring ecosystem health. Biological Conservation, 192: 85-197.

James, A.B.W., A. Suren. 2009. The response of invertebrates to a gradient of flow reduction - an instream channel study in a New Zealand lowland river. Freshwater Biology 54: 2225-2242.

Jones, J.I., J.F. Murphy, A.L. Collins, D.A. Sear, P.S. Naden, P.D. Armitage. 2011. The impact of fine sediment on macro-invertebrates. River Research and Applications. DOI: 10.1002/rra.1516.

Lake, P.S. 2000. Disturbance, patchiness, and diversity in streams. Journal of North American Benthological Society 19(4): 573-592.

Lange, K.A., C.R. Townsend, C.D. Matthaei. 2014. Can biological traits of stream invertebrates help disentangle the effects of multiple stressors in an agricultural catchment? Freshwater Biology 59: 2431-2446.

Magbanua F.S., C.R. Townsend, K.J. Hageman, C.D. Matthaei. 2013. Individual and combined effects of fine sediment and the herbicide glyphosate on benthic macroinvertebrates and stream ecosystem function. Freshwater Biology 58: 1729-1744.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, New Jersey.

Matthaei, C.D., F. Weller, D.W. Kelly, C.R. Townsend. 2006. Impacts of fine sediment addition to tussock, pasture, and deer farming streams in New Zealand. Freshwater Biology 51: 2154-2172.

Matthaei C. D., J.J. Piggott, C.R. Townsend. 2010. Multiple stressors in agricultural systems: interactions among sediment addition, nutrient enrichment and water abstraction. Journal of Applied Ecology 47: 639 -649.

McCune, B., J.B. Grace. 2002. Analysis of ecological communities. MjM Software, Gleneden Beach, Oregon, USA.

McCune, B. and M. J. Mefford. 2011. PC-ORD. Multivariate Analysis of Ecological Data. Version 6. MjM Software, Gleneden Beach, Oregon, U.S.A.

McDonald, J.H. 2014. Handbook of Biological Statistics (3rd ed.). Sparky House Publishing, Baltimore, Maryland.

MEA (Millennium Ecosystem Assessment). (2003). Ecosystems and human well-being: a framework for assessment. Washington DC: Island Press.

Merritt R.W., Cummins K.W., and Berg M.B. 2008. An Introduction to the Aquatic Insects of North America. Dubuque, Iowa: Kendall-Hunt.

Miller, S.W., D. Wooster, J. Li. 2007. Resistance and resilience of macroinvertebrates to irrigation water withdrawals. Freshwater Biology 52: 2494-2510.

Moore A.A., M.A Palmer. 2005. Invertebrate biodiverstiy in agricultural and urban headwater streams: implications for conservation and management. Ecological Applications 15(4): 1169-1177.

Nickerson, C., R. Ebel, A. Borchers, F. Carriazo. 2011. Major Uses of Land in the United States, 2007, EIB-89, U.S. Department of Agriculture, Economic Research Service.

Niyogi D.V., M. Koren, C.J. Arbuckle. 2007. Stream communities along a catchment land-use gradient: subsidy-stress responses to pastoral development. Environmental Management 39: 213-225.

Northwest Power and Conservation Council. “Umatilla/Willow Subbasin Plan.” In: Columbia River Basin Fish and Wildlife Program. Portland, Oregon, 2004.

Oki, T. and S. Kanae. 2006. Global hydrologic cycles and world water resources. Science 313:1068-1072.

Piggott J.J., K. Lange, C.R. Townsend, C.D. Matthaei. 2012. Multiple stressors in agricultural streams: a mesocosm study of interactions among raised water temperature, sediment addition, and nutrient enrichment. Plos One 7(11): e49873.

Poff, N.L. 1992. Why disturbances can be predictable: a perspective on the definition of disturbance in streams. Journal of the North American Benthological Society 11(1): 86-92.

Poff, N.L., T. Wellnitz, J.B. Monroe. 2003. Redundancy among three herbivorous insects across an experimental current velocity gradient. Oecologia 134: 262-269.

Poff, N.L, J.D. Olden, N.K.M. Vieira, D.S. Finn, M.P. Simmons, and B.C. Kondratieff. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society, 25(4): 730-755.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Resh, V.H., A.V. Brown, A.P. Covich, M.E. Gurtz, H.W. Li, G.W. Minshall, S.R. Reice, A.L. Sheldon, J.B. Wallace, R.C. Wissmar. 1988. The role of disturbance in stream ecology. Journal of the North American Benthological Society 7(4): 433-455.

Rosenberg D.M., V.H. Resh. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates, pp. 1-9. In Rosenberg D.M. and V.H. Resh (eds). Freshwater biomonitoring and benthic macroinvertebrates. Routeledge, Chapman and Hall Inc., New York. 488 pp.

Scherr, M.A., D.E. Wooster, S. Rao. 2010. Effects of temperature on growth rate and behavior of Epeorus albertae (Ephemeroptera: Heptageniidae) nymphs. Physiological Ecology 39(6): 2017-2024.

Statzner, B., L.A. Beche. 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biology 55: 80-119.

Strayer, D.L., D. Dudgeon. 2010. Freshwater biodiversity conservation: recent progress and future challenges. Journal of the North American Benthological Society 29: 344-358.

Sutherland, A.B., J.L. Meyer, E.P. Gardiner. 2002. Effects of land cover on sediment regime and fish assemblage structure in four southern Appalachian streams. Freshwater Biology 47: 1791-1805.

Thorp, J.H. and A.P. Covich (eds.). 2010. Ecology and Classification of North American Freshwater Invertebrates, 3rd ed. Academic Press, San Francisco, CA, USA.

Townsend C.R., S.S. Uhlmann, C.D. Matthaei. 2008. Individual and combined responses of stream ecosystems to multiple stressors. Journal of Applied Ecology 45: 1810-1819.

Vorosmarty, C.J., P.B. McIntyre, M.O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S.E. Bunn, C.A. Sullivan, C.R. Liermann, P.M. Davies. 2010. Global threats to human water security and river biodiversity. Nature 467: 555-561.

Wagenhoff A., C.R. Townsend, N. Phillips, C.D. Matthaei. 2011. Subsidy-stress and multiple stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. Freshwater Biology 56: 1916-1936.

Wagenhoff A., C.R. Townsend, C.D. Matthaei. 2012. Macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients: a stream mesocosm experiment. Journal of Applied Ecology 49: 892-902.

Waite, T.A. & L.G. Campbell. 2006. Controlling the false discovery rate and increasing statistical power in ecological studies. Ecoscience, 13(4): 439-442.

Waters, T.F. 1965. Interpretation of invertebrate drift in streams. Ecology 46: 327-334.

Waters T.F. 1995. Sediment in streams: sources, biological effects and control. American Fisheries Society Monograph 7. American Fisheries Society, Bethesda, Maryland.

Webb, J.A., K.A. Miller, E.L. King, S.C. Little, M.J. Stewardson, J.K. Zimmerman, N.L. Poff. 2013. Squeezing the most out of existing literature: a systematic re-analysis of published evidence on ecological responses to altered flows. Freshwater Biology 58: 2439-2451.

Whittier, T.R., and J. Van Sickle. 2010. Macroinvertebrate tolerance values and an assemblage tolerance index (ATI) for western USA streams and rivers. Journal of the North American Benthological Society, 29(3): 852-866.

Wood, P.J., P.D. Armitage. 1997. Biological effects of fine sediment in the lotic environment. Environmental Management 21(2): 203-217.

Zweig, L.D., C.F. Rabeni. 2001. Biomonitoring for deposited sediment using benthic invertebrates: a test on 4 Missouri streams. Journal of the North American Benthological Society 20(4): 643-657.

APPENDIX

This appendix provides additional analyses that were completed as part the experiment that was completed investigating the impacts of reduced discharge and increased sediment on aquatic invertebrate communities in the Umatilla River. Analysis of study blocks included the decomposition rate of leaves from the leaf packs, temperature between groups, and analysis of invertebrate communities based on study block. Other analyses included the influence of the rock dams on invertebrate drift, the similarity of the communities found in the experimental channels to that found in nearby un-manipulated areas of the river, and the influence of reduced discharge and increased sediment on a taxa of caddisfly (family: Hydropsychidae). In addition, an analysis of the influence of discharge on pre-treatment samples of channel communities was completed.

LEAF DECOMPOSITION IN LEAF PACKS

Methods

After removing invertebrates, leaf litter from leaf packs was dried at 60ºC for 24 hours and weighed. Percent leaf litter decomposition was calculated based on dried weight of leaf litter in leaf packs before placing them in the stream and the dry weight of leaf litter remaining in leaf packs after picking out invertebrates. Leaf decomposition between groups was analyzed using one-way ANOVA and Tukey's HSD.

Results

There were no significant differences in leaf pack decomposition in pre-treatment samples by treatment or study block (Table A1). In post-treatment samples, there was a significant difference in leaf pack decomposition by study block ($p = 0.0001$, one-way

ANOVA), but no significant difference by treatment (Table A1 & A2). In post-treatment leaf packs, block 3 ($p=0.003$, Tukey HSD) and block 2 ($p < 0.001$, Tukey HSD) had significantly greater decomposition than block 1.

TEMPERATURE ANALYSIS

There were no significant differences in mean temperature or mean weekly high temperature between channel types or study blocks (one-way ANOVA; Table A3).

STUDY BLOCK COMMUNITY STRUCTURE

Multi-Response Permutation Procedures (MRPP) showed evidence that invertebrate communities differed by study block ($A = 0.049$, $p < 0.001$). Pairwise comparisons show significant differences between block 1 and block 2 ($p = 0.021$), block 1 and block 3 ($p < 0.001$), and block 2 and block 3 ($p = 0.001$).

COMPARING CHANNEL INVERTEBRATE COMMUNITIES TO NATURAL COMMUNITIES

Methods

Benthic aquatic invertebrate samples were collected in September 2014 in areas within the study reach but outside of channels. These samples were used to assess if the aquatic invertebrate communities found within the channel samples were consistent with natural communities outside the channels. Kick nets were used to collect invertebrates while agitating and scrubbing rocks in the streambed to dislodge invertebrates. Invertebrates were collected from areas of each study block in deeper regions of the river

and shallower regions near the bank. Invertebrate samples were stored in 95% ethanol. Subsamples of each benthic sample were identified, with at least 300 individuals identified per sample. The community structure of the benthic and control samples were compared using Sorenson's Distance.

Results

There were detectible differences between the invertebrate communities collected in the benthic samples and the invertebrate communities collected from the experimental channel samples (Figure A1). However, there were still many similarities among the dominant taxa in these invertebrate communities (Table A4).

INFLUENCE OF ROCK DAMS

Methods

Rock dams not only reduced discharge, but they also had the potential to act as a physical barrier to drifting invertebrates. In order to assess if there were any significant impacts from dams on the abundances of drifting invertebrates and on the channel invertebrate community abundances, drift samples were collected. Drift samples were collected in September 2014 in each of 24 channel and at nine locations within the study reach outside of the channels at areas of low discharge. Drift nets were 1m long with 30cm opening and with 500µm mesh. Drift samples were collected at sunrise for one hour, with a start time a half hour before sunrise and ending a half hour after sunrise.

Multiple linear regression models were used to determine if drift abundance and channel community abundance were influenced by the presence of dams and if there were any interactions between dams and discharge.

A “full” model was developed and used initially on the data:

Response variable ~ Discharge + Dam + Discharge*Dam + ε

As with the other regression models, if the interaction term was not significant it was dropped and the following “reduced” model was used:

Response variable ~ Discharge + Dam + ε

Discharge values used in this analysis were calculated from day-of drift sampling.

Results

A significant model was found for drift invertebrate abundance, with a positive relationship with discharge, negative relationship with the presence of dams, and positive relationship with the interaction between discharge and dams (Table A5; Figure A2). However, this didn’t translate to a direct impact on the abundance of invertebrates in the channels. There were no significant models found for the effect of discharge and dams for leaf pack and rock pack invertebrate abundance (Table A5; Figures A3 & A4). Even though the rock dam treatments effectively reduced channel discharge by approximately 75%, channels with dams still had a wide range of discharges and abundances, comparable to channels without dams (Figures A3 & A4).

HYDROPSYCHIDAE IN CHANNEL COMMUNITIES

Methods

Abundances of Hydropsychidae caddisflies were recorded from community data from leaf packs and rock packs sampled from the Umatilla River. Hydropsychidae caddisflies were selected for analysis due to a preliminary analysis that showed that this taxa may have been sensitive to treatments. Multiple linear regression models were used

to assess how community metrics and functional traits were impacted by water discharge and sediment level as independent and interacting stressors. The following "full" model was used initially:

Response variable ~ Discharge + Sediment + Discharge*Sediment + ε

However, if the interaction term was not significant it was removed from the model and the data were analyzed with a "reduced" model:

Response variable ~ Discharge + Sediment + ε

Datasets for percent Hydropsychidae showed signs of heteroscedasticity, so robust standard errors were calculated using the Car package in R (Fox & Weisberg 2011). The sediment explanatory variable was the log transformed sediment weight (g) collected from community samples. The discharge explanatory variable was the average discharge for each channel taken over the four weeks after treatments were implemented. R statistical software, version 0.99.486 was used to run all models (R Core Team 2013). Response variable data sets were transformed when necessary in order to fit assumptions of multiple linear regression models.

Results

In rock packs, percent Hydropsychidae had a significant positive relationship with discharge and a significant interaction between sediment and discharge (Table A6; Figure A5). In leaf packs, percent Hydropsychidae had a significant positive relationship with discharge. In leaf packs, a significant model was also found for Hydropsychidae abundance, with a significant positive relationship with discharge (Table A6).

THE INFLUENCE OF PRE-TREATMENT DISCHARGE ON AQUATIC INVERTEBRATE COMMUNITIES

Methods

Simple linear regression models were used to determine the impact of discharge on pre-treatment aquatic invertebrate communities in the experimental channels.

Response variable ~ Discharge + ε

The discharge explanatory variable was the average discharge from each channel during the pre-treatment period of the experiment. R statistical software, version 0.99.486 was used to run all models (R Core Team 2013). Response variable data sets were transformed when necessary in order to fit assumptions of multiple linear regression models.

Results

For rock pack community metrics, there was a significant positive relationship between pre-treatment discharge and % EPT taxa and diversity, and a significant negative relationship between pre-treatment discharge and assemblage tolerance index (ATI) (Table A7). For rock pack functional traits, there was a significant negative relationship between the proportion of burrowers and pre-treatment discharge, while the proportion of clingers had a significant positive relationship with pre-treatment discharge (Table A7). There were no significant models found for the relationship between pre-treatment discharge and community metrics or functional traits in leaf pack communities (Table A8).

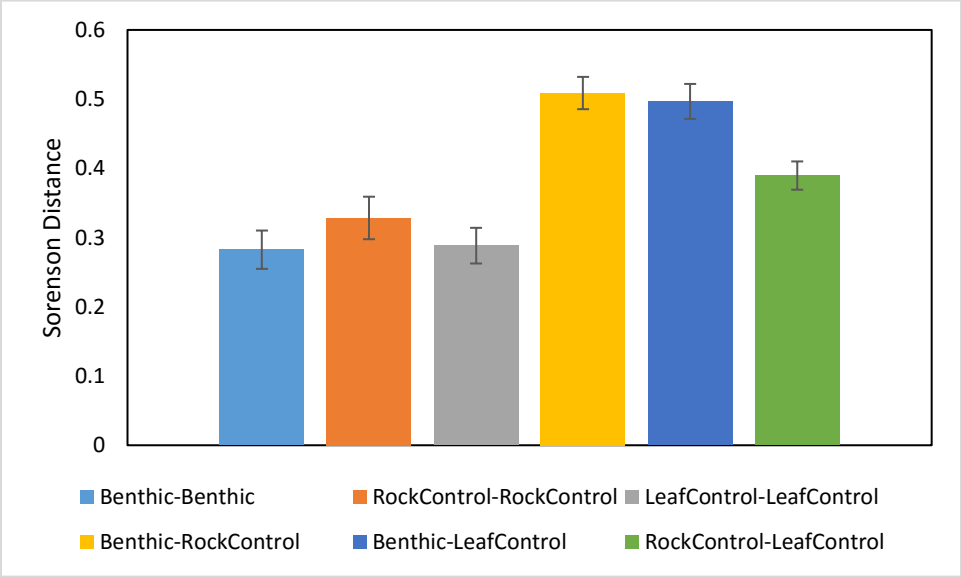

Figure A1: Sorenson distance values comparing benthic samples to post-treatment control samples (leaf packs and rock packs).

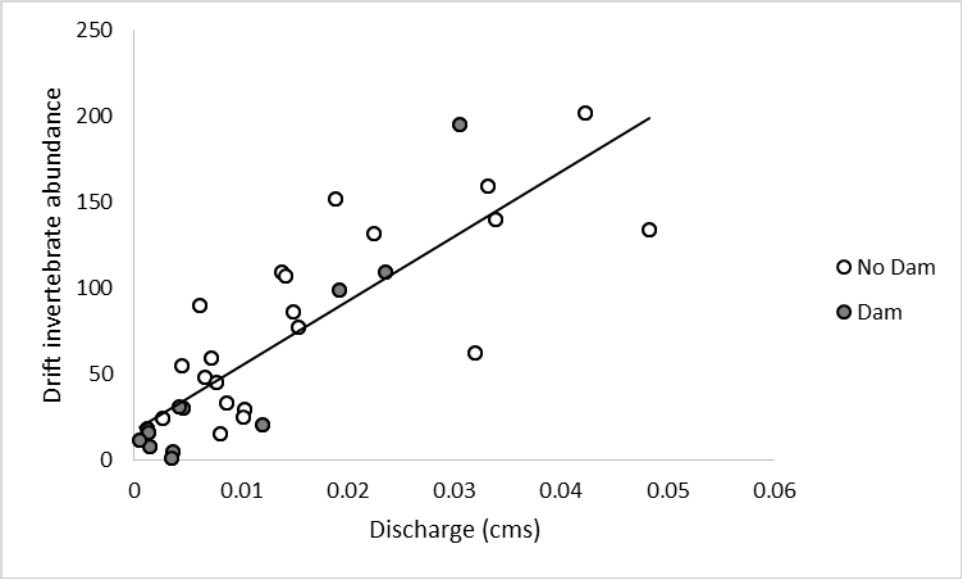

Figure A2: Relationship between drift invertebrate abundance and discharge. Points coded according to whether dam was added (dark grey) or not (white).

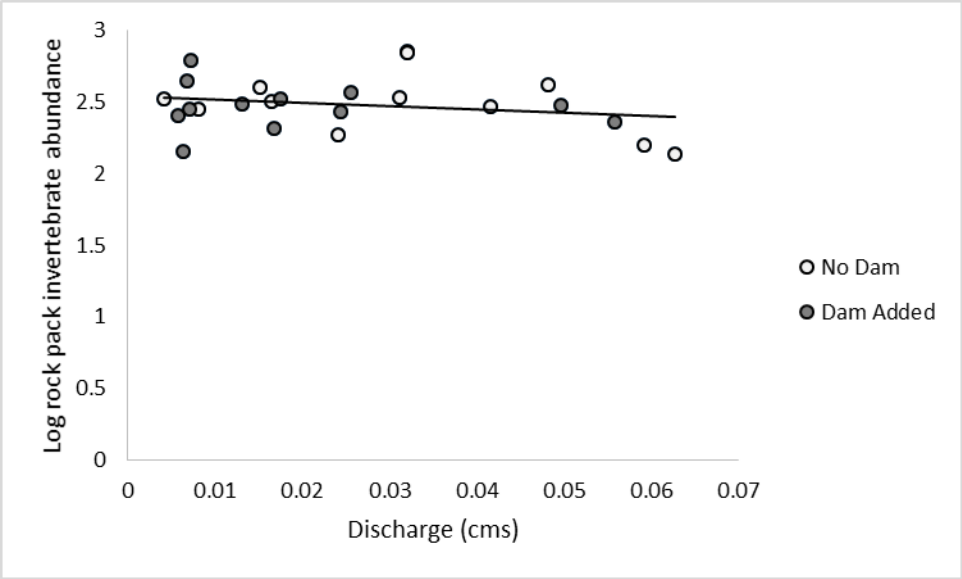

Figure A3: Relationship between post-treatment rock pack invertebrate abundance (log transformed) and discharge (cms). Points coded according to whether dam was added (dark grey) or not (white).

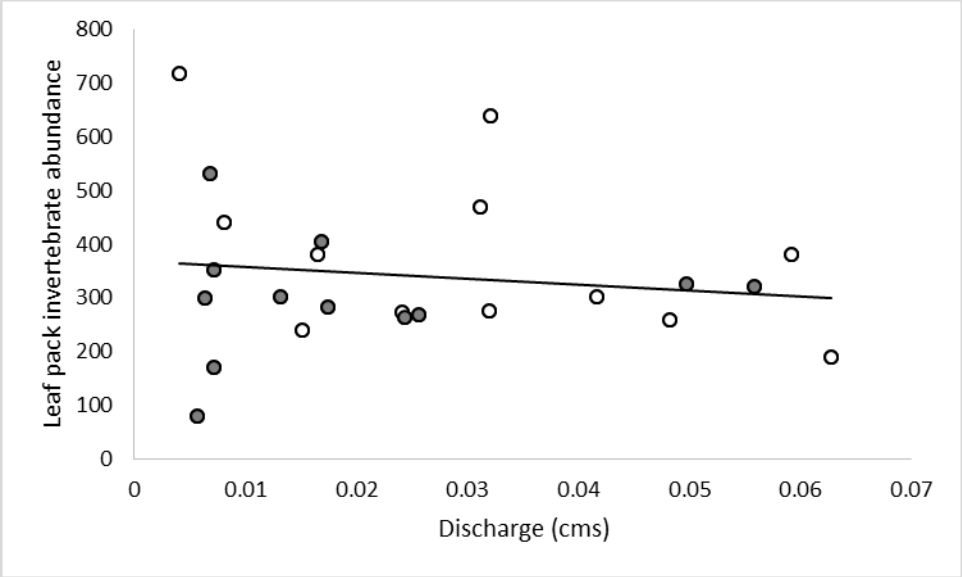

Figure A4: Relationship between post-treatment leaf pack invertebrate abundance and discharge (cms). Points coded according to whether dam was added (dark grey) or not (white).

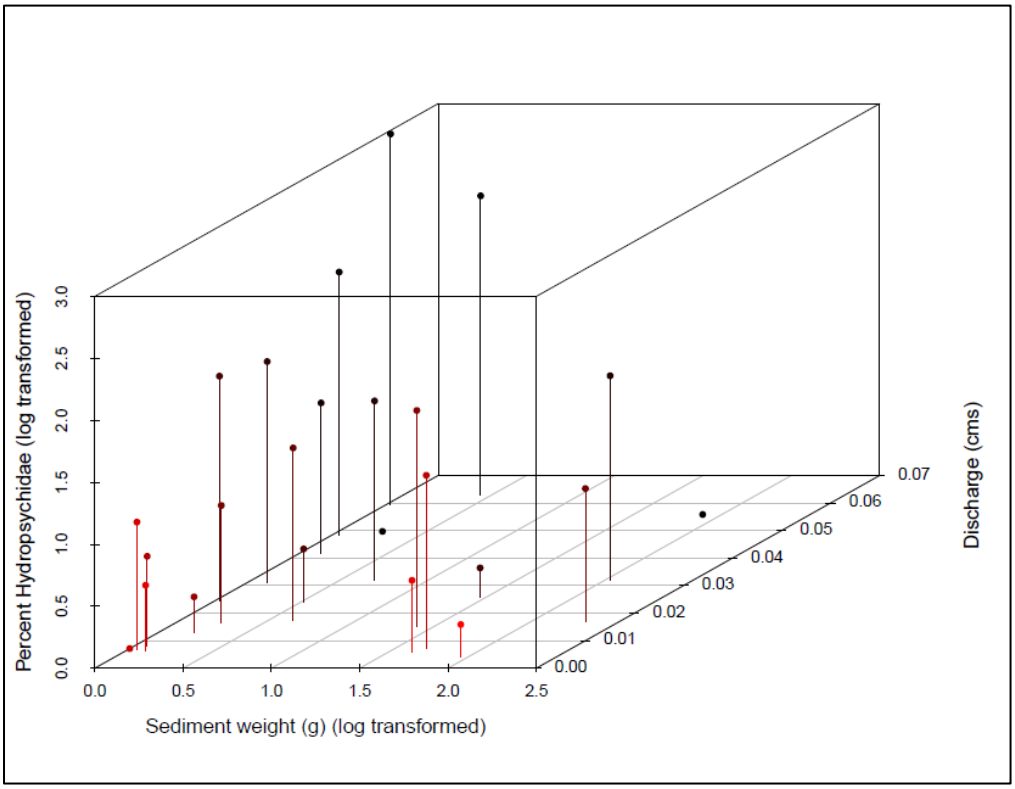

Figure A5: 3D scatterplot of the relationship between percent Hydropsychidae and the interaction between discharge and sediment in rock pack communities. Percentages of Hydropsychidae and sample sediment weights were log transformed. Discharge values are calculated mean post-treatment discharges from each channel.

Table A1: Treatment decomposition organized by sampling period.

Sampling Period	Treatment	Mean Decomposition (%)	Std Err
Pre-treatment	Control	53.718	4.228
	Increased sediment	53.656	2.854
	Reduced discharge	57.056	5.331
	Increased sediment & reduced discharge	54.350	5.621
Post-treatment	Control	84.415	3.466
	Increased sediment	88.016	3.506
	Reduced discharge	80.086	5.226
	Increased sediment & reduced discharge	87.325	2.921

Table A2: Study block decomposition organized by sampling period.

Sampling Period	Block	Mean Decomposition (%)	Std Err
Pre-Treatment	1	52.684	3.089
	2	52.628	4.951
	3	58.772	3.051
Post-treatment	1	75.420[a]	3.130
	2	92.075[b]	1.819
	3	87.387[b]	1.488

Table A3: Mean temperature and mean weekly high temperature by channel type and study block.

Channel Type	Mean Temperature (ºF)	Std Err	Mean Weekly High Temperature (ºF)	Std Err
Deep no dam	63.065	0.089	68.243	0.045
Deep with dam	62.978	0.022	68.389	0.180
Shallow no dam	63.156	0.134	68.793	0.407
Shallow with dam	63.069	0.110	68.381	0.254

Study Block	Mean Temperature (ºF)	Std Err	Mean Weekly High Temperature (ºF)	Std Err
1	63.153	0.072	68.752	0.309
2	63.085	0.097	68.438	0.141
3	62.963	0.051	68.164	0.034

Table A4: Dominant taxa for benthic samples and post-treatment control leaf and rock samples.

Sample	Taxa	Life stage	Mean abundance	Std Err
Benthic	Chironomidae	Larvae	83.500	17.339
	Elmidae	Larvae	61.167	4.942
	Hydropsychidae	Larvae	43.833	14.053
	Annelida	Adult	22.667	7.796
	Oecetis	Larvae	18.500	3.566
Control Leaf	Chironomidae	Larvae	165.000	23.780
	Annelida	Adult	52.667	20.141
	Leptohyphidae	Larvae	39.333	7.787
	Elmidae	Larvae	25.000	3.066
	Hydropsychidae	Larvae	21.333	13.032
Control Rock	Chironomidae	Larvae	125.333	19.423
	Annelida	Adult	31.500	17.251
	Chironomidae	Pupae	30.500	11.766
	Gastropoda	Adult	28.167	8.264
	Leptohyphidae	larvae	16.500	4.667

Table A5: Multiple linear regression results of the influence of discharge and dams on the abundances of invertebrates in drift samples, leaf pack communities, and rock pack communities.

Response variable	Explanatory variables	Coefficient estimate	Std Err	t-value	p-value	Multiple R^2	p-value
Log drift abundance	Discharge	16.101	4.715	3.415	**0.002****	0.692	**<0.001**
	Dam present	-0.604	0.148	-4.088	**<0.001*****		
	Discharge*Dam present	28.912	9.479	3.050	**0.005****		
Leaf pack abundance	Discharge	-1986.000	1633.680	-1.216	0.238	0.143	0.197
	Dam present	-103.480	59.460	-1.740	0.096		
Log rock pack abundance	Discharge	-2.940	2.279	-1.290	0.211	0.081	0.413
	Dam present	-0.068	0.083	-0.815	0.424		

Table A6: Multiple linear regression results of the influence of discharge and sediment on Hydropsychidae caddisfly percentage and abundance in rock pack and leaf pack communities. Response variable datasets were transformed when appropriate.

Response variable	**Explanatory variables**	**Coefficient estimate**	**Std Err**	**t value**	**P-value**	**Multiple R2 value**	**P-value**
Rock pack communities							
% Hydropsychidae	Discharge	33.789	10.398	3.250	**0.004****	0.370	**0.024***
	Sediment	0.415	0.301	1.380	0.183		
	Discharge *Sediment	-23.739	10.402	-2.282	**0.034***		
Hydropsychidae abundance	Discharge	15.650	13.731	1.140	0.267	0.063	0.504
	Sediment	-0.053	0.320	-0.167	0.869		
Leaf pack communities							
% Hydropsychidae	Discharge	25.181	14.593	2.743	**0.012***	0.283	**0.030***
	Sediment	-0.157	0.293	-0.606	0.551		
Hydropsychidae abundance	Discharge	33.956	14.335	2.369	**0.028**	0.230	0.064
	Sediment	-0.236	0.405	-0.583	0.566		

Table A7: Simple linear regression results of the influence of pre-treatment discharge on rock pack aquatic invertebrate communities. Response variables were transformed when appropriate. Benjamini-Hochberg procedure applied with false discovery rate of 0.20.

	Pre-treatment discharge				
Response variables	**Coefficient estimate**	**Std Err**	**t value**	**R^2 value**	**p-value**
Community Metrics					
ATI	-0.484	0.159	-3.046	0.297	**0.006 ****
Total Abundance	-0.190	0.252	-0.754	0.025	0.459
EPT Taxa Abundance	16.910	21.790	0.776	0.027	0.446
% EPT Taxa	19.411	8.278	2.345	0.200	**0.029 ***
Total Taxa Richness	0.827	1.169	0.707	0.022	0.487
EPT Taxa Richness	0.578	0.850	0.680	0.021	0.504
Diversity	0.144	0.060	2.408	0.209	**0.025 ***
Functional Traits					
Gills	0.028	0.037	0.749	0.025	0.462
Burrow	-0.297	0.103	-2.899	0.276	**0.008 ****
Climb	-0.081	0.069	-1.176	0.059	0.252
Cling	0.275	0.091	3.021	0.293	**0.006 ****
Collector-gatherer	-0.022	0.076	-0.290	0.004	0.775
Collector-filterer	0.026	0.055	0.475	0.010	0.640
Scraper	0.094	0.074	1.267	0.068	0.218
Predator	0.015	0.022	0.677	0.020	0.505

Table A8: Simple linear regression results of the influence of pre-treatment discharge on leaf pack aquatic invertebrate communities. Response variables were transformed when appropriate. Benjamini-Hochberg procedure applied with false discovery rate of 0.20.

	Pre-treatment discharge				
Response variable	**Coefficient estimate**	**Std Err**	**t value**	**R^2 value**	**p-value**
Community Metrics					
ATI	-0.226	0.131	-1.729	0.120	0.098
Total Abundance	-29.770	69.450	-0.429	0.008	0.672
EPT Taxa Abundance	-21.500	41.290	-0.521	0.012	0.608
% EPT Taxa	3.353	6.350	0.528	0.013	0.603
Total Taxa Richness	-1.521	1.412	-1.077	0.050	0.293
EPT Taxa Richness	-0.209	0.877	-0.238	0.003	0.814
Diversity	0.233	0.125	1.873	0.138	0.074
Functional Traits					
Gills	-0.033	0.047	-0.702	0.022	0.490
Burrow	-0.032	0.054	-0.595	0.016	0.558
Climb	0.034	0.044	0.769	0.026	0.450
Cling	0.035	0.056	0.622	0.017	0.540
Collector-gatherer	-0.046	0.067	-0.694	0.021	0.495
Collector-filterer	0.026	0.031	0.840	0.031	0.410
Scraper	* Did not analyze - only found in one sample				
Predator	-0.018	0.013	-1.371	0.079	0.184