

The Effects of Natural Wildlife Crossings on Macroinvertebrates and Algae in a  
Northwest Montana Stream

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## **Abstract**

Water is often one of the most important resources in an environment. For large herbivores in a dry ecosystem, access to water is essential to survival. Bison, deer, and elk often create crossings as they use rivers and streams, and can have impacts on aquatic communities as a result. Increased disturbance and sedimentation can adversely affect abundances of key invertebrate groups and algae. Characteristics of these animal crossings, such as substrate type and width, may also play a role in the growth of aquatic communities. In this study, I analyzed the effects of natural animal crossings on abundances of Ephemeroptera, Plecoptera, Trichoptera, and algae. Abundances of invertebrates increased downstream of crossings, while mud substrate was found to have negative effects on algae growth. Crossing width was also positively correlated with differences in Ephemeroptera abundances between upstream and downstream locations. These results suggest that nutrient additions due to stirring of organic sediment and animal wastes may play a role in stimulating invertebrate population growth. Furthermore, algae growth may be more dependent on substrate than disturbance and sedimentation. Crossing width may have a variety of effects on aquatic communities as well. Overall, large herbivores were shown to have broad effects on stream environments, and may be highly influential in the health of aquatic ecosystems.

## Introduction

For many mammals, streams are a highly important source of water. To access these streams, mammals may follow discrete paths through vegetation. In the case of larger herbivores, these paths soon become semi-permanent with frequent use. ensures that the paths are clearly defined, and continually serve a purpose. Once at the streams, however, these paths may not end. They may create visible stream crossings, that are likely to have some influence on stream ecosystems. I examined whether these crossings have measurable effects on stream communities, particularly on aquatic macroinvertebrates and algae at the National Bison Range, in Northwest Montana.

Due to their unique grazing habits, bison are especially influential in the creation and maintenance of stream crossings. Bison often move as a herd and utilize the same set areas for feeding, sometimes referred to as "grazing lawns" (McNaughton 1984). To access these plots of food resources, bison often employ specific paths, which tend to remain generally consistent over time (Catchpole 1996; Fritz et al. 1999). When these paths cross a stream, an identifiable water crossing can be formed. Such a crossing is also clearly defined because bison may cross in single file, so each successive animal provides another disturbance to the same route (Fritz et al. 1999). These bison crossings may be used by deer or elk as well, further increasing their use. However, deer and elk make use of a wide variety of crossings and often have other, much smaller, established stream crossing sites. These sites may not show quite the level of disturbance as found at a bison crossing, but still are evidently well used.

Animal crossings may have an effect on stream organisms, particularly small or non-motile inhabitants such as macroinvertebrates and algae. Macroinvertebrates in particular can be negatively affected by increased sedimentation and disturbance. Higher levels of substrate sedimentation have been shown to reduce macroinvertebrate numbers (McClelland and Brusven 1980; Matthaei et al. 2006). Additionally, increased sedimentation in the moving water above the substrate can also lead to up to an 80% reduction in macroinvertebrate counts only 30 cm below the water's surface (Richards and Bacon 1994). Invertebrate drift can also be amplified as a result of water sedimentation (Doeg and Milledge 1991). Essentially, this means that invertebrates are more likely to leave the substrate and drift downstream in search of more ideal waters when higher levels of sediments are present. It is likely, then, that bison have an effect on macroinvertebrates due to the fact that sedimentation increases at a bison crossing (Fritz et al. 1999). Crossings by other animals may have similar effects. Algal growth may also be affected by sedimentation. In sandy sediments, such as those often kicked up at a river crossing, increased sediment density is shown to be negatively correlated with algae growth (Barko and Smart 1986).

As disturbance and sedimentation increases at a crossing, macroinvertebrates may face other potentially negative impacts. When substrate containing macroinvertebrates was subjected to tumbling, most taxa saw major reductions (between 21 and 95%) in population density (Reice 1985). Additionally, these disturbed populations took roughly four weeks to fully recover to pre-disturbance densities, and were equally affected by a second disturbance event. A group of bison crossing a stream could be analogous to just such a disturbance.

To determine whether macroinvertebrates and algae in Mission Creek are negatively affected by stream crossings, macroinvertebrates and algae can be measured at varying distances up and downstream from a crossing. Mayfly (Order: Ephemeroptera), stonefly (Order: Plecoptera), and caddisfly (Order: Trichoptera) larvae can be used to help identify any detrimental effects on macroinvertebrates, as these three taxa are some of the most intolerant macroinvertebrate groups, and commonly used as an index (EPT) for stream health (Reice 1985; Lenat 1988). Along with substrate analyses and measures of other crossing characteristics (such as width and depth), these data can aid in determining the exact effects of stream crossings.

I predicted that areas upstream of a crossing would not be negatively affected by the crossing, and serve as a control. Especially for bison, crossings tend to have spatially limited effects, and may not have large consequences up and downstream (Fritz et al. 1999). At the crossing, I expected a reduction in EPT densities due to high levels of disturbance and sedimentation. I predicted that algal growth would also be reduced, as a result of increased sandy sedimentation. Downstream from the crossing, I expected to see a similar reduction in invertebrate and algal densities, when compared to upstream values. However, I would expect less pronounced negative affects due to distance from the actual disturbance.

As muddier substrates tend to produce more sandy sedimentation, I predicted that mud substrates would have the lowest algae growth. Mud and stone mixed substrate should fare slightly better, while stony substrate should have the highest levels of algae. Furthermore, I expected that wider crossings (measured from upstream to downstream) would indicate higher or more frequent disturbance, leading to higher

sedimentation. Wider crossings should then correlate with larger differences between upstream and downstream densities of macroinvertebrates and algae, with downstream densities being higher.

## Methods

This study took place on the National Bison Range (NBR), a nearly 75 km<sup>2</sup> refuge located in northwestern Montana. The NBR receives on average only about 33 centimeters of rain a year (U.S. Fish and Wildlife Service), meaning that water can be limiting. One benefit that animals on the NBR have, however, is multiple rivers and streams that travel across the refuge property. One such stream, Mission Creek, runs along the northern boundary of the NBR. On the NBR, a variety of large herbivores often cross Mission Creek. Whitetail deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), elk (*Cervus elaphus*), and bison (*Bison bison*) all make use of the stream, and contribute to the creation of discrete crossings.

Ten crossings were identified along Mission Creek. Width of the each crossing (measured along the bank from upstream to downstream) was measured. Transects were established at the crossing, 5m upstream and downstream from the crossing, and 15m upstream and downstream from the crossing, to create 5 transects.

Macroinvertebrates were analyzed at eight of the ten crossings. At each crossing, a Surber sampler net was used to take two random substrate samples. After collection, each sample was analyzed in the field for EPT counts. This process was then repeated two days later, for a total of four samples per transect. Algae were analyzed by

placing three tiles at each transect, at all ten crossings. Substrate and depth were recorded for each tile location. Algae growth was allowed to accumulate for three weeks before tiles were collected, and then quantified using a ranking system. Tiles were assigned a score of one through five, based on algae coverage of the tile: 0-25% cover received a one, 25-75% cover received a two, 75-100% thin cover received a three, full coverage with 0-50% thick (opaque) coverage received a four, and full coverage with greater than 50% thick coverage received a five.

Statistical analyses were performed using the program R. Separate one-way Analysis of Variance (ANOVA)s were used to analyze the effect of location relative to stream crossings (upstream, at the crossing, or downstream; data from five and fifteen meter transects was pooled) on Ephemeroptera and Trichoptera abundance. As data could not be normalized, a Kruskal-Wallis test was used to test Plecoptera abundance. A Kruskal-Wallis test was also used to test the effects of location relative to crossing on algal growth. Algal growth was also analyzed with respect to substrate type using a one-way ANOVA. Using a two-way ANOVA, relationship to crossing and substrate type were simultaneously compared with algal growth. For all ANOVAs, Tukey's HSD post-hoc tests were performed (where applicable). A series of four linear regressions were also used to compare crossing width to difference between upstream and downstream densities of Ephemeroptera, Plecoptera, Trichoptera, and algae.

## Results

I found significant effects of distance from crossings on stream invertebrate abundances, but not on algae. Distance from crossing had a significant effect on Ephemeroptera ( $p=0.0000171$ ,  $F=11.78$ ,  $df=2$ ; Fig. 1), with post-hoc tests showing significant differences between upstream and downstream locations ( $p=0.0000087$ ). Downstream samples had significantly higher abundances. Plecoptera abundance varied significantly with distance from crossing ( $p=0.01021$ , KW chi-squared=9.1693,  $df=2$ ; Fig. 2), with higher abundances downstream. Significance was also found for Trichoptera ( $p=.0000134$ ,  $F=12.06$ ,  $df=2$ ; Fig. 3), with post-hoc tests indicating significant differences between upstream locations and both at crossing ( $p=0.0037659$ ) and downstream transects ( $0.0000182$ ). Downstream locations had the highest abundance, followed by locations at the crossings, and finally upstream locations. No significant differences were found for algae growth ( $p=0.4221$ , KW chi-squared=1.7249,  $df=2$ ).

Algae growth varied significantly among substrates ( $p=0.000278$ ,  $F=8.69$ ,  $df=2$ ; Fig. 4). Mud substrate was significantly lower than both stone-mud mixed substrate ( $p=0.0132519$ ) and stone substrate ( $p=0.0001732$ ). The two-way ANOVA comparing both location relative to crossing and substrate to algae growth found similar results, with no significant differences as a result of distance ( $p=0.676280$ ,  $F=.3923$ ,  $df=2$ ) and significant differences due to substrate ( $p=0.001075$ ,  $F=7.1988$ ,  $df=2$ ). Post-hoc tests again showed that mud substrate was significantly different from both stone-mud mixed substrate ( $0.0419988$ ) and stone substrate ( $p=0.0011758$ ). The interaction between distance and substrate was not significant ( $p=0.150893$ ,  $F=1.7126$ ,  $df=4$ ).

Differences between upstream and downstream abundances of Ephemeroptera increased significantly with crossing width ( $p=0.056$ ,  $R^2=0.4822$ ,  $t_{(6)}=2.364$ ; Fig. 5). An  $\alpha$  value of 0.056 was accepted as significant due to a robust  $R^2$  value. Plecoptera showed a similar trend but was not significant ( $p=0.141$ ,  $R^2=0.3237$ ,  $t_{(6)}=1.695$ ; Fig. 6). There was no relationship between crossing width and Trichoptera abundance ( $p=0.4982$ ,  $R^2=0.07968$ ,  $t_{(6)}=-0.721$ ) or algae growth ( $p=0.497$ ,  $R^2=0.05941$ ,  $t_{(8)}=0.711$ ). Data for all regressions is summarized in Table 1.

## Discussion

EPT densities were all affected by location of transect in relation to the crossing. All three taxa had significantly higher densities downstream than upstream, in an effect opposite to that hypothesized. This indicates that direct trampling and disturbance effects, as well as increased sedimentation, were not strong enough to have a measurable negative effect on invertebrate numbers. If crossing use did have a negative effect at all, it was overshadowed by significant positive effects. These positive effects may have been a consequence of nutrient inputs resulting from large herbivore use. Bison, deer, and elk may have released organic sediments from the substrate, creating a more nutritious growth environment for invertebrates. Inputs to the stream from herbivore feces and urine may have added to the nutrient pool as well. One recent study found that increased bison density in a stream can lead to elevated level of phosphorus, a chemical often found in animal wastes (Larson et al. 2013). Along with potential additions from deer and elk, it is possible that this nutrient input was carried

downstream from the crossing and allowed for increased invertebrate density. Due to the generally swift current of Mission Creek, these nutrients may not have been able to affect any transects upstream. Furthermore, the sediments and waste deposition may have been carried downstream too swiftly for them to have the strongest impacts at the crossing, accounting for the fact that for all invertebrates the downstream transects had the highest densities.

Algae, however, were not significantly affected by transect location relative to crossings. In this case, my hypothesis that algal biomass would be lowest downstream from crossings, and increasingly higher upstream, was rejected because no difference was found when all distances were compared. This indicates that disturbance and sedimentation did not have measurable negative effects, but also that algal growth was not positively affected by any potential nutrient inputs from herbivores. It is possible that algae already received a sufficient baseline of nutrients to grow, and that new inputs did not improve habitat condition. Further, the nutrients provided by herbivores (for example, phosphorus) may not have been the nutrients that limit algal growth in Mission Creek. In that case, additions of nutrients already in quantities sufficient for algal growth may not have a measurable effect.

Algae did respond to substrate differences significantly. Mud substrate provided the least responsive growth environment, and any substrate with some or all stone present was significantly better. Low algal biomass on mud partially supported the hypothesis that algae would produce the least growth on mud, with increasing growth rates as more stone is added, although no measurable difference was found between stone-mud mixed and completely stone substrates. This deficit in growth on mud

substrate was likely due to increased sandy sedimentation, as well as a shortage of physical anchor points for algae, such as rocks. The effects of substrate type were further supported when substrate was combined with transect location relative to crossings, and simultaneously compared to algal growth. No significant interaction was found between substrate and location relative to crossings, and only substrate significantly affected algal densities. This suggests that the results of substrate analysis were not compounded by other measured factors.

The results of relating crossing width to differences between mean upstream and downstream densities of EPT and algae were less clear. Of the taxa examined, only Ephemeroptera exhibited a significant relationship between crossing width and abundance. For Plecoptera, Trichoptera, and algae, no significant trends were discovered. However, the regression of differences in mean Plecoptera densities against width (Fig. 6) does suggest a similar trend to that seen in Ephemeroptera. Overall, then, effects of crossing width seems to be mixed. Ephemeroptera (and to a lesser extent Plecoptera) data suggest that perhaps wider crossings may mean more sediments and nutrients added to the stream environment, leading to stronger responses by these taxa. This would account for differential growth rates as downstream populations fared better than upstream populations. However, it could also be argued based on Trichoptera and algae data that wider crossings may dilute the effects of frequent use. Inputs may be spread over a larger area, and so provide less direct benefit to populations of invertebrates and algae. Further research on the subject may be able to better shed light on this topic.

There were a number of potential issues with this study that further research should also take into account. A larger sample size, in terms of number of crossings, invertebrate samples, and algae tiles, may better indicate actual trends. Abiotic factors of streams such as dissolved oxygen, pH, conductivity, turbidity, and temperature may also play a role in growth of invertebrate and algae growth, but were not measured due to a lack of equipment. Also, substrate could have been properly correlated with invertebrate samples. Substrate at each sample location could be recorded, allowing for analysis of potential substrate effects on macroinvertebrate densities. This would also allow for the possibility of testing combined effects of substrate and location relative to crossings on EPT densities, as was done with algae in this study.

Going forward, future studies should consider using multiple streams or rivers that vary in size, substrate, flow rate, canopy cover, abiotic factors, or other conditions. Since all samples in this study came from the same stream, they may have been correlated in unknown ways as a result of Mission Creek's characteristics. Predator densities could also be measured at each sample location, to account for any potential predatory effects on population densities. Finally, other large herbivores could be used in similar studies. Cattle have been shown to have stronger effects on stream environments than bison (Li et al. 1994; Fritz et al. 1999). This may be partly due to the fact that intensity of cattle grazing declines with distance from a water source, while bison typically spend less than 6% of their time within 10 m of streams (Valentine 1947; Larson et al. 2013). A direct comparison of cattle and bison (or other large herbivore) effects could determine the mammals that have the greatest impacts on stream environments.

## Acknowledgments

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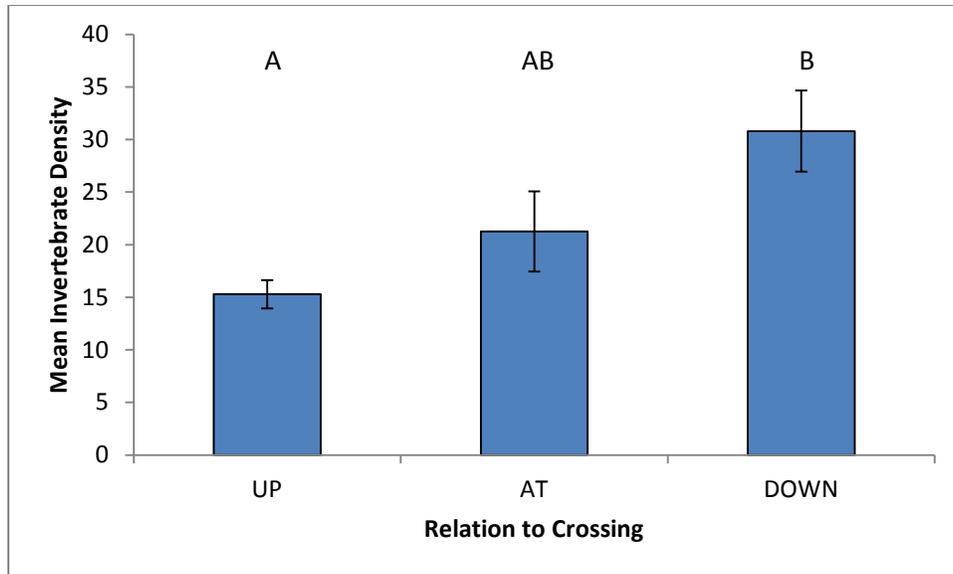
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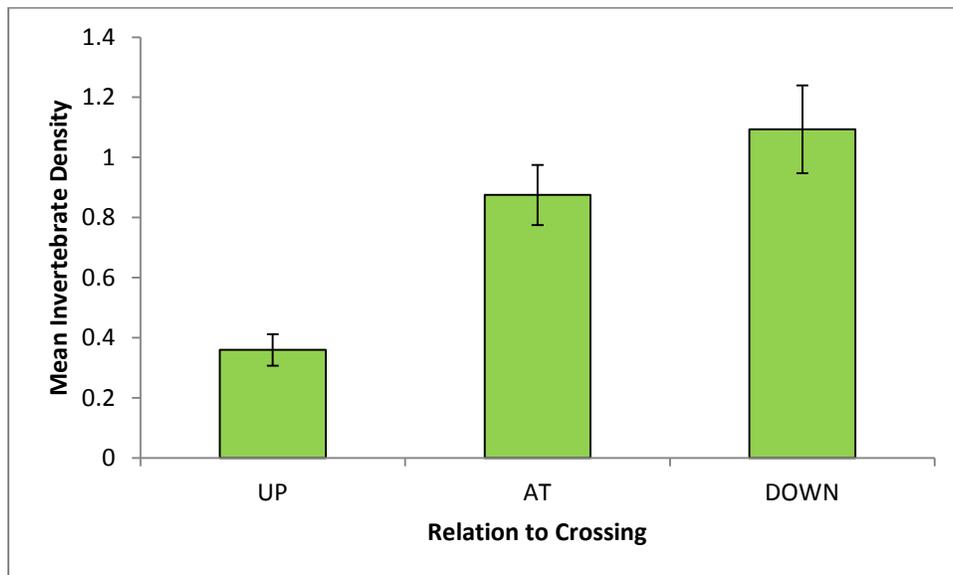
**Table 1:** Statistical results of linear regressions comparing width of crossings to differences between upstream and downstream EPT and algae densities.

Ephemeroptera showed significant results.

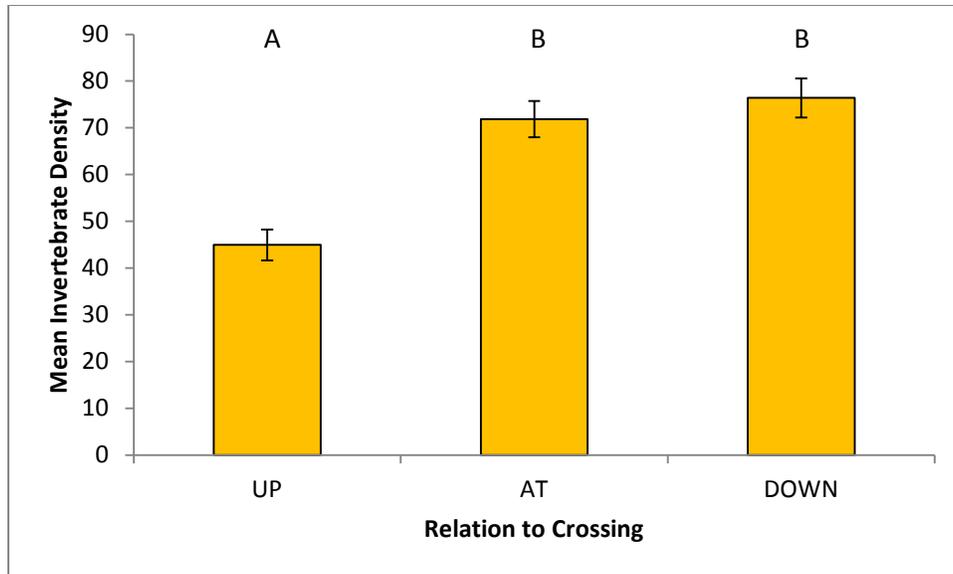
	Coefficient	Standard Error	R <sup>2</sup>	t	p
Ephemeroptera	0.5558	0.2351	0.4822	2.364	<b>0.056</b>
Plecoptera	0.012626	0.007451	0.3237	1.695	0.141
Trichoptera	-0.2757	0.3825	0.07968	-0.721	0.4982
Algae	.007085	0.009967	0.05941	0.711	0.497



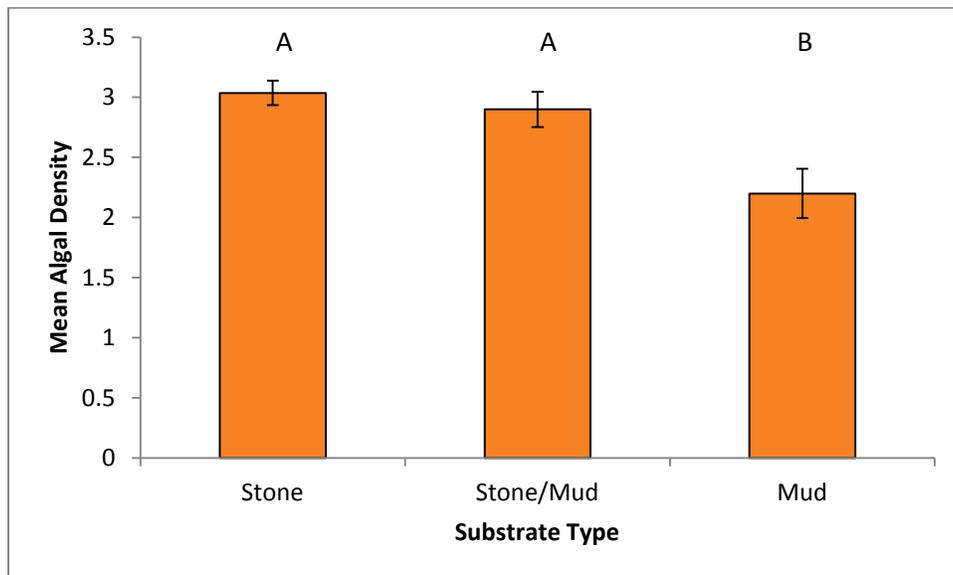
**Figure 1.** The effects of distance from crossing on mean Ephemeroptera density. Location of transect had a significant effect on invertebrate density ( $p=0.0000171$ ,  $F=11.78$ ,  $df=2$ ). Standard error included.



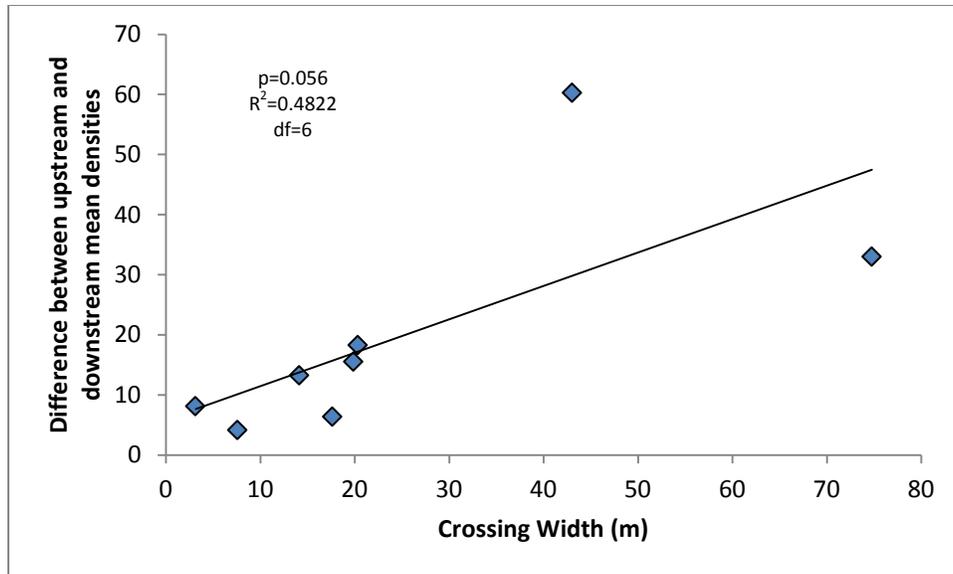
**Figure 2.** The effects of distance from crossing on mean Plecoptera density. Location of transect had a significant effect on invertebrate density ( $p=0.01021$ ,  $\chi^2=9.1693$ ,  $df=2$ ). Standard error included.



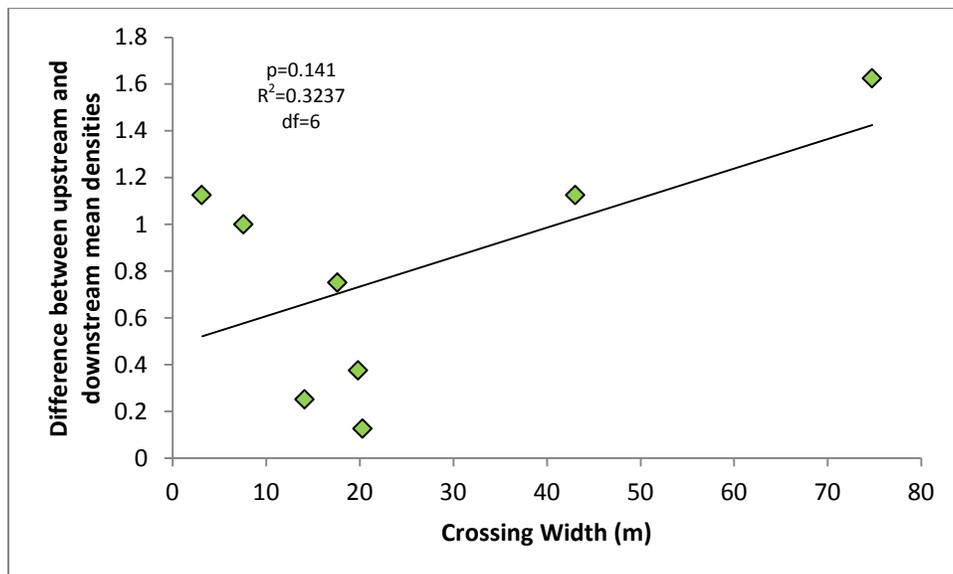
**Figure 3.** The effects of distance from crossing on mean Trichoptera density. Location of transect had a significant effect on invertebrate density ( $p=0.0000134$ ,  $F=12.06$ ,  $df=2$ ). Standard error included.



**Figure 4.** The effects of substrate type on mean algal density. Substrate had a significant effect on algal density ( $p=0.4221$ ,  $F=8.69$ ,  $df=2$ ). Standard error included.



**Figure 5.** The effects of crossing width on difference between mean upstream and downstream Ephemeroptera densities. Results show a significant positive correlation between crossing width and mean upstream and downstream Ephemeroptera densities.



**Figure 6.** The effects of crossing width on difference between mean upstream and downstream Plecoptera densities. No significant correlation was found between crossing width and mean upstream and downstream Plecoptera densities.