

PROJECT SUMMARY

Tubifex tubifex is the oligochaete host of *Myxobolus cerebralis*, the parasite that causes whirling disease in several salmonid species and is a serious threat to trout fisheries in some areas of the United States. Recent research has shown that *T. tubifex* consists of several distinct lineages that vary in their physiology and susceptibility to the *M. cerebralis* parasite. Lineage III worms are highly susceptible to *M. cerebralis* and are associated with highly infected sites. Conversely, lineage V worms are not susceptible to the parasite, may out-compete lineage III worms, and are associated with decreases in infectivity in the field.

The physical habitat variables influencing the distribution and abundance of *T. tubifex* in stream habitats are not well understood and could have implications for the success of using resistant *T. tubifex* lineages to manage whirling disease. However, few studies have been done on substrate preferences of *T. tubifex*. Exploratory oligochaete sampling and PCR analyses conducted by the Colorado Division of Wildlife in the Williams Fork River indicated that lineage V predominated on one reach, but on other reaches various proportions of lineages III, V, and VI were found. Preliminary observations indicated that lineage V was associated with somewhat coarser substrates; however, no quantitative sediment samples were collected to quantify possible habitat associations for the various lineages.

Our primary objective was to evaluate if sediment size distribution and other physical microhabitat factors were associated with lineage composition of *T. tubifex*. The three lineages differed in their relative abundances among sites and sediment characteristics best explained differences in lineage composition among the sites. Lineage V *T. tubifex* were more abundant in substrates with coarse sand and gravel, while lineage III worms were more abundant in substrates with fine sand and high core bulk density.

The spatial distribution of lineages did not follow a predictable longitudinal pattern and this suggests that habitats do not differ in a systematic way over the length of river we sampled. In the present study, lineage V was dominant on all stream reaches with interspersed samples being dominated by lineage III. Exploratory sampling by the Colorado Division of Wildlife indicated greater relative abundance of lineage III and the dominance of lineage V in this study may indicate that lineage V has been expanding. The Williams Fork River was documented as positive for *M. cerebralis* in 1994 by myxospore detection in trout but in this study only three worm samples tested positive for *M. cerebralis*, indicating that the severity of the infection may be declining. The potential declining severity of infection may be due to the increasing abundance of lineage V *T. tubifex*, although this remains to be tested.

Project Title: The role of sediment size distribution and other microhabitat factors in the abundance and relative dominance of various *T. tubifex* lineages

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ABSTRACT

The physical variables influencing the distribution and abundance of *Tubifex tubifex* in stream habitats are not well understood and could have implications for the success of using resistant *T. tubifex* lineages to manage whirling disease. Our primary objective was to evaluate if sediment size distribution and other physical microhabitat factors were associated with lineage composition of *T. tubifex*. Our second objective was to randomly sample stream habitats to gain a more complete understanding of *T. tubifex* distribution across a wide variety of stream habitat types.

Canonical Correspondence Analysis showed that lineage III, V, and VI differed in their relative abundances among sites and that depth and sediment characteristics were the physical habitat variables that best explained the differences in lineage composition among the sites. Lineage V worms were associated with higher levels of coarse sand and gravel, as well as somewhat deeper habitats than lineage III worms. Lineage VI worms appear to be associated with somewhat deeper habitats than either lineage III or V.

The spatial distribution of lineages did not follow a predictable longitudinal pattern. Lineage V was the dominant lineage at all stream reaches with interspersed samples being dominated by lineage III. The *T. tubifex* composition at 23 of 29 targeted sampling sites was dominated by lineage V and 3 of 29 samples were dominated by lineage III. Two samples had about equal proportions of lineage III and V. Lineage VI was represented at 27 of the sites but was dominant in only one sample.

T. tubifex CPUE was not significantly related to either depth or flow velocity; however, flow velocity was more strongly related to CPUE than depth. A MANOVA indicated that the overall difference in depth and flow between sites with and without *T. tubifex* was not significantly different.

INTRODUCTION

The oligochaete worm *Tubifex tubifex* is distributed worldwide and inhabits sediments in lakes and rivers (Gilbert and Granath 2003). *T. tubifex* can live in variety of habitats and water quality conditions and are able to thrive in eutrophic environments (Robbins et al. 1989). *T. tubifex* is the oligochaete host of *Myxobolus cerebralis*, the parasite that causes whirling disease in several salmonid species (Markiw and Wolf 1983, Wolf and Markiw 1984, Markiw 1986). Whirling disease is a serious threat to trout fisheries in the United States (Hedrick et al. 1998; Thompson et al. 1999; Gilbert and Granath 2003) and has increased interest in understanding the ecology of *T. tubifex* to evaluate management strategies to control the parasite.

Recent research has shown that *T. tubifex* consists of several distinct lineages that vary in their susceptibility to the *M. cerebralis* parasite (Beauchamp et al. 2001, 2002, 2005; Nehring et al. 2005; DuBey et al. 2005; DuBey and Caldwell 2004). Beauchamp et al. (2002, 2005) showed that lineage V worms were associated with less infected sites and that lineage III dominance was associated with highly infected sites. Nehring et al. (2005) reported declines in TAM densities and a shift in lineage composition over six years (1998-2004) at Windy Gap Reservoir from 82.8 percent lineage III (no lineage V) to 76.1 percent lineage V (1.8 percent lineage III). Lineage VI also appears to be resistant to *M. cerebralis* infection (DuBey et al. 2005; DuBey and Caldwell 2004).

The existence of *M. cerebralis*-resistant lineages of *T. tubifex* has rekindled interest in exploiting resistant lineages as a control for *M. cerebralis*. One management option being pursued is the introduction of resistant lineages into infected streams that are dominated by lineage III *T. tubifex*. However, the physical variables influencing the distribution and abundance of *T. tubifex* in stream habitats are not well understood and could have implications for the success of using resistant *T. tubifex* lineages to manage whirling disease. Some studies suggest that lineages differ in their growth performance and reproductive success as a function of temperature (Anlauf 1994, 1997; Anlauf and Neumann 1997; Sturmbauer et al. 1999; de la Hoz Franco and Budy 2004). Flow velocity may also influence lineage composition (de la Hoz Franco and Budy 2004), but DuBey and Caldwell (2004) did not detect any lineage composition differences related to water quality, velocity, or percent organic matter in the San Juan River (NM).

It is generally accepted that *T. tubifex* are found in backwaters, pools, and stream margins where water velocity is low and allows accumulation of sand, silt, and organic sediments (McMurtry et al. 1983; DuBey and Jacobi 1996; Bergersen and Anderson 1997; DuBey and Caldwell 2004). Due to the high densities of *T. tubifex* occurring in these habitats, it is thought that substrate variables may also influence *T. tubifex* distribution, abundance, and lineage composition. However, few studies have been done on substrate preferences of *T. tubifex*. McMurtry et al. (1983) suggest that the benthic microbial community may be more important in influencing substrate selection than the substrate's physical and chemical properties. Lazim and Learner (1987) indicate that *T. tubifex* distribution is most strongly correlated with leaf litter in a small stream. However, their experiments also indicate that worms were attracted to the leaves because of the associated bacteria.

Preliminary oligochaete sampling and PCR analyses conducted by the Colorado Division of Wildlife in the Williams Fork River indicated that lineage V predominated on one reach, but on other reaches various proportions of lineages III, V, and VI are found. Preliminary observations indicated that lineage V was associated with somewhat coarser substrates; however, no quantitative sediment samples were collected to quantify possible habitat associations for the various lineages.

Our primary objective was to evaluate if sediment size distribution and other physical microhabitat factors were associated with lineage composition of *T. tubifex*. Most oligochaete sampling has focused on detecting the presence of *T. tubifex* and *Mxyobolus cerebralis* using sampling focused on habitats thought to have high abundances of *T. tubifex*. Therefore, our second objective was to randomly sample stream habitats to gain a more complete understanding of *T. tubifex* distribution across a wide variety of stream habitat types.

METHODS AND MATERIALS

Study Site

The Williams Fork River is located in Grand County Colorado. We focused our sampling on the portion of the Williams Fork River below the Williams Fork Reservoir to its confluence with the Colorado River near Parshall, CO (Figure 1). The river below the reservoir is regulated and discharge varies depending on the season and water needs in the Colorado River. It is a popular trout fishery and is known to be positive for *M. cerebralis*. Sampling conducted by the Colorado Division of Wildlife indicated that there were 3 lineages of *T. tubifex* (III, V, and VI) present in the river. The importance of the Williams Fork fishery and the variation in *T. tubifex* lineage composition made the Williams Fork an ideal locale to study the potential influence of physical habitat variables on lineage composition.

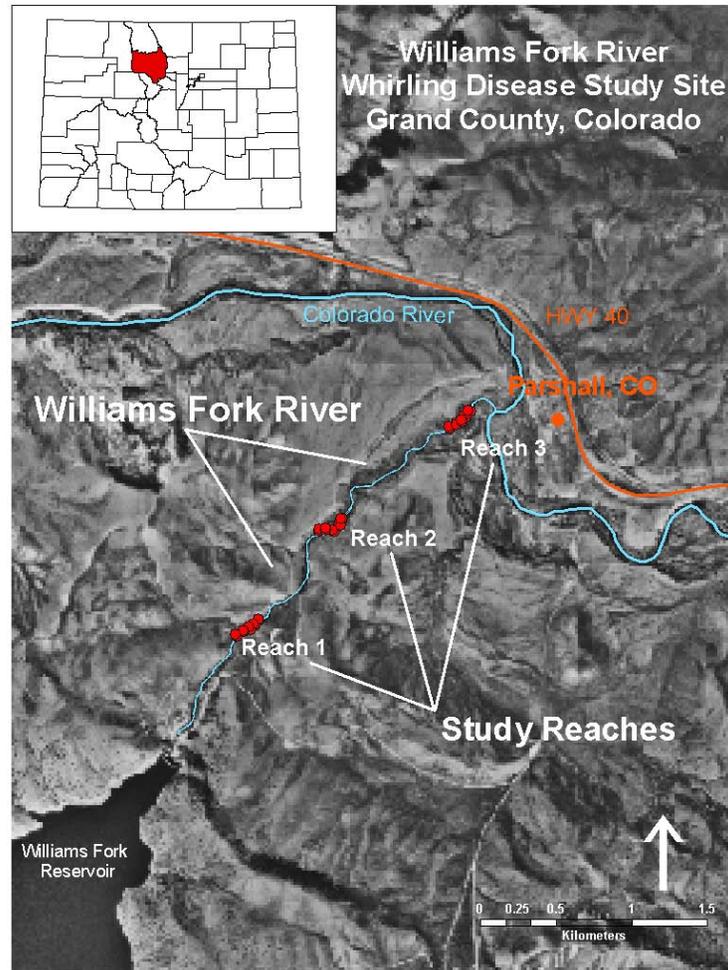


Figure 1. The Williams Fork River, CO. Study reaches used for random *T. tubifex* sampling and stream habitat transects are indicated. More detailed maps for the three reaches and other sampling areas are in Appendices I-III.

We took two approaches to estimating the relative abundance and density of *T. tubifex*. First, we used a random sampling approach to assess *T. tubifex* density for all stream habitat types. Secondly, we used a targeted sampling approach to look at the relative dominance of lineages in habitats that contained high densities of *T. tubifex*.

Random Samples

We located sites at the upper (reach 1), middle (reach 2), and lower (reach 3) sections of the Williams Fork River from the dam to the confluence with the Colorado River (Figure 1, Appendix I). At each sampling reach we established a stream section that was 200 meters long (Figure 2, Appendix 1). We established sampling transects every 10 meters along the stream section (spatially referenced anchor points are shown in Appendix 1) and along each transect we measured depth, flow velocity, and dominant substrate at 1 meter intervals. We also estimated substrate composition using pebble count methodology (Kondolf 1997). The number of points sampled varied on each

transect due to variation in stream width but overall we collected data at 629 habitat sample points (reach 1=228, reach 2=219, and reach 3=182). Finer substrate composition was analyzed from sediment collected with a core sampler (see below). A *T. tubifex* sample was taken on each transect by randomly choosing one of the sampling points on each transect, resulting in 20 *T. tubifex* samples from each stream section. *T. tubifex* samples consisted of a paired core and kicknet sample (see oligochaete sampling below).

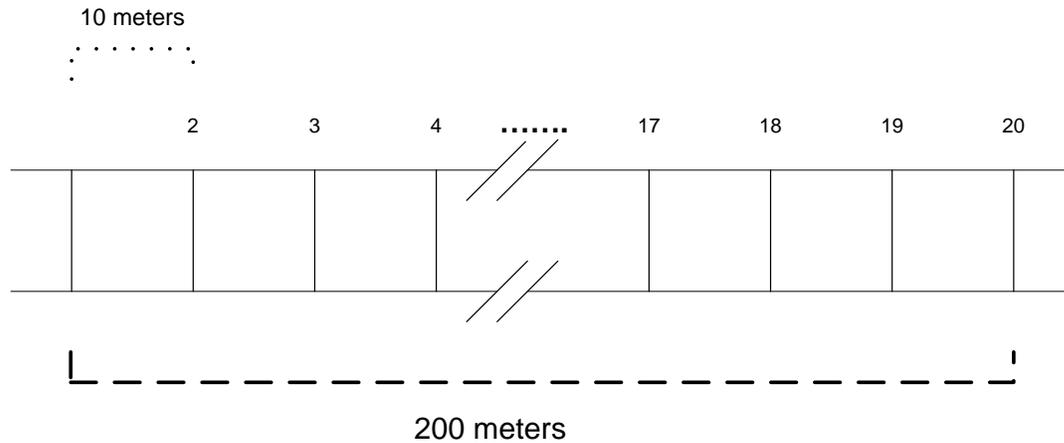


Figure 2. Schematic of sampling reaches. Each reach was 200 meters long and transects were established every 10 meters. Depth, flow velocity, substrate type, and pebble counts were made at one meter intervals along each transect. A *T. tubifex* sample was taken at one randomly selected interval on each transect.

Targeted Samples

Since the majority of the Williams Fork River consisted of riffle and run type stream habitat (Winkelman, unpublished data), we were concerned that random sampling would result in too few samples containing *T. tubifex*. Therefore, we used a targeted sampling strategy to ensure that we would have sufficient samples to analyze for lineage composition. To select targeted sampling sites we initially stratified the stream into habitat types based on flow and depth. We arbitrarily selected three depths (shallow, medium, deep) and flow velocities (slow, medium, fast), resulting in 9 possible habitat types. Since our random sampling resulted in sufficient samples from habitats with medium to fast flow velocity and shallow depth, we concentrated our targeted sampling on habitats with slow flow velocity and medium to deep depths. Targeted samples were collected in all three reaches, as well as in the intervening stream segments which we labeled as Reach 1-2 and Reach 2-3 (Appendices II and III). This sampling resulted in a total of 50 samples (Appendices II and III). *T. tubifex* samples were taken at each site, consisting of core and timed kicknet samples (described below).

Oligochaete monitoring

For each random and targeted sample an oligochaete sample was taken, consisting of 41.85 cm² core sample taken to a depth of 10 cm, if possible, and a 0.5 m² kicknet

sample taken over 60 seconds duration. The core sample was taken first from the center of the 0.5 m² kicknet sampling frame. The corer was a commercially made (AMS Company, American Falls, Idaho) stainless steel, 7.3cm inside diameter, “sludge sampler” equipped with a “cone head” so that the opening of the corer was unobstructed. The corer was fitted with a slide hammer for driving into the substrate. The target depth for the core was to penetrate 10cm of substrate. This depth was marked on the outside of the core sampler body with electrical tape to provide both a visual and tactile reference. If a 10cm depth could not be reached, the depth was measured and recorded. Actual depth of the core plus depth of water at the core location were recorded. The content of the corer was emptied into a double set of heavy duty sealable plastic bags, placed on ice, and returned to the lab for processing.

Initial processing of all core material was completed in the lab the day after core collection. The core was washed in a # 60 (250um screen opening) sieve and all material and invertebrates retained by the sieve preserved in a whirlpack in 70% ethanol. The wash water and sediment that passed the sieve was retained in a bucket and allowed to settle for 3 to 5 days. After the settling period, supernate liquid was removed from the bucket without disturbing the settled material, the volume and specific gravity of supernate recorded, and the settled material allowed to air dry in the bucket. Once dry, the material in the bucket was placed in a smaller, covered container for eventual reunification with the coarse fraction of the core sample.

Invertebrates were removed from the ethanol preserved portion of the sample and identified. The remaining material in the preserved portion of the sample was air dried and combined with the “fine” fraction of the sample that had been air dried. The sample consisting of the two reunited fractions was then subjected to standard techniques (ASTM method D-422-63) to estimate particle size distribution and other characteristics by first weighing and dry sieving the entire sample and then subjecting a 100 gram (approximate weight) sub sample to hydrometer analysis (sieve analysis of this 100 gram sample is termed “wet sieving”). As part of the dry sieving process, “large organic material” (mats of macrophyte roots, as well as sticks large enough to be retained by a number 8 sieve but small enough to pass a number 1 sieve along both axes of the stick) were separated from the sample, weighed, and retained for further analysis. “Bulk specific gravity” of the core was defined as dry weight (gm) of the core material divided by core volume, where core volume equals core depth times the surface area of the corer. For this report, we only used data from the dry sieve analysis and grouped the sieved fractions into four categories; gravel (4.75-25.4 mm), coarse sand (2-4.75 mm), medium sand (0.3-2 mm), and fine sand (0.075-0.3 mm).

In other research on lineage composition we collect 2 50-worm samples for lineage analyses; however, we analyzed only one 50-worm sample in this study because of the number of samples that we had to analyze. Previous sampling and positive identifications of oligochaetes in Willow Creek (Zendt and Bergersen 2000), the Poudre River (Allen and Bergersen 2002), and Williams Fork River and Spring Creek (K. Thompson unpublished data) indicate that haired oligochaetes in the study areas on these streams are overwhelmingly *T. tubifex* (>99%). Consequently we felt confident that separating haired worms was a reasonable surrogate for actual physical identification in our study areas.

The worm samples were subjected to a multiplex quantitative PCR assay to estimate the relative proportions of DNA present representing the differing lineages of *T. tubifex* (Wood et al. 2004). This assay is based on the *T. tubifex* mitochondrial DNA sequences determined by Beauchamp et al. (2001, 2002), and utilizes two non-lineage specific “universal” primers to amplify a segment of the *T. tubifex* mitochondrial 16S rDNA from all the tubifex worms in a sample. Four lineage-specific, differently colored fluorogenic probe oligonucleotides and a real-time PCR instrument designed for multiplex qPCR (Stratagene Mx4000) were used to measure the relative proportion of worm DNA in lineages I, III, V, and VI in the 50-worm samples.

Statistical Analyses

Relationships among physical habitat variables (sediment particle size distribution, core bulk specific gravity, depth, and flow velocity) and *T. tubifex* lineage composition were examined using Canonical Correspondence Analyses (CCA, ter Braak 1996; Leps and Smilauer 2003). The relationship of depth and flow velocity to *T. tubifex* density (CPUE) was examined using multiple linear regression. To compare differences in depth and flow velocity among sites with and without *T. tubifex* present we used a MANOVA.

RESULTS

T. Tubifex Lineage Composition and Habitat Relationships

The spatial distribution of lineages did not follow a predictable longitudinal pattern. Lineage V was the dominant lineage at all stream reaches with interspersed samples being dominated by lineage III (Figure 3). The *T. tubifex* composition at 23 of 29 targeted sampling sites was dominated by lineage V DNA and 3 of 29 samples were dominated by lineage III DNA (Figure 3). Two samples had about equal proportions of lineage III and V DNA. Lineage VI DNA was represented at 27 of the sites but was dominant in only one sample (Figure 3).

The CCA showed that the three lineages differed in their relative abundances among sites and that depth and sediment characteristics were the physical habitat variables that best explained the differences in lineage composition among the sites (Figure 4). The first canonical axis (eigenvalue=0.198) explained 43.7% of the variation in lineage composition and the second axis (eigenvalue=0.076) explained an additional 16.7% for a total of 60.4% of the variation in lineage composition. Both canonical axes were significant based on a permutation test (F ratio = 16.28, p=0.008 and F ratio=4.57, p=0.006 for axes 1 and 2 respectively). Lineage V worms were associated with higher levels of coarse sand and gravel, as well as somewhat deeper habitats than lineage III worms. Lineage VI worms appear to be associated with somewhat deeper habitats than either lineage III or V (Figure 4).

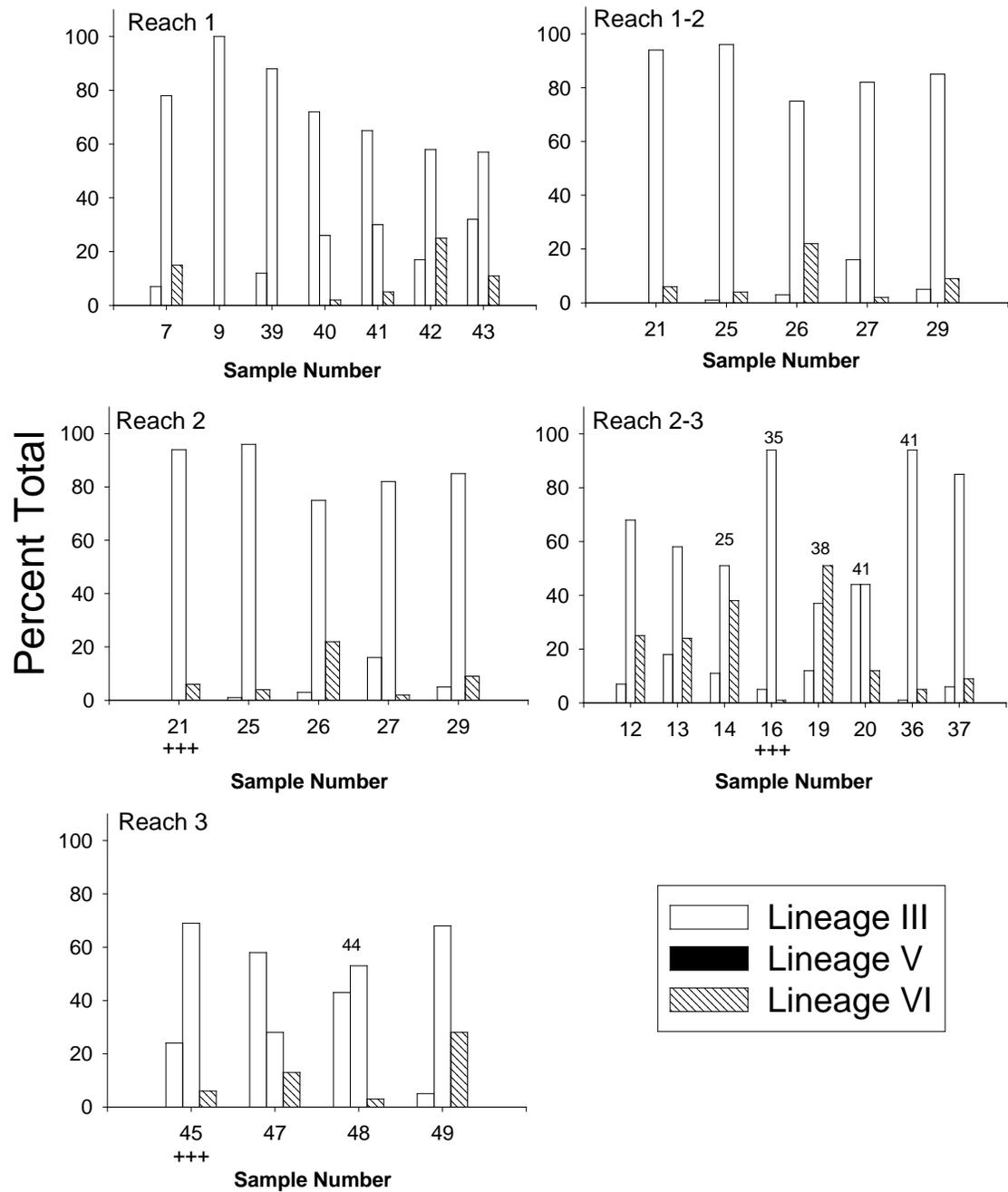


Figure 3. The percentage of lineages III, V, and VI in each targeted sample for each sampling reach. Sampling locations are shown for all sampling reaches in appendices I-III. +++ indicates that the PCR test indicated a high level of *M. cerebralis* was present (this occurred in sample 16, 21, and 45). All other samples showed no *M. cerebralis* present. Sample sizes are indicated above the bars when less than 50 worms were available for the PCR analyses.

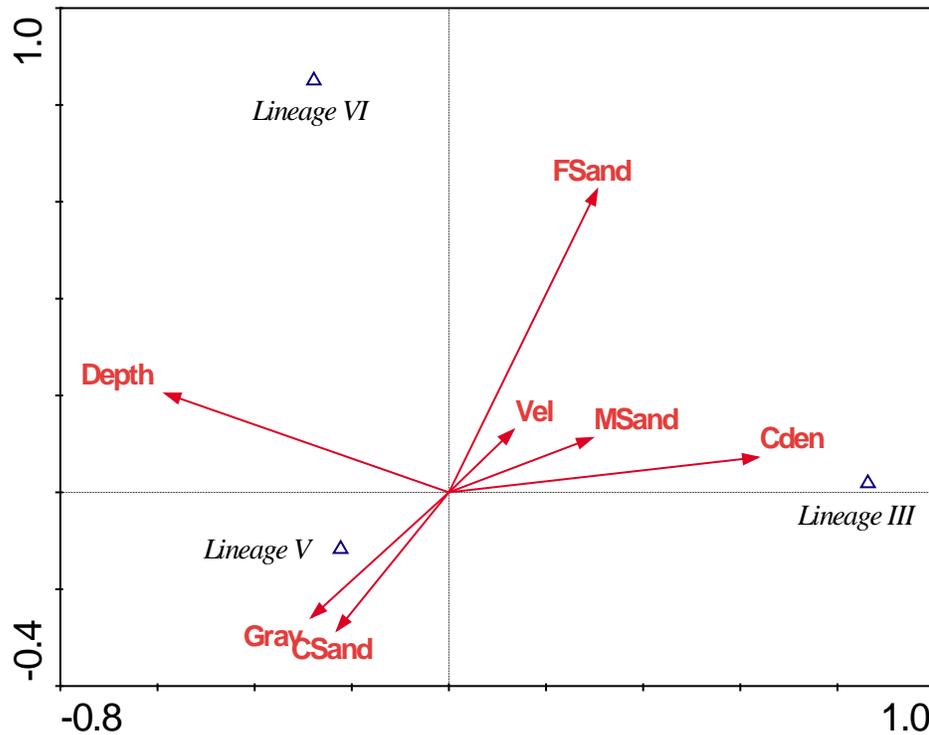


Figure 4. CCA biplot of each lineage, including vectors for environmental variables. Depth=depth of sample in meters, Vel=Flow velocity in M/sec, Cden=Core bulk specific gravity, Fsand=Fine sand, MSand=medium sand, CSand=coarse sand, and Grav=gravel (sediment characteristics are defined in text).

Oligochaete CPUE for random samples

T. tubifex CPUE was not significantly related to either depth or flow velocity; however, flow velocity was more strongly related to CPUE than depth (Table 1). We feel the relatively weak relationship with flow velocity was due to samples in low velocity habitats that had no *T. tubifex* present (Figure 5).

Depth was not significantly different between sites with and without *T. tubifex* present ($p=0.778$, Figure 6); however, flow velocity did differ among these sites ($p=0.046$, Figure 6). However, the MANOVA indicated that the overall difference in depth and flow between sites with and without *T. tubifex* was not significantly different ($p=0.089$, Pillai's trace= 0.086, $F=2.53$ d.f.=2,54).

Table 1. Parameter estimates from multiple regression model predicting CPUE (worms/minute).

Parameter	Estimate	Standard Error	T-value	P
Intercept	4.38	2.49	1.76	0.084
Depth	2.94	11.19	0.26	0.794
Velocity	-9.49	5.27	-1.80	0.077

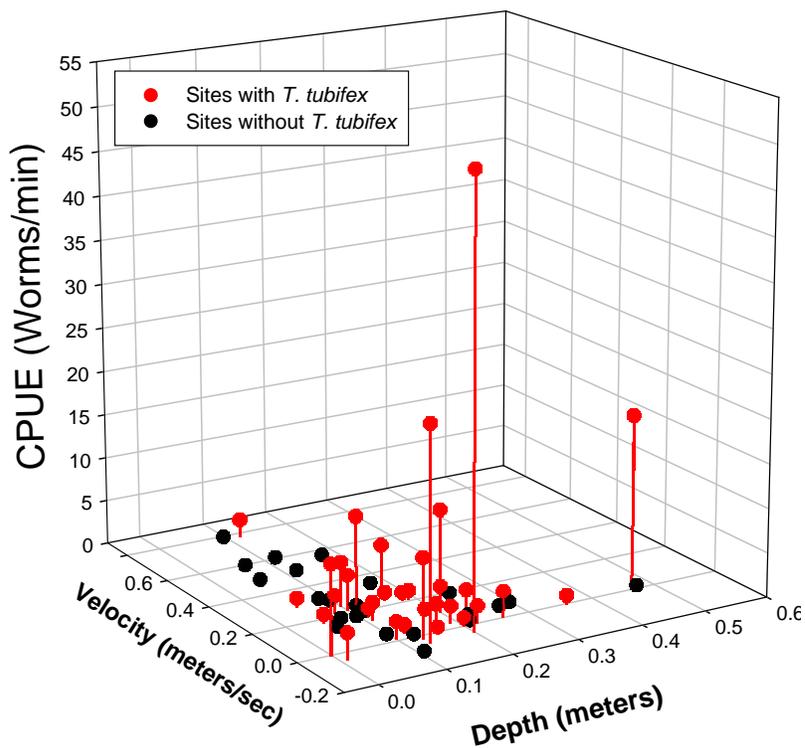


Figure 5. CPUE of *T. tubifex* in kicknet from random samples as a function of depth (meters) and flow velocity (meters/sec).

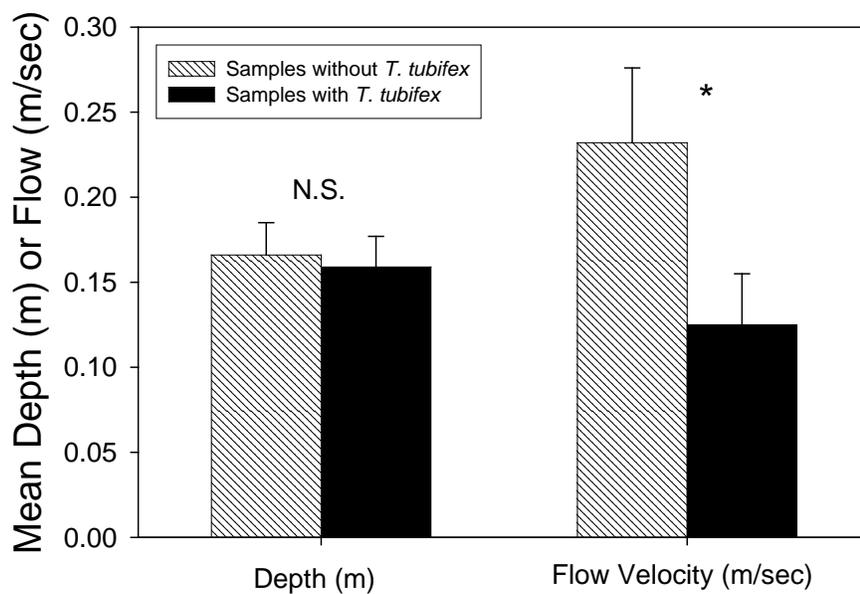


Figure 6. Mean depth and flow for random sites with and without *T. tubifex* present.

DISCUSSION

Lineage composition appears to be influenced by physical habitat variables, particularly substrate composition. Lineage V worms were associated with coarser substrates than lineage III and lineage VI appears to be more prevalent in deeper habitats, although lineage VI has been associated with shallow habitats in other studies (DuBey and Caldwell 2004). McMurtry et al. (1983) found no relationship between tubificid abundance and sediment particle size; however, they did not measure sediment particle size above 0.06 mm. Lazim and Lerner (1987) suggest that *T. tubifex* prefer fine silt-clay substrates but are more abundant in coarser grained sand when finer substrates are not available. The relationship of lineage composition to sediment characteristics could have important implications for management strategies involving *T. tubifex* and ongoing research on introducing resistant lineages of worms may be facilitated by an understanding of lineage specific substrate preferences. For instance, resistant worms might be introduced into habitats in which they would have better survival and reproduction, thereby increasing the likelihood that the introduction would be successful.

We did not find any strong relationships between depth or flow velocity on *T. tubifex* abundance, which is similar to findings in other studies (DuBey and Caldwell 2004). We feel that depth is probably not an important variable in stream and river ecosystems. *T. tubifex* are known to occur in lakes and reservoirs that are significantly deeper than any habitats in the Williams Fork River and other similar systems and depth does not seem to be a likely limiting factor for *T. tubifex*. Although our flow velocity data did not predict *T. tubifex* density very well, we believe that flow velocity may be important. The predictive power of flow velocity in our study was hampered by the presence of many low velocity sites where *T. tubifex* density was zero. In other words, *T. tubifex* density was low in high velocity habitats but was not necessarily high in low velocity habitats. Other studies suggest that stream flow or discharge may be important in predicting the severity of *M. cerebralis* infection (de la Hoz Franco and Budy 2004). Flow velocity influences sediment deposition, may concentrate myxospores (Kerans and Zale 2002), and may result in dilution or destruction of TAMS (Kerans and Zale 2002; MacConnell and Vincent 2002).

In addition to the physical habitat factors that have been discussed above, we suggest that studies should investigate long-term habitat stability as a factor in the regulation of *T. tubifex* populations. In streams and rivers that have a high degree of variability in discharge, it is likely that small-scale habitat characteristics vary substantially, particularly flow and sedimentation. This variation could have important consequences for the abundance and distribution of *T. tubifex*. For instance, many low velocity habitats when measured at low discharge may not exist at higher stream discharges. We suggest that areas that remain low velocity at a wide range of stream discharges are probably the habitats with the highest *T. tubifex* densities.

The spatial distribution of lineages did not follow a predictable longitudinal pattern and this suggests that habitats do not differ in a systematic way over the length of river we sampled. In the present study, lineage V was dominant in all stream reaches with interspersed samples being dominated by lineage III. Exploratory sampling by the Colorado Division of Wildlife indicated greater relative abundance of lineage III and the dominance of lineage V in this study may indicate that lineage V has been expanding.

The Williams Fork River was documented as positive for *M. cerebralis* in 1994 by myxospore detection in trout but in this study only three worm samples tested positive for *M. cerebralis*, indicating that the severity of the infection may be declining. The potential declining severity of infection may be due to the increasing abundance of lineage V *T. tubifex*, although this remains to be tested.

CONCLUSIONS

Lineage composition appears to be influenced by physical habitat variables, particularly in habitats that vary in the proportion of fine and coarse sand. We did not find any strong relationships between depth or flow velocity on *T. tubifex* abundance. However, there are indications that flow velocity is an important variable in determining overall *T. tubifex* abundance. The spatial distribution of lineages did not follow a predictable longitudinal pattern, suggesting that habitats do not differ in a systematic way over the length of river we sampled. The lack of a spatial pattern is also due to the dominance of lineage V in almost every sample. The increase of samples dominated by lineage V, compared to samples collected earlier by Colorado Division of Wildlife indicates that the lineage composition is changing and may be related to declines in the severity of infection.

FURTHER RESEARCH

We suggest that future research should focus on long-term monitoring of the Williams Fork River and other areas in which lineage composition is variable and potentially changing. This monitoring should also include collection of data on *M. cerebralis* infectivity, such as TAM counts in water samples and myxospore prevalence and abundance in fish. Such monitoring would help us understand the relationship between lineage composition and changes in the prevalence of *M. cerebralis*. An understanding of the relationship between lineage composition and *M. cerebralis* would help guide management and research efforts in using resistant lineages as a management strategy for controlling whirling disease.

In addition to physical habitat factors, we suggest that studies should investigate long-term habitat stability as a factor in the regulation of *T. tubifex* populations. We believe that areas with low flow velocity at a wide range of stream discharges are probably the habitats with the highest *T. tubifex* densities.

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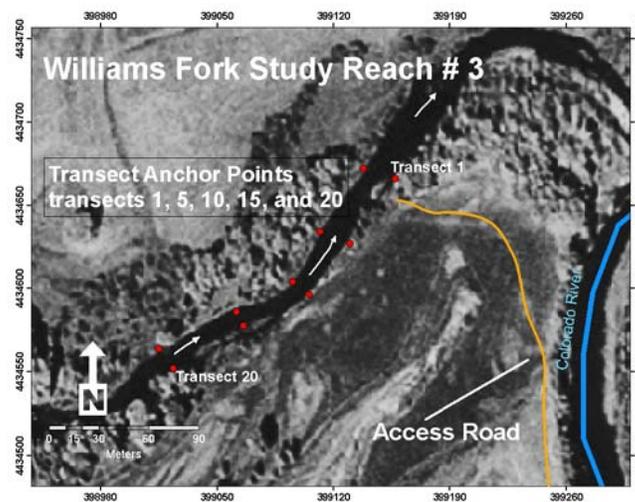
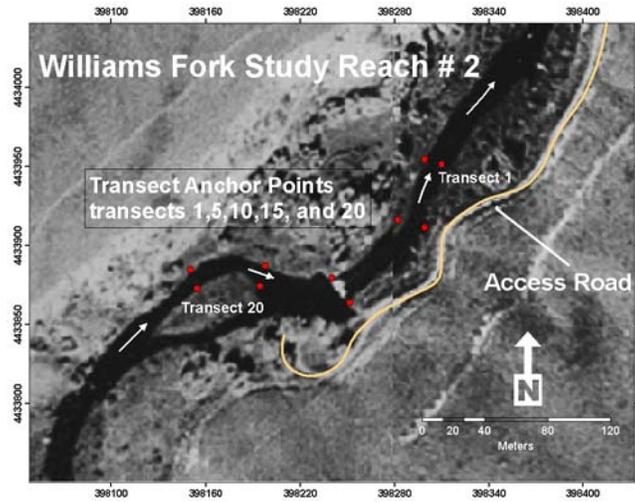
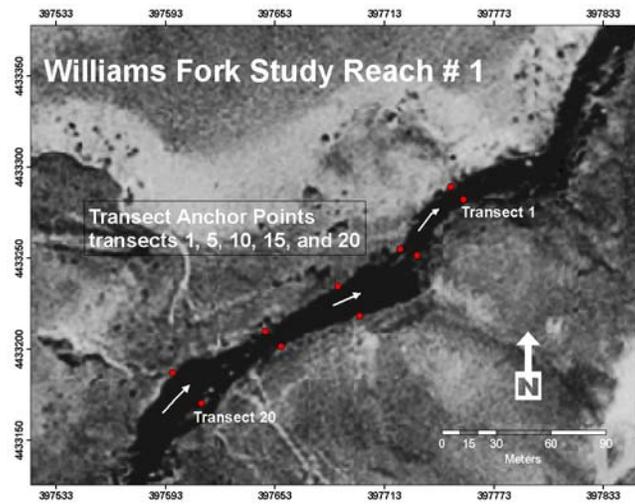
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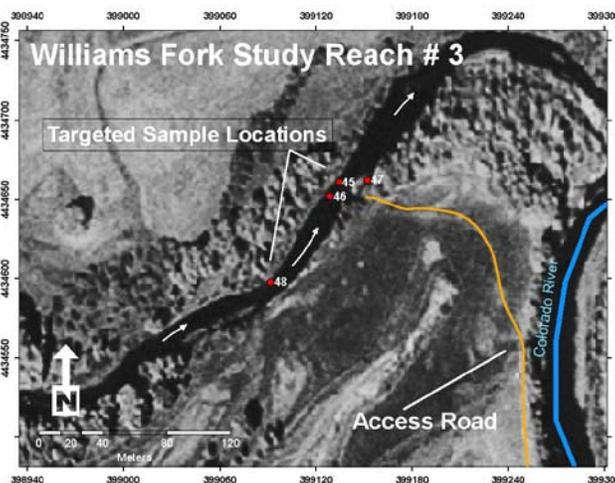
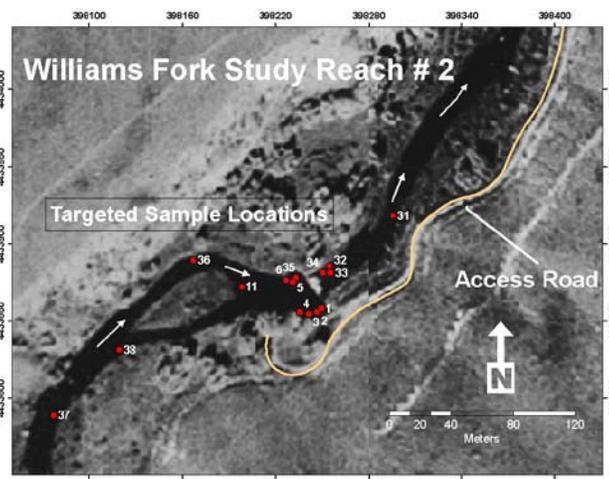
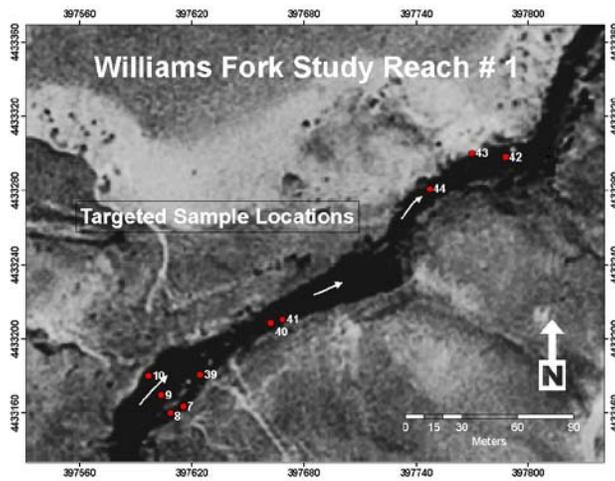
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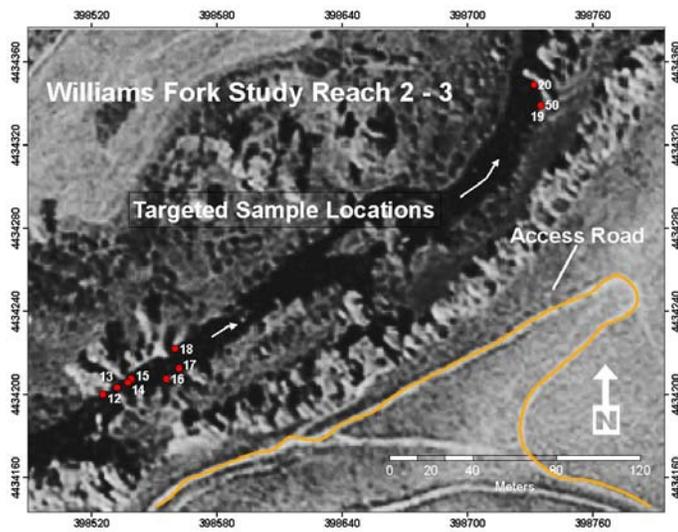
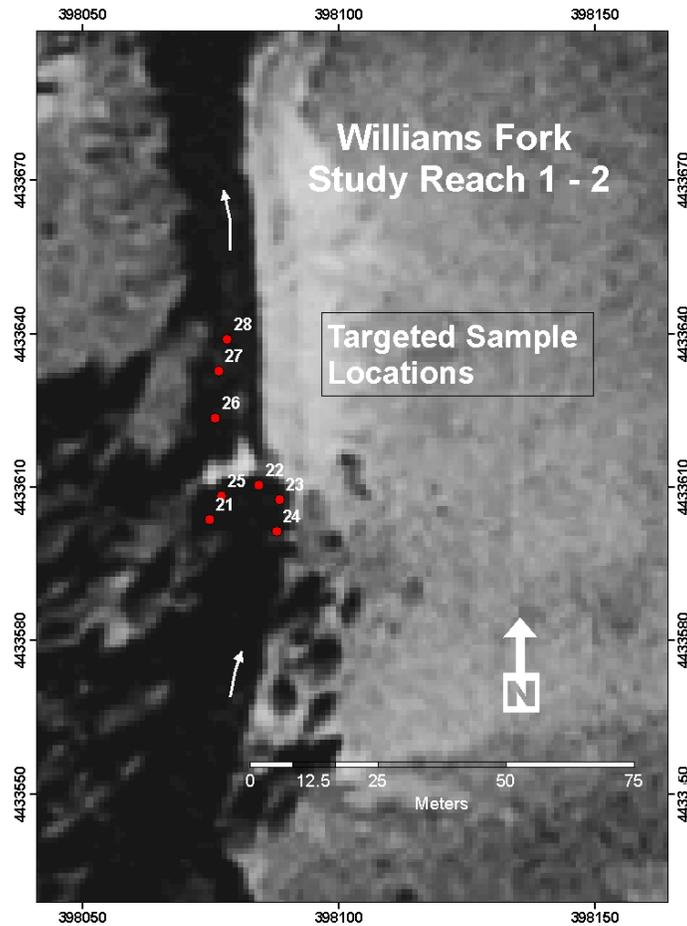
Appendix I. Williams Fork transect sampling sites for each study reach



Appendix II. Williams Fork targeted sampling sites for each study reach

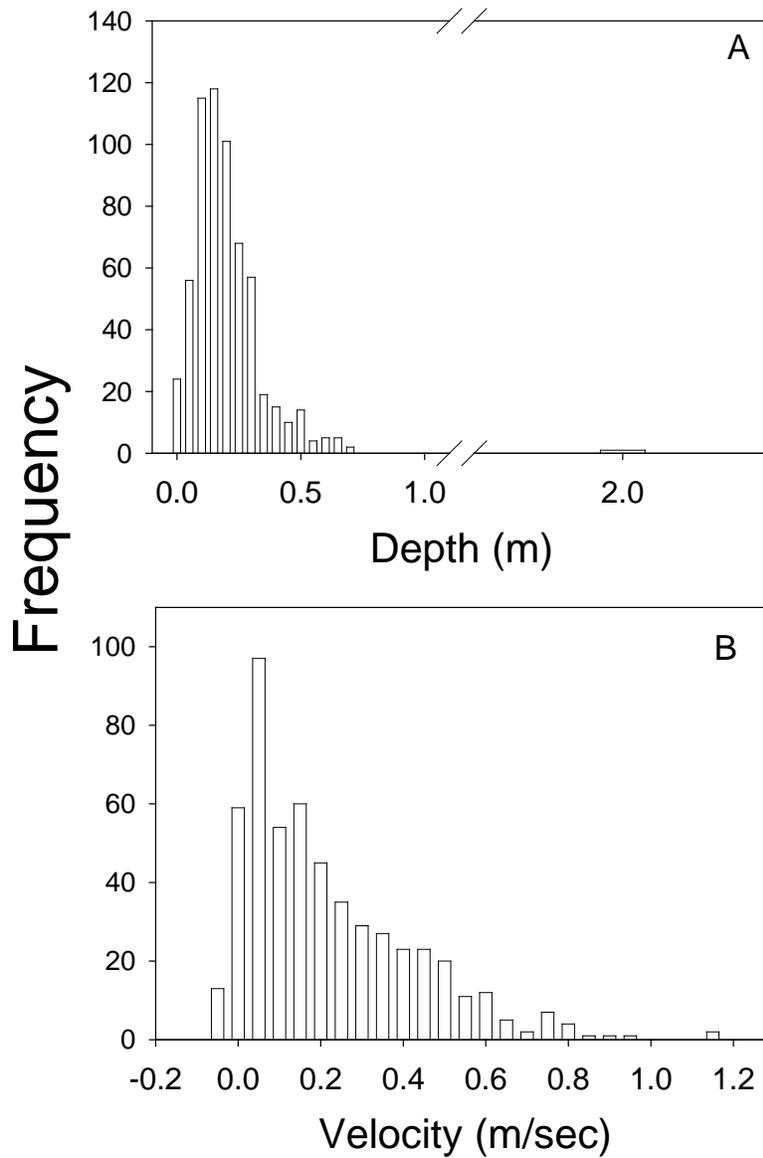


Appendix III. Additional targeted sampling locations. Reach 1-2 is between reaches 1 and 2. Reach 2-3 is between reaches 2 and 3.



Appendix IV. Depth, flow velocity, and substrate characteristics of the Williams Fork River, CO.

In general, the Williams Fork River is dominated by riffle and run mesohabitat types, characterized by relatively shallow depths and high flow velocities (Figure A1). We sampled the Williams Fork River at 15 CFS. Depths ranged from 0-2 meters (Figure A1A) and flow velocities ranged from -0.10-1.15m/sec (Figure A1B). Pebble count data indicated that the 3 sampling reaches were similar in substrate characteristics (Figure A2). The majority of habitat in the Williams Fork consists of riffle mesohabitats, followed by glide/run mesohabitats, with relatively few pools.



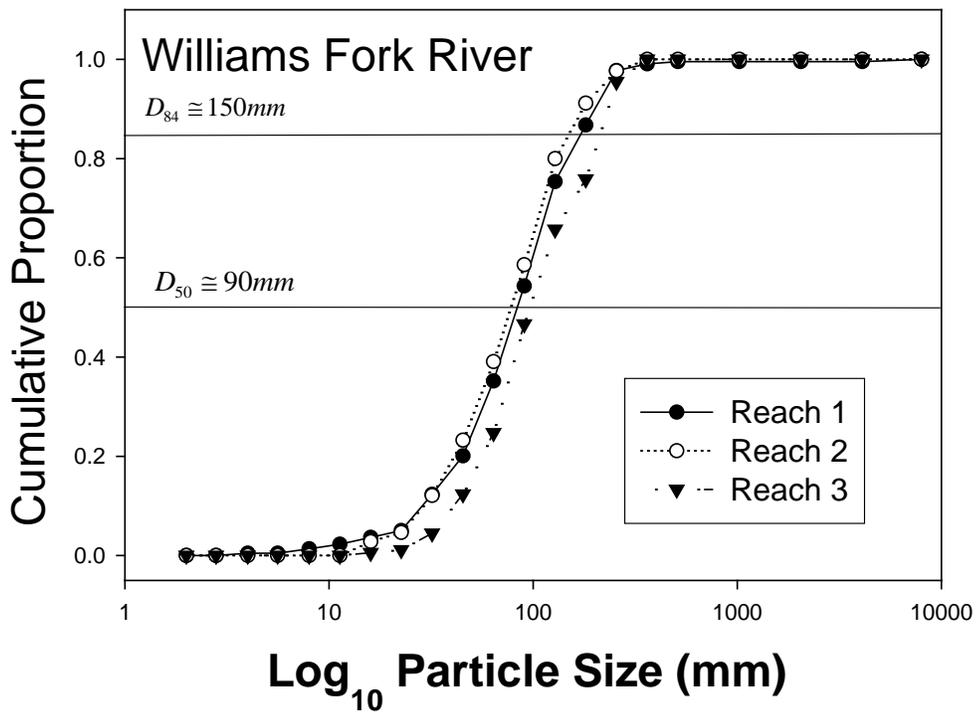
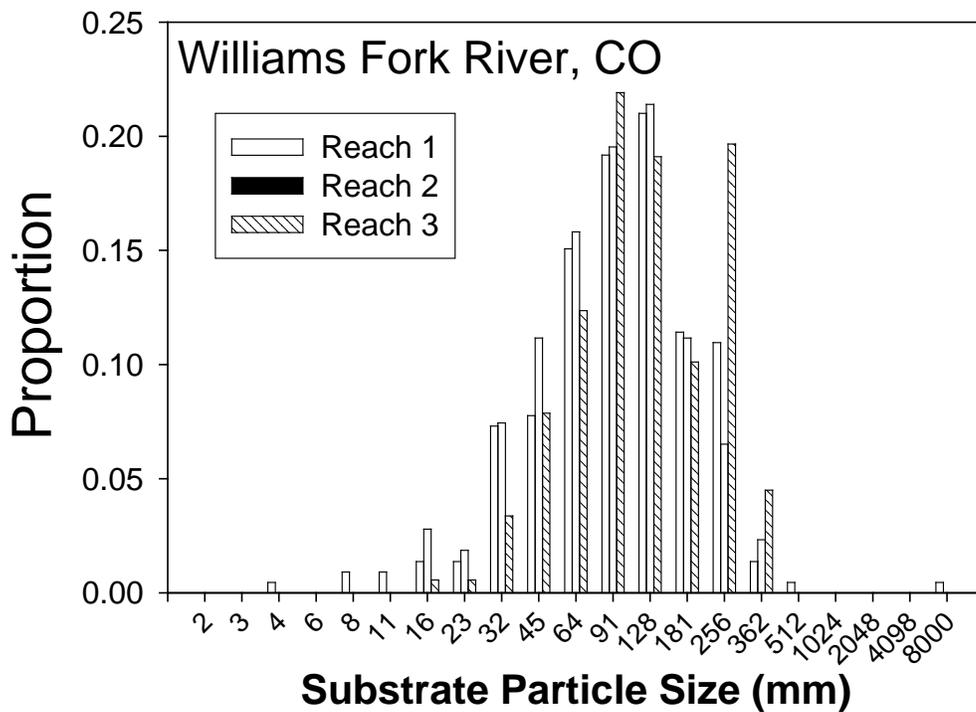


Figure A2. The particle size frequency distribution and the cumulative particle size distribution for the Williams Fork River, CO. The D50 and D84 values are indicated in the cumulative distribution figure.