

EVALUATION OF DISTRIBUTION AND FISH PASSAGE IN RELATION TO
ROAD CULVERTS IN TWO EASTERN MONTANA PRAIRIE STREAMS

by

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ABSTRACT

Road culverts can restrict passage of fish migrating between seasonal habitats. The development of new roads, as well as the repair and upgrade of existing roads, has led to research addressing the effects culverts have on fish populations. The majority of this research has focused on salmonid species, and the effect of culverts on movements of small-bodied, weak swimming species is largely unknown. Fish passage within an assemblage of prairie fishes was examined in two tributaries of the lower Yellowstone River having a variety of culvert types. Passage restriction at culverts was determined using a combination of existing fish passage models, downstream displacement experiments, and patterns of longitudinal fish distribution above and below culverts. Fish movement during experiments was not significantly different through culvert versus natural reaches for most species ($P > 0.05$), however longnose dace passage was significantly restricted. Additionally, few differences were observed in relative abundance and species richness above and below culvert crossings. FishXing modeling also revealed that study culverts were capable of passing some species for a portion of the study period. A survey of culverts throughout much of eastern Montana showed that the conditions observed in study culverts were typical of many low gradient, prairie streams. Most culverts in the survey had small outlet drops, low gradients, contained natural substrate, and low water velocities similar to those of natural reaches. Our results suggest that in these conditions, culverts may allow for adequate passage of most prairie species. However, more research is needed to determine what thresholds in these variables negatively influence passage of prairie fishes.

INTRODUCTION

Culverts are among the most common structures used to convey water where roads intersect small streams (Baker and Votapka 1990). The large number of tributaries found in both mountain and prairie stream systems, coupled with the increasing number of road systems, can equate to large numbers of culvert crossings fish populations may encounter (Coffman 2005). Concern over the implications of potential barriers to movement has led to considerable research and an increased emphasis on providing adequate passage for fish species (Baker and Votapka 1990).

Studies have shown that culverts can act as upstream migration barriers to various species of fish for a host of reasons (Belford and Gould 1989; Warren and Pardew 1998; Rajput 2003; Burford 2005; Gibson et al. 2005). Hydraulic characteristics that can limit upstream movement of fish include high water velocities, large outlet drops, low water depths, lack of resting habitats both within the culvert and in the downstream plunge pool, and disorienting turbulent flows (Furniss et al. 1991). The biological repercussions of these hydraulic factors are likely different for each of the species that inhabit small streams as body type and swimming capability differ among species and fish size (Katapodis and Gervais 1991).

Historically, much of the emphasis of road culvert studies has been placed on salmonid species (Belford and Gould 1989; Harper and Quigley 2000; Kane et al. 2000; Burford 2005). This is likely due to the economic and ecological implications of restricted fish passage to such highly migratory species. In contrast, relatively few studies have examined the effects of culvert barriers on prairie fish assemblages (Warren

and Pardew 1998; Toepfer et al. 1999; Rajput 2003). However, because prairie fish evolved in low gradient systems, it may be reasonable to assume them to have lower swimming and jumping capabilities than large-bodied, migratory salmonids. Therefore culverts likely represent an even greater challenge to smaller-bodied, weaker swimming species (Katapodis 2005). While small prairie fishes may not hold the same direct economic value as salmonids, they do contribute to the overall biologic diversity of prairie aquatic ecosystems. Additionally, several species of prairie fish, including the Topeka shiner (*Notropis topeka*) and leopard darter (*Percina pantherina*), are listed as threatened or endangered, thereby justifying the need for research addressing the effects of culvert crossings (Toepfer et al. 1999; Schaefer et al. 2003). More specifically, small prairie fishes comprise a considerable portion of the native species (22 out of 56) found in Montana (Montana Fish, Wildlife and Parks 2007).

Fish movement in warm water streams has been documented. Cyprinids, which were once assumed to be relatively stationary in regards to their home ranges, have been shown to migrate for spawning purposes (Linfield 1985; Lucas 2000; Bonneau and Scarnecchia 2002), and also to move from resting to feeding habitats (Clough and Ladel 1997). Recent studies have also identified the importance of habitat connectivity at multiple scales for the conservation of small, nonsalmonid stream fishes (Labbe and Fausch 2000; Fausch 2002; Dodds et al. 2004).

Upstream or downstream migration barriers could initiate the loss of species in areas that chronically dewater. Winston et al. (1991) identified the extirpation of four cyprinids as a result of dam construction on a prairie stream in Oklahoma. While there are large differences between dams and culverts, this example shows that a total barrier

can result in local extirpation and a decrease in species richness above that barrier. More specifically, Rajput (2003) found significantly lower species richness as well as fish abundance upstream of low-water bridges, which were constructed of several small diameter metal culverts with a concrete slab poured over the top. Because seasonal dewatering is common in prairie systems, recolonization is a key factor affecting species persistence (Dodds et al. 2004). Therefore, culverts, like other landscape disturbances that prevent or impede movement, could potentially affect the rate of recolonization after local extinctions (Sheldon and Meffe 1994).

Research on passage abilities of small-bodied fish species has typically been achieved through direct observation, laboratory studies, and indirect measures such as software modeling and comparisons of upstream and downstream fish assemblages. Direct observation techniques include ‘passive’ release and recapture of marked fish (Warren and Pardew 1998; Rajput 2003; Coffman 2005) as well as ‘active’ displacement experiments (Belford and Gould 1989; Burford 2005). These studies provide useful information regarding the passage capabilities of multiple fish species as well as identifying the types of crossings that restrict passage. For example, Warren and Pardew (1998) used ‘passive’ mark-recapture techniques to examine the effects of four different types of stream crossings on movement of 15 small stream fish species in Arkansas. In their study, movement was found to be an order of magnitude lower through culverts than through open box and ford crossings and natural reaches. However, mark-recapture studies of this type are typically conducted over long periods of time, and therefore the exact conditions that either permitted or prohibited passage are not known. In addition, it is unclear whether restriction of fish passage is due to actual physical conditions at the

road crossing or else a lack of motivation to move upstream (Coffman 2005). ‘Active’ fish passage experiments using downstream displacement can be effective at overcoming the motivation question and in quantifying the conditions during time of passage. However, such experiments are limited by providing only information about passage efficiency during relatively short time intervals, and therefore results may not be representative of culvert conditions and passage success during other times of the year (Burford 2005).

Passage success has also been estimated from laboratory studies to define thresholds in fish swimming and jumping capabilities. These thresholds can then be compared to hydraulic conditions commonly produced by culverts to assess fish passage (Toepfer et al. 1999; Gardner 2006). For example, Toepfer et al. (1999) found that water velocities of 25 cm/s produced the greatest amount of swimming activity of leopard darters in a controlled flume. This velocity equated to the maximum velocity and distance these fish could travel to traverse the length of culverts. Many of the culverts found in their study area produced water velocities greater than 25 cm/s indicating that these culverts may restrict upstream passage. Limitations of laboratory-based swimming capacity tests are typically related to differences between tightly controlled environments and conditions observed in field settings (Castro-Santos 2004). For example, motivation to swim upstream in natural settings involves many different cues, from physiological to chemical. In contrast, many laboratory studies use prodding and electrical stimulation to provide motivation which may not be directly comparable (Castro-Santos 2004; Gardner 2006). Additionally, laboratory flumes can have more uniform water velocities than are typical of actual stream crossing structures (Castro-Santos 2004).

The software model FishXing has also been widely used to estimate fish passage (Rajput 2003; Burford 2005). The model combines field measurements of culvert characteristics with burst and prolonged swimming and jumping abilities from fisheries literature to estimate fish passage (Six Rivers Watershed Interactions Team 1999). Field measurements and swim speeds are input into the model via a user-friendly input screen complete with pull-down menus for many of the entries. Once the appropriate information has been entered into the model, the range of flows considered passable is calculated. The model also displays the factor involved in listing a culvert as a barrier. These factors include excessive velocities, excessive leap height, insufficient water depth, and shallow plunge pool depth. However, I found only a few studies that have used this model to assess culvert passage of small prairie fishes. Rajput (2003) used FishXing to evaluate the passage status of 28 low-water bridge crossings in Arkansas. This study found that the model was congruent with direct measurement of passage success based on passive mark-recapture estimates in 71% of the cases. Advantages of this assessment technique include the ability to assess passage status of a large number of culverts with relatively easily measured physical characteristics, and the ability to estimate the amount of time a culvert may be passable to a species of interest. However, model use is limited by the general lack of swimming capability information on many prairie fishes. Additionally, literature on field validation of this model appears to be limited. A combination of displacement experiments and FishXing modeling was used to examine fish passage through culverts for salmonid species in the Clearwater River drainage in western Montana (Burford 2005). In several cases, culverts were determined to be

barriers by the model, but readily passed fish upon direct observation suggesting the need for more field testing.

Differences in fish assemblages above and below barriers have also been used to examine passage restriction (Winston et al. 1991; Rajput 2003; McLaughlin et al. 2006). This approach can give a long term perspective on effects of passage restriction. For example, Winston et al. (1991) examined fish assemblages in the North Fork Red River in Oklahoma and found 25 species in reaches above Altus Dam versus 34 species below. This study occurred 40 years after construction of the dam, and shows that barriers to migration can have long term consequences that can be observed by simply examining species composition. McLaughlin et al. (2006) also documented lower minnow and sucker abundance upstream of low-head sea lamprey barriers in the Laurentian Great Lakes basin. This example shows how even partial barriers can affect fish distribution. Rajput (2003) also found significantly lower species richness and fish abundance in reaches upstream of culvert crossings in Arkansas, indicating that culverts can not only act as barriers, but can consequently affect the overall distribution of fishes. However, limitations to this type of assessment exist. Longitudinal differences in fish assemblage are common in prairie stream systems (Ostrand and Wilde 2002), making it difficult to determine whether differences in assemblage above and below culverts are related to passage restriction directly or else a result of the natural longitudinal changes in species composition (Schlosser 1990).

This study employed the combination of a variety of passage assessment tools - fish distribution patterns, displacement experiments, and FishXing modeling - to examine the effects of culverts on fish passage of eastern Montana prairie fishes. Fish movement

and culvert passage studies are not uncommon to Montana (Belford and Gould 1989; Schmetterling and Adams 2004; Burford 2005). However, to my knowledge, there have been no studies specifically addressing passage capabilities of eastern Montana prairie fishes. Recent interest in prairie fish conservation has led Montana Fish, Wildlife and Parks to conduct a prairie stream inventory throughout much of eastern Montana (MT FWP 2003). Additionally, knowledge of fish assemblages in this region is vital for assessing potential impacts of high road densities and water quality and quantity changes associated with proposed energy development. The proposed oil and gas development in eastern Montana includes over 24,000 km of roads which could add significantly to the number of road crossings (Bureau of Land Management 2003). The overall goal of this study is to enhance knowledge of prairie systems and to examine how road crossings affect little-known prairie fish assemblages.

The specific objectives for this study were to: 1) identify and quantify the types and characteristics of stream crossings common to eastern Montana prairie streams; 2) examine the passage capabilities of common prairie fish species and the physical and hydraulic conditions that may influence their passage; and 3) examine the longitudinal distribution of prairie fishes in systems in relation to culvert crossings. Because culverts have been shown to restrict the upstream migration of many species, I hypothesized that fish movement through culverts would be lower than through natural reaches (Belford and Gould 1989; Warren and Pardew 1998; Rajput 2003; Burford 2005). Additionally, I hypothesized that species richness and relative abundance would be lower for fish assemblages above culverts relative to those below.

STUDY AREA

The Yellowstone River drainage near Glendive, Montana, was chosen for this project for the species-rich tributaries found in its lower reaches and variety of road crossing structures. The geography of this area is typified by relatively low precipitation, and soils that have little capacity to hold water (Morris et al. 1981). As a result, hydrographs regularly show flashy responses from storm and runoff events, and culverts must typically be sized to accommodate large amounts of water. Peak runoff in the small creeks generally occurs in late winter to early spring peak as a result of lowland snowmelt (Elser et al. 1980).

Sand Creek and Clear Creek, two direct tributaries of the Yellowstone River, were chosen as study streams because both streams had sufficient water flow to ensure fish presence during both high and low flow periods. Preliminary surveys revealed that many eastern Montana prairie streams remain dewatered for a majority of the year, so only streams that flowed for much of the summer were considered for this study. Study streams were located in Dawson County, and flow southerly, crossing Interstate 94 before joining the Yellowstone River several kilometers downstream (Figures 1 and 2).

Irrigated agriculture is the predominant land use in the study area. The Glendive Unit of the Buffalo Rapids Irrigation Project controls an irrigation canal system that runs from Fallon to Glendive, Montana (U.S. Department of the Interior, Bureau of Reclamation 2007). Built between 1939 and 1941, the canal intersects all tributaries that flow southerly into the Yellowstone River in this area. Most streams have siphons installed, and the canal has no direct interaction with the actual stream. However, head-

gates are installed upstream of the siphons in the canal, and water is periodically released back into the tributaries for irrigation, as well as to accommodate the channel constrictions at the siphon entrance. This periodic release of water to the tributaries can be quite large depending on water demands, and appears to have altered the channel morphology of stream reaches below the canal intersection. Additionally, the number of fish entering the system via the canal, and the effect this irrigation water has on streamflow and chemical properties remains unknown (Morris et al. 1981). However, advantages of this canal system to this project were the ability to investigate fish passage at varying water flows and for a longer duration than most prairie streams which may only contain water for short periods during spring runoff.

The fish assemblage within the study area is typical of many prairie streams in southeastern Montana (Elser et al. 1980). Both Sand and Clear Creek contain rich fish assemblages, with up to 19 species occupying reaches in the study area (Montana Rivers Information System, MFISH). Although non-native fish such as green sunfish (*Lepomis cyanellus*) and black bullhead (*Ameiurus melas*) are commonly found in both streams, the majority of the assemblage is comprised of native species.

Throughout the rest of this document, fish species are referred to by their common name. Table 1 identifies the formal name and abbreviation of each species mentioned.

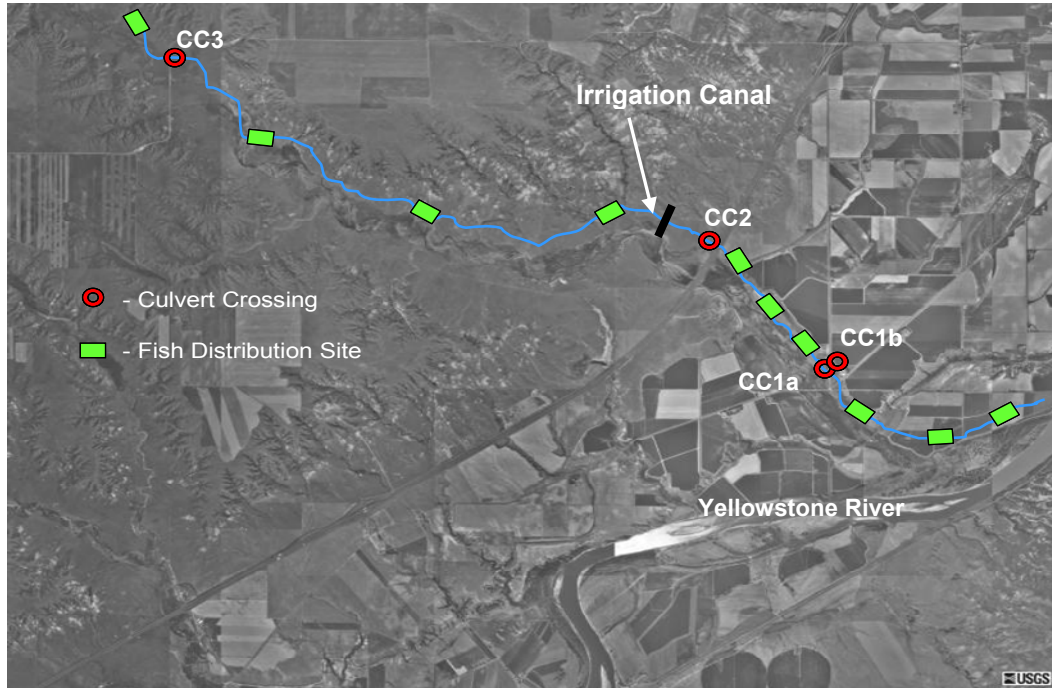


Figure 1: Aerial view of Clear Creek study area.



Figure 2: Aerial view of Sand Creek study area.

Table 1: Common names, formal names, and abbreviations of fish species.

Common Name	Formal Name	Abbreviation
Black Bullhead	<i>Ameiurus melas</i>	BLBU
Blacknose Dace	<i>Rhinichthys atratulus</i>	BLDA
Bluehead Chub	<i>Nocomis leptcephalus</i>	BLCH
Brassy Minnow	<i>Hybognathus hankinsoni</i>	BRMI
Brook Stickleback	<i>Culaea inconstans</i>	BRST
Channel Catfish	<i>Ictalurus punctatus</i>	CHCA
Brown Trout	<i>Salmo trutta</i>	BRTR
Common Carp	<i>Cyprinus carpio</i>	COCA
Creek Chub	<i>Semotilus atromaculatus</i>	CRCH
Emerald Shiner	<i>Notropis atherinoides</i>	EMSH
Fathead Minnow	<i>Pimephales promelas</i>	FAMI
Flathead Chub	<i>Platygobio gracilis</i>	FLCH
Goldeye	<i>Hiodon alosoides</i>	GOEY
Goldfish	<i>Carassius auratus</i>	GOFI
Green Sunfish	<i>Lepomis cyanellus</i>	GRSU
Leopard Darter	<i>Percina pantherina</i>	LEDA
Longnose Dace	<i>Rhinichthys cataractae</i>	LODA
Longnose Sucker	<i>Catostomus catostomus</i>	LOSU
Mountain Sucker	<i>Catostomus platyrhynchus</i>	MOSU
Northern Redbelly Dace	<i>Phoxinus eos</i>	NORD
Northern Plains Killifish	<i>Fundulus kansae</i>	NOPK
River Carpsucker	<i>Carpiodes carpio</i>	RICS
Sand Shiner	<i>Notropis stramineus</i>	SASH
Shorthead Redhorse	<i>Moxostoma macrolepidotum</i>	SHRE
Stonecat	<i>Noturus flavus</i>	STCA
Topeka Shiner	<i>Notropis topeka</i>	TOSH
White Sucker	<i>Catostomus commersonii</i>	WHSU

METHODS

Culvert Survey

To identify the types of road crossing structures common to eastern Montana prairie streams, an initial survey of tributaries of the upper Musselshell River, the Little Missouri River, and the lower Yellowstone River was conducted in February 2005. This survey was led by Montana Department of Transportation personnel, and included stream crossings listed as important to agency biologists. All of the stream crossings examined occurred on Montana county road crossings. At each crossing, height, width, length, and slope of the structure as well as the outlet drop were measured. Additionally, the structure material and amount of substrate present in the structure was noted, and photographs and GPS coordinates were also taken to document culvert type and conditions at each site. It is important to note that this survey was not a random sample of all culverts found in eastern Montana, but rather comprised a range of crossings with passage concerns from local agency biologists. Surveyed culverts were limited to those that occurred on county road crossings and state and federal highways due to private landowner access considerations, and how representative they are of all road crossings is uncertain.

Fish Passage Assessment

Following the initial survey, a combination of direct and indirect assessment techniques were used to examine passage capabilities for prairie fish species. Three different techniques were used to assess five culvert crossings on both Sand and Clear

Creek during 2005 and 2006. To assess potential large-scale effects of passage restriction from road crossings on fish distribution, I examined longitudinal distribution at a series of sites positioned downstream and upstream of road crossings within each study stream. At each culvert, I then examined fish passage directly using fish displacement experiments at varying flows throughout the summer of 2006. Fish passage was also assessed indirectly using the FishXing model to quantify the amount of time and under what flow conditions each crossing may be passable for various species.

Longitudinal Distribution

Species composition and relative abundance were measured by sampling several reaches downstream and upstream of all culverts throughout each study stream. Fish sampling reaches were 300 m in length to ensure that all species were collected (Patton et al. 2000). Two to three, 300-m long sites were established above and below each road crossing starting at the junction of the Yellowstone River (Figures 1, 2). Only one site was sampled above the uppermost culvert crossing due to private landowner access considerations (Clear Creek) or lack of water (Sand Creek). Site locations were chosen roughly equidistant from one another throughout the stream. At each site, fish were captured using 6.35-mm mesh seines. Seining was conducted downstream, with individual hauls occurring about every 10 to 20 meters. Fish were placed in aerated live-wells, counted and identified to species, and released. A random subset of 20 fish per species was measured for total length. Voucher samples of up to five fish per species were preserved in a 10% formalin solution, and retained for later identification. Each site was sampled during spring (May-June) and summer (July-August) to account for

temporal variation in distribution. Data from the spring and summer samplings were combined at each site to compensate for fish recruiting to the seine mesh size as summer progressed.

Habitat measurements were also taken at each site following the protocol developed for eastern Montana prairie streams by Bramblett (2003) (Appendix A). Variables measured included: thalweg depth, channel width, dominant substrate, water temperature, and water turbidity. Thalweg depths and dominant substrate type were recorded every 3 meters, and channel widths were measured every 30 meters while moving downstream, starting at the upstream end of the site. Water temperature and turbidity were measured at the midpoint of each site prior to entering the stream so that sediments were not disturbed.

To examine whether road crossings were affecting overall distribution of fish within the study area, I first used Spearman's rank correlation analysis (Quinn and Keough 2002) to examine the relationship between stream distance from the mouth and relative abundance of the five most common fish species collected. Correlation coefficients closer to the value 1 would indicate a strong relationship between distance from the mouth and species abundance. Relative abundance was calculated as the total number of fish collected in both spring and summer samplings for each site. Stream distance from the mouth was measured using global positioning system (GPS) waypoints measured at the center of each site and a geographic information system (Arc GIS). Stream distance (in meters) was measure between each GPS waypoint using the manual measuring tool in Arc GIS.

Mean species richness and mean relative species abundance were compared between sites immediately upstream and downstream of each crossing using Mann-Whitney U-tests. Species richness was calculated as the total number of species collected at each site. Mean species richness and species abundance were calculated using the three sites immediately upstream and downstream of each crossing. To account for potential differences in fish distribution related to habitat features, habitat variables including mean thalweg depth and mean wetted width were also compared upstream and downstream of culvert crossings using Mann-Whitney tests.

Fish Passage Experiments

Actual passage success through culverts was examined through the use of a fish displacement experiment. Stream sections at each study culvert were divided into treatment and reference reaches (Figure 3). The treatment reach was separated into upstream and downstream segments by the culvert whereas a 'reference culvert' separated the reference reach into upstream and downstream segments. The reference culvert was a length of natural stream channel equal to the length of the study culvert in the treatment reach. The length of the upstream and downstream segments was generally equivalent to the length of the plunge pool downstream of the study culvert. Block nets constructed of black plastic netting (6.35-mm mesh size) and supported by metal t-posts, were positioned at the upstream and downstream end of the treatment and reference reaches to ensure a closed system during each trial.

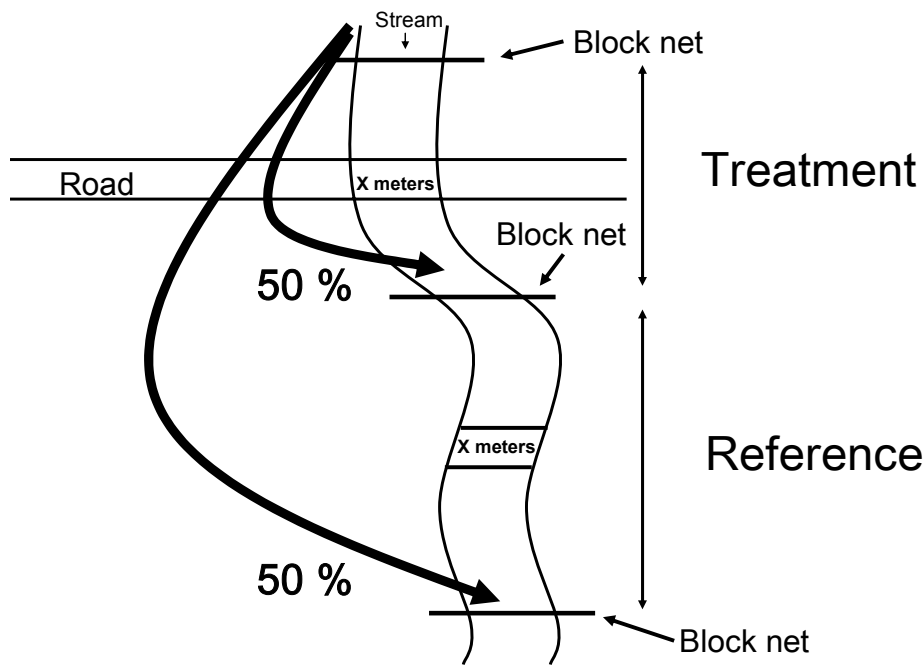


Figure 3: Fish passage experiment study design.

At the start of a trial, fish were collected upstream of the study area using 6.35-mm mesh seines. Seining was conducted downstream following similar protocols as in the longitudinal distribution surveys. Fish were placed in an aerated live well to avoid incidental mortality. After at least 20 fish of each of the predominant species had been collected, fish were identified to species, measured to total length, randomly split into treatment and reference groups, and marked using a Visible Implant Elastomer (VIE) tag, with tag color unique to each group. VIE tags were chosen as the method of marking because of their adaptability to a number of species and size classes, and likely low effect on swimming capability (Northwest Marine Technology 2006). Although fish as small as 20 mm have been successfully tagged with VIE tags (Frederick 1997), I tagged only

fish greater than 60 mm. Initial trials showed that the proximal margin of the anal fin was a suitable tag location in most species found within the study area. Because, species, tag color, and tagging error can influence tag retention (Roberts and Angermeier 2004), I conducted a pilot study to determine the retention of VIE tags. Tag retention was assessed by marking 30 fish representing the predominant species (creek chub and white sucker) and size classes with VIE tags. Tagged fish were then placed with unmarked fish in an in-stream cage for 48 hours. Fish were then examined independently for tag presence by a field technician not involved with the initial tagging, and retention was determined as the proportion of fish retaining tags. The pilot study retention was 100%, indicating that this method of tagging was appropriate for this study. Additionally, no mortality associated with the tagging procedure was observed.

Fish were randomly placed into reference and treatment groups during each trial so that fish length was similar between groups. Mann-Whitney U-tests were used to test whether the lengths of fish used in treatment groups differed from those in reference groups ($\alpha = 0.05$). Tests were conducted for all species combined as well as individual species. Of the fish that were marked, creek chub lengths were not significantly different in six of eight trials (Mann-Whitney $P = 0.28 - 0.97$). However, in two trials creek chub length was significantly larger for marked treatment fish (+ 8.5 – 19.8 mm) than for reference fish (Mann-Whitney $P = 0.005$). For all other species, marked fish lengths were not significantly different between treatment and reference groups for all ten trials (Mann-Whitney $P = 0.08 - 0.85$).

Following marking, test fish were allowed to recover in an aerated live well, and then released near the downstream block net in both the treatment and reference reaches

(Figure 3). Fish were displaced downstream to use the homing instinct as motivation to swim through the reach of interest. Evidence in the literature suggests that stream fishes use the homing tendency to return to natal streams for spawning (Linfield 1985), and to return to resting areas after migration to and from feeding areas (Clough & Ladle 1997). Additionally, downstream displacement has been used successfully as a measure of passage efficiency in other fish passage studies (Belford and Gould 1989; Burford 2005). Each trial was allowed to run for 48 hours.

Due to an abundance of organic material found in these types of streams, screens were occasionally installed upstream of each block net to reduce deposition. Additionally, block nets were periodically monitored and debris was removed when necessary. At the end of the 48-hour test period, additional block nets were erected to isolate each upstream and downstream segment above and below the treatment and reference culvert. Fish were then removed from each segment using multiple pass seining and backpack electrofishing until no further fish were collected. Fish were examined for VIE tags, identified to species, and measured to total length. Any fish with VIE tags collected upstream of the actual or reference culvert were considered to have passed through the reach of interest. Fish captured below either of these culverts were assumed to have remained stationary. Attempts were made to capture fish in both the treatment and reference culverts segments, but sampling inside the culvert barrel was inefficient. Therefore efforts to sample the culvert segments were abandoned. Experiments were run twice at each road crossing within the study streams with efforts made to represent local flow variation. Attempts were made to conduct a third trial

during high flow events, but were unsuccessful when block nets collapsed from debris accumulation.

Upon completion of each experiment, water depth and velocity were measured in both the treatment and reference culvert. Measurements were taken at five equidistant points (left bank, left-center, center, right-center, and right bank) along four equidistant transects located in each reach. These values were then averaged and compared using Mann-Whitney U-tests ($\alpha = 0.05$). Additionally, mean thalweg depth and mean channel width were calculated for the upstream and downstream segments for treatment and reference reaches. Thalweg depth was measured at 1.5-m intervals, whereas wetted width was measured at 3-m intervals progressing downstream from the top of each segment. Data from the upstream and downstream segments were combined, and comparisons between treatment and reference reaches were made using Mann-Whitney tests. Habitat variables measured at crossing CC3 were conducted when the stream was dry. Therefore thalweg depth and wetted width were measured as bankful depth and width respectively.

The proportion of fish that passed through reference and treatment reaches was compared using 2x2 chi-square contingency tables and an odds ratio test (Quinn and Keough 2002). The odds ratio is the proportion of fish that moved through the treatment culvert divided by the proportion of fish that moved through the reference culvert. Odds ratio values ≥ 1 suggest that fish movement through study culverts equals or exceeds that of reference culverts, and therefore no restriction in passage occurred. Odds ratio values < 1 suggest restricted passage, with less movement occurring through study culverts than

through natural reaches. Odds ratios are considered statistically significant if 95% confidence intervals do not contain 1 (Quinn and Keough 2002, McLaughlin et al. 2006).

Simple linear regression was used to examine which physical and hydraulic characteristics associated with culverts may be influencing fish passage. Fish species having restricted passage as identified by odds ratios were used in this analysis. The ratio of marked fish recaptured upstream of the culvert to those captured downstream was used as a passage index, and plotted against culvert slope, culvert length, and mean water velocity and examined for significant relationships ($\alpha = 0.05$). To compare species with and without restricted passage, a fish species not considered as having restricted passage by the odds ratio test was also analyzed using the same methods.

Recapture efficiency using seines and backpack electrofishing appeared to vary in relation to in-stream habitat and turbidity. Therefore, recapture efficiency was measured at a subset of study sites. To determine recapture efficiency, segments upstream and downstream of the culvert were closed at each end using 6.35-mm block nets. Thirty fish comprised of the dominant species captured at a site were then marked with a pelvic fin clip, and placed in test segments. After 48 hours, the same method of recapture (seining and electrofishing) was used to collect the fish in each segment. Fish were counted and examined for fin clips after each pass with the seine and with the electrofisher. Percent recapture efficiency was calculated as the total proportion of fish recaptured after three passes of seining and three passes of electrofishing. The experiment resulted in an average recapture of 56.7% (± 6.9 SE) in 4 trials. Because I found no difference in recapture efficiency with respect to the side of the culvert, the fact that not all fish were

typically recaptured in any reach should not bias results based on upstream-downstream comparisons.

Modeling Using FishXing

The fish passage experiments I conducted were limited to two flow periods. To assess fish passage over the full range of flow conditions during a spring-summer hydrograph, I used the FishXing software model. FishXing analysis was conducted for each study culvert with each of the species used in the displacement experiments (creek chub, flathead chub, longnose dace, and white sucker). FishXing uses prolonged and burst swim speed data from the literature to predict if fish will pass through a culvert at a particular flow. However, as a result of limited information on the swimming capabilities of these species, I used surrogate species for some of these analyses (Table 2). For white suckers, I used the prolonged swim speed provided in FishXing, but used burst speed data from longnose suckers since this information was lacking for white suckers. Similarly, for flathead chub I used the prolonged swimming speed supplied by the model, but used burst speed data for goldfish. I chose goldfish burst swimming speed data for all of the cyprinids because they were the cyprinid species having swim speed data in FishXing most similar in body size to the minnows encountered in the study area. Additionally, for longnose dace, I used the prolonged speed for blacknose dace and the burst speed data for goldfish. The only species I could not find a suitable surrogate for was creek chub. For this species, I used the critical speed for bluehead chub of 0.86 cm/s (Gardner 2006) and goldfish burst speed data.

Table 2: Swimming capabilities and surrogate species used in the FishXing model.

Fish Species Modeled	Prolonged Swim Speed Surrogate Species	Prolonged Swim Speed (cm/sec)	Burst Speed Surrogate Species	Burst Speed (cm/sec)
White sucker	White sucker	51	Longnose sucker	182
Flathead chub	Flathead chub	44	Goldfish	137
Longnose dace	Blacknose dace	38	Goldfish	137
Creek chub	Bluehead chub	86*	Goldfish	137

* From Gardner (2006)

The physical and hydraulic measurements required by FishXing were collected at each culvert crossing. Measurements included culvert shape and dimensions, culvert material, corrugation dimensions, culvert entrance loss coefficient, plunge pool and tailwater depth, and culvert outlet elevation. Culvert length, slope, and tailwater and channel cross sections were recorded using survey equipment and the assistance of an engineer.

To determine stream discharge, I installed stream gauging equipment at each road crossing. Water height data loggers (TruTrack, New Zealand) were mounted inside PVC stilling wells (Rantz et al. 1982), and set to record water height and stream temperature once per hour. Stilling wells were installed as close as possible to the culverts, and followed USGS stream gauging guidelines (Carter and Davidian 1968). Discharge was then measured at each stream crossing using a Marsh-McBirney velocity meter in conjunction with a standard top-setting rod. The USGS Six-tenths-depth method for measuring discharge was used because the majority of study sites routinely experienced water depths between 9.1 and 76.2 cm (Buchanan 1969; Rantz et al. 1982). Discharge was measured a minimum of five times throughout each summer, and was measured at a

variety of flows to represent the range of conditions found throughout the year. The resulting data were used to create stage-discharge relationships which produced estimated hydrographs for the duration of the study (Carter and Davidian 1968). These hydrographs were used to obtain the high and low passage flows necessary for the FishXing model.

Literature suggests that without model calibration, FishXing may not accurately reflect the environmental conditions observed at an individual culvert (Karle 2005). To address this potentially confounding factor, I calibrated the model using field measurements of water depth taken at the inlet and outlet of the study culverts and compared observed depth data to that predicted by the FishXing model. Mannings-n values within the culvert and in the downstream cross section were then adjusted in the model until the predicted water depths were within 15.2 cm of field observations.

To assess the amount of time study culverts were passable I ran the calibrated model for each of the study species. Passage 'windows' were then calculated to identify the amount of time each culvert crossing was passable for the four species. To determine the upper and lower flows that bracket the estimated passage windows, hourly data from stream gauging equipment was used in conjunction with the range of flows predicted as passable by FishXing to quantify the percentage of time a culvert was passable from late March to November 2006. Additionally, the highest flows observed passing fish during displacement experiments were included to adjust upper discharge boundaries where the flow rate during the passage event was outside the range deemed passable by the FishXing model. In contrast, I did not adjust passage window boundaries lower where passage was predicted but not observed as in these cases, failure to observe passage may have been a result of recapture inefficiency.

Simple linear regression was used to examine the relationship between culvert characteristics and the amount of time a culvert was considered passable. The amount of time considered passable using the passage window (including the displacement experiment) discharges was plotted against culvert length and slope. This analysis was conducted for each species used in the FishXing analyses.

RESULTS

Culvert Survey

Descriptions of the 34 stream crossings examined during the February 2005 culvert survey are listed in Table 3. Of the 34 stream crossings, 15 were multiple culvert, 10 were single culvert, 6 were low-water bridges (2-3 small culverts with concrete poured over the top), 2 were bridges, and 1 was a concrete box culvert crossing. Single culvert crossings consisted of mostly corrugated metal pipe (CMP) materials (8 out of 10), whereas multiple culvert crossings were a mixture of CMP and structural steel plate (SSP) materials. All of the culverts found in low-water bridges were constructed of CMP (Table 2). The 34 stream crossings comprised a total of 58 culverts. Mean culvert length was $28.9 \text{ m} \pm 3.4 \text{ SE}$ (standard error), however most culverts (46 of 58, 79%) were less than 30 m in length (Figure 4). Culvert slopes were relatively low with a mean value of $1.2\% \pm 0.1 \text{ SE}$ (Figure 4). Mean outlet drop height was $17.2 \text{ cm} \pm 4.9 \text{ SE}$, although most culverts (41 of 58, 71%) had outlet drop heights of 0 cm (Figure 4).

The study sites selected from the culvert survey were generally representative of the 58 culverts examined, other than the fact that most culverts observed during the survey were dry. Study culvert length ranged from 14.0 – 70.7 m with a mean of $26.7 \text{ m} \pm 7.5 \text{ SE}$. Culvert slopes ranged from 0 – 1.8% with a mean of $1.1\% \pm 0.3 \text{ SE}$. Most study culverts (6 of 7, 86%) had an outlet drop of 0 cm, and the greatest outlet drop was only 5.1 cm (Table 4).

Table 3: Location and characteristics of culverts at selected stream crossings during survey of eastern Montana streams, February 2005. CMP refers to corrugated metal pipe, and SSP to structural steel plate.

Crossing	Drainage	Structure Type	Material	# of Culverts	Latitude / Longitude
1	Musselshell River	Multiple Culvert	CMP	3	N 46.594 W 109.684
2	Musselshell River	Single Culvert	CMP	1	N 46.240 W 109.136
3	Musselshell River	Single Culvert	CMP	1	N 46.256 W 109.052
4	Musselshell River	Bridge	Concrete	0	N 46.264 W 108.951
5	Musselshell River	Multiple Culvert	CMP	2	N 46.648 W 108.008
6	Musselshell River	Multiple Culvert	SSP	2	N 46.681 W 108.008
7	Musselshell River	Multiple Culvert	CMP	2	N 46.683 W 108.008
8	Yellowstone River	Single Culvert	SSP	1	N 45.958 W 108.343
9	Yellowstone River	Multiple Culvert	CMP	2	N 45.956 W 108.364
10	Yellowstone River	Multiple Culvert	CMP	3	N 47.134 W 104.945
11	Yellowstone River	Bridge	Concrete	0	N 47.096 W 104.763
12	Yellowstone River	Single Culvert	CMP	1	N 47.035 W 104.796
13	Yellowstone River	Multiple Culvert	SSP	3	N 47.040 W 104.810
14	Yellowstone River	Multiple Culvert	SSP	2	N 47.010 W 104.834
15	Yellowstone River	Multiple Culvert	SSP	3	N 46.962 W 104.839
16	Yellowstone River	Multiple Culvert	CMP	3	N 46.976 W 104.857

Table 3 continued

17	Yellowstone River	Multiple Culvert	CMP	3	N 46.924 W 104.935
18	Yellowstone River	Multiple Culvert	SSP	3	N 46.935 W 104.941
19	Yellowstone River	Single Culvert	CMP	1	N 47.037 W 104.391
20	Yellowstone River	Box Culvert	Concrete	1	N 47.036 W 104.358
21	Little Missouri River	Low Water Bridge	CMP	3	N 46.948 W 104.182
22	Little Missouri River	Low Water Bridge	CMP	2	N 46.860 W 104.180
23	Little Missouri River	Low Water Bridge	CMP	2	N 46.816 W 104.217
24	Little Missouri River	Low Water Bridge	CMP	2	N 46.772 W 104.222
25	Little Missouri River	Low Water Bridge	CMP	2	N 46.709 W 104.234
26	Little Missouri River	Low Water Ford	CMP	1	N 46.584 W 104.108
27	Yellowstone River	Multiple Culvert	SSP	2	N 46.185 W 104.733
28	Yellowstone River	Single Culvert	CMP	1	N 46.031 W 104.796
29	Yellowstone River	Multiple Culvert	CMP	2	N 45.978 W 106.644
30	Yellowstone River	Single Culvert	CMP	1	N 46.079 W 106.939
31	Yellowstone River	Single Culvert	SSP	1	N 46.094 W 106.935
32	Yellowstone River	Single Culvert	CMP	1	N 46.098 W 106.933
33	Yellowstone River	Single Culvert	CMP	1	N 46.229 W 106.944
34	Yellowstone River	Multiple Culvert	CMP & SSP	2	N 46.258 W 106.935

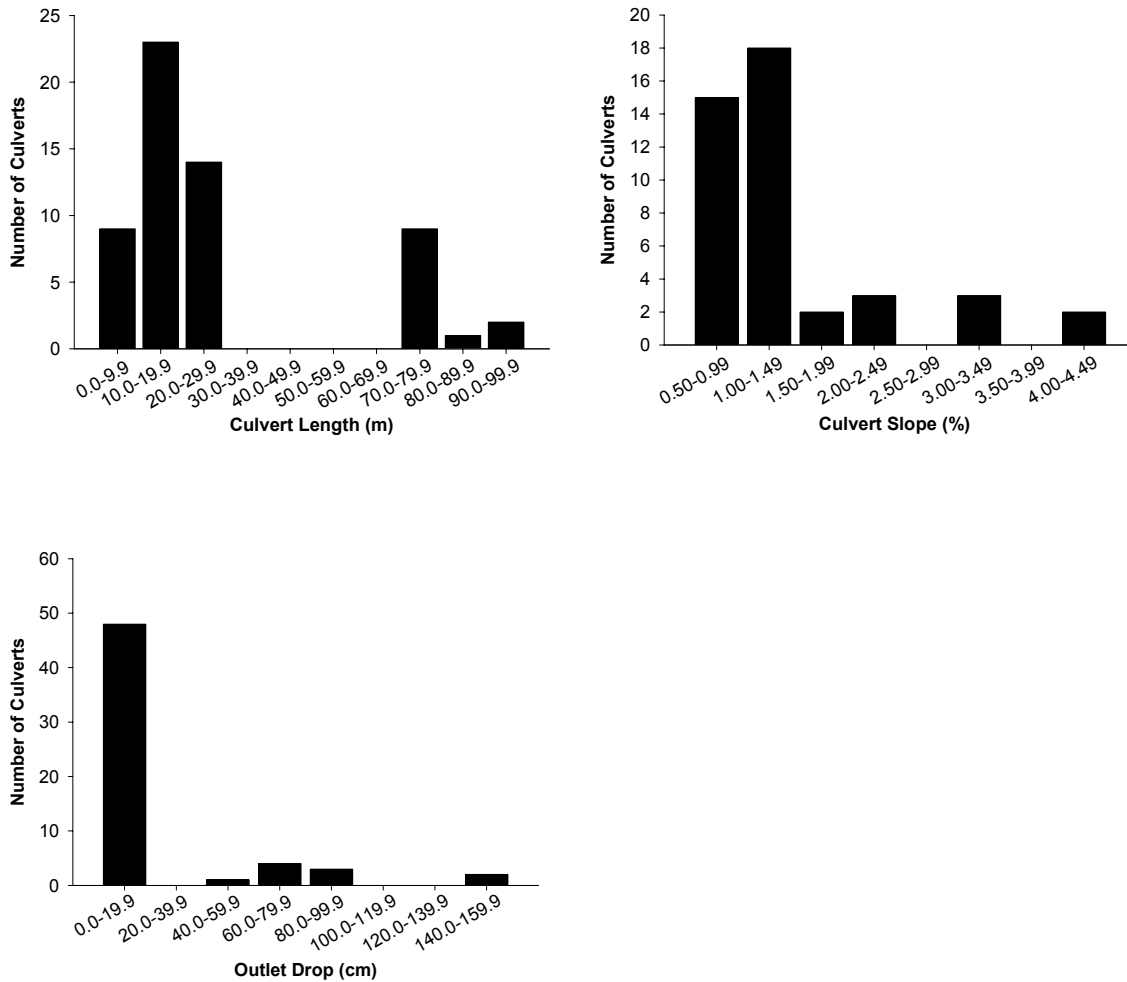


Figure 4: Histograms of culvert length, slope, and outlet drop for the 58 culverts examined during the eastern Montana tour (February 2005).

Table 4: Characteristics of the five study culvert crossings. Letters in parentheses indicate left, center, and right culverts at crossing CC3.

Stream	Crossing	Length (m)	Width (m)	Height (m)	Slope (%)	Outlet Drop (cm)
Clear	CC1a	19.7	3.4	2.1	0.37	0.0
Clear	CC1b	14.0	1.5	1.5	0.0	0.0
Clear	CC2	70.7	4.6	3.0	0.55	5.1
Clear	CC3 (l)	18.4	1.2	1.2	1.66	0.0
Clear	CC3 (c)	18.4	1.2	1.2	1.59	0.0
Clear	CC3 (r)	18.4	1.2	1.2	1.85	0.0
Sand	SC1	27.1	2.4	2.4	1.58	0.0

Fish Passage Assessment

Longitudinal Distribution

Distributional sites for Clear and Sand creeks were sampled twice during the summers of 2005 and 2006. Clear Creek contained 21 fish species with creek chub, fathead minnow, longnose dace, sand shiner, brook stickleback and white sucker the six most common species (Figure 5). Brook stickleback were captured in numbers similar to that of sand shiner, but were only captured in occasional pool habitats, and therefore were not included in further analyses. Sand Creek contained 10 species with creek chub, fathead minnow, flathead chub, longnose dace, and sand shiner the five most common although overall abundances were much lower than in Clear Creek (Figure 5). Stream length was relatively limited in Sand Creek, resulting in too few sites above and below stream crossings for statistical analyses. Therefore, analyses were confined to Clear Creek data. Additionally, because the 2005 Clear Creek data did not include several of the sites established in 2006, I used only 2006 data in the analysis.

In general, species richness declined as distance from the stream mouth increased. The lowest reach on Clear Creek contained 18 species of fish, while the uppermost site contained 8 (Figure 6). However, mean species richness was similar above and below all road crossings (Mann-Whitney $P = 0.07 - 0.82$). In contrast no decrease in species richness was observed above the culvert crossing in Sand Creek. In that stream, species richness above the culvert was nearly twice that of below (Figure 7).

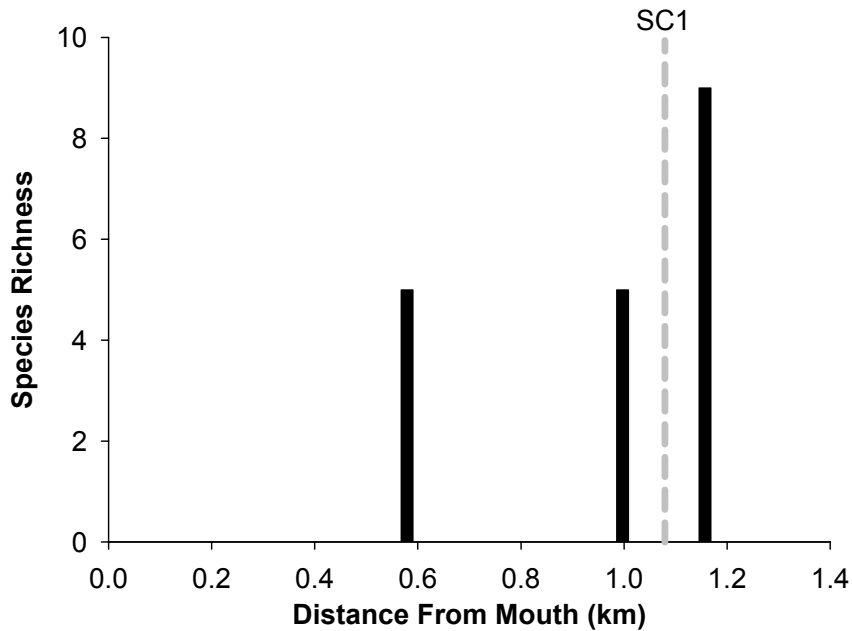


Figure 7: Species richness by distance from mouth for Sand Creek 2006.

A total of 5,103 creek chubs, 358 fathead minnows, 414 longnose dace, 147 sand shiners, and 1,312 white suckers were collected in 2006 in Clear Creek. Creek chubs were typically the most abundant species at all sites, however white sucker were collected in similar numbers in upper reaches (Figure 8). Few obvious patterns in longitudinal abundance relative to culverts are evident in Figure 8, however both fathead minnow and sand shiner abundances decrease as distance from the stream mouth increases, with sand shiners only found in the lower 6.0 km. Several patterns were observed for rarer species that were not used in statistical analyses. Northern redbelly dace and brook stickleback abundance increased as distance from the stream mouth increased. Brook stickleback abundance increased 70-fold between the lowest reach and the uppermost reach. Similarly, Northern redbelly dace were not collected in the lowest three sites, and then

increased 30-fold upstream of site number four (stream km 4.3). In contrast, flathead chub, channel catfish, longnose sucker, river carpsucker, emerald shiner, and common carp were limited to the lower three reaches (below stream km 2.5).

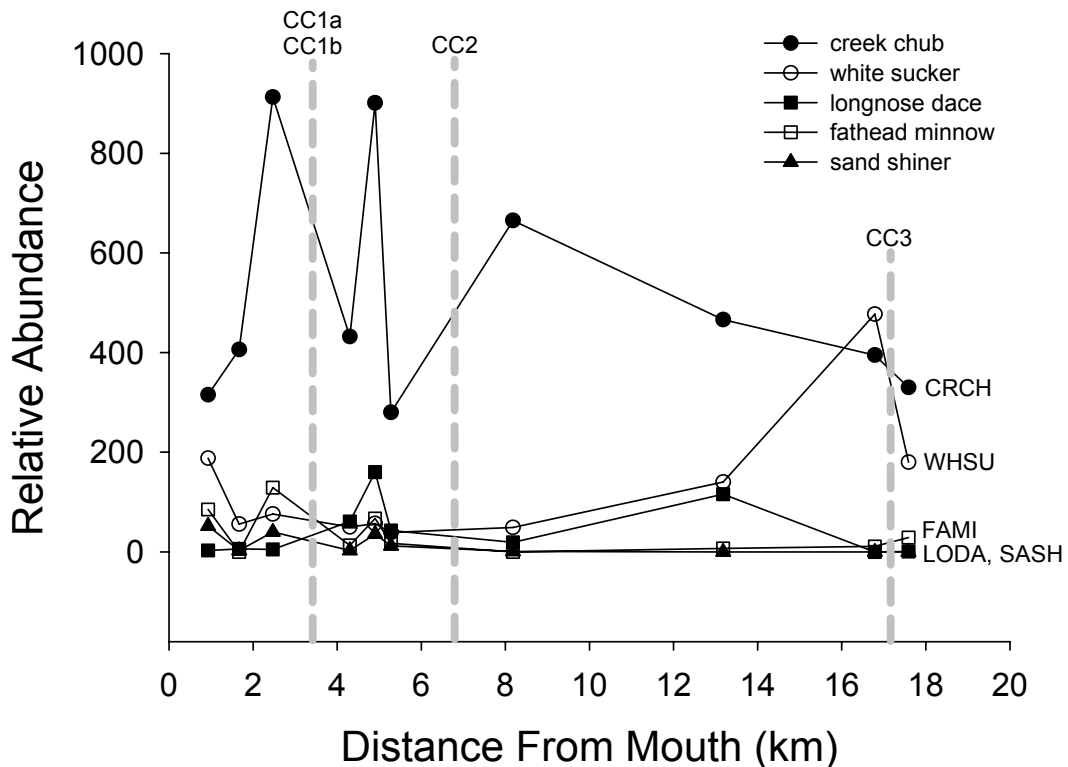


Figure 8: Relative abundance of the five most common Clear Creek fish species by distance from the stream mouth. Relative abundance was calculated as the total number of fish captured during both spring and summer samplings.

Sand shiner relative abundance was negatively correlated with distance from the stream mouth ($r_s = -1.00$, $P < 0.0001$). Negative trends in abundance with stream distance were also observed for creek chub ($r_s = -0.09$, $P = 0.80$), fathead minnow ($r_s = -0.29$, $P = 0.41$) and longnose dace ($r_s = -0.04$, $P = 0.92$) although the trends were not significant. In contrast, white sucker relative abundance showed a general positive trend

with distance from the stream mouth ($r_s = 0.19$, $P = 0.60$) although this trend was also not significant (Figure 9).

Pairwise comparisons above and below culvert crossings on Clear Creek revealed that in most cases, species abundance did not appear to be influenced by road crossings. No differences in relative abundance were detected for both creek chub (Mann-Whitney $P = 0.18 - 0.83$) and white sucker (Mann-Whitney $P = 0.13 - 0.65$). Longnose dace abundance was significantly higher above crossings CC1a and CC1b (Mann-Whitney $P = 0.05$) and was similar above and below other crossings (Mann-Whitney $P = 0.28 - 0.65$) indicating that culverts were not influencing fish numbers. In contrast, relative abundance was significantly lower above crossing CC2 for both fathead minnow (Mann-Whitney $P = 0.05$) and sand shiner (Mann-Whitney $P = 0.05$) suggesting that this crossing may be affecting fish distribution.

Significant differences in species abundance above and below crossings were likely not related to the habitat variables measured. The stream channel generally was narrower and deeper in the lower reaches and wider and shallower with distance upstream (Figure 10). Mean thalweg depth ranged from $0.41 \text{ m} \pm 0.01 \text{ SE}$ to $0.22 \text{ m} \pm 0.01 \text{ SE}$. Mean thalweg depth was similar above and below crossings CC2 and CC3 (Mann-Whitney $P = 0.27 - 0.35$), but was significantly deeper below crossings CC1a and CC1b (Mann-Whitney $P = 0.05$). Similarly, mean wetted width ranged from $2.71 \text{ m} \pm 0.18 \text{ SE}$ to $7.06 \text{ m} \pm 0.63 \text{ SE}$, and was similar above and below all stream crossings (Mann-Whitney $P = 0.18 - 0.51$). Only crossing CC2 appeared to significantly affect abundances of fathead minnow and sand shiner. Because mean thalweg depth and mean

channel width were similar above and below this crossing, it may be reasonable to assume that the crossing is limiting their distribution.

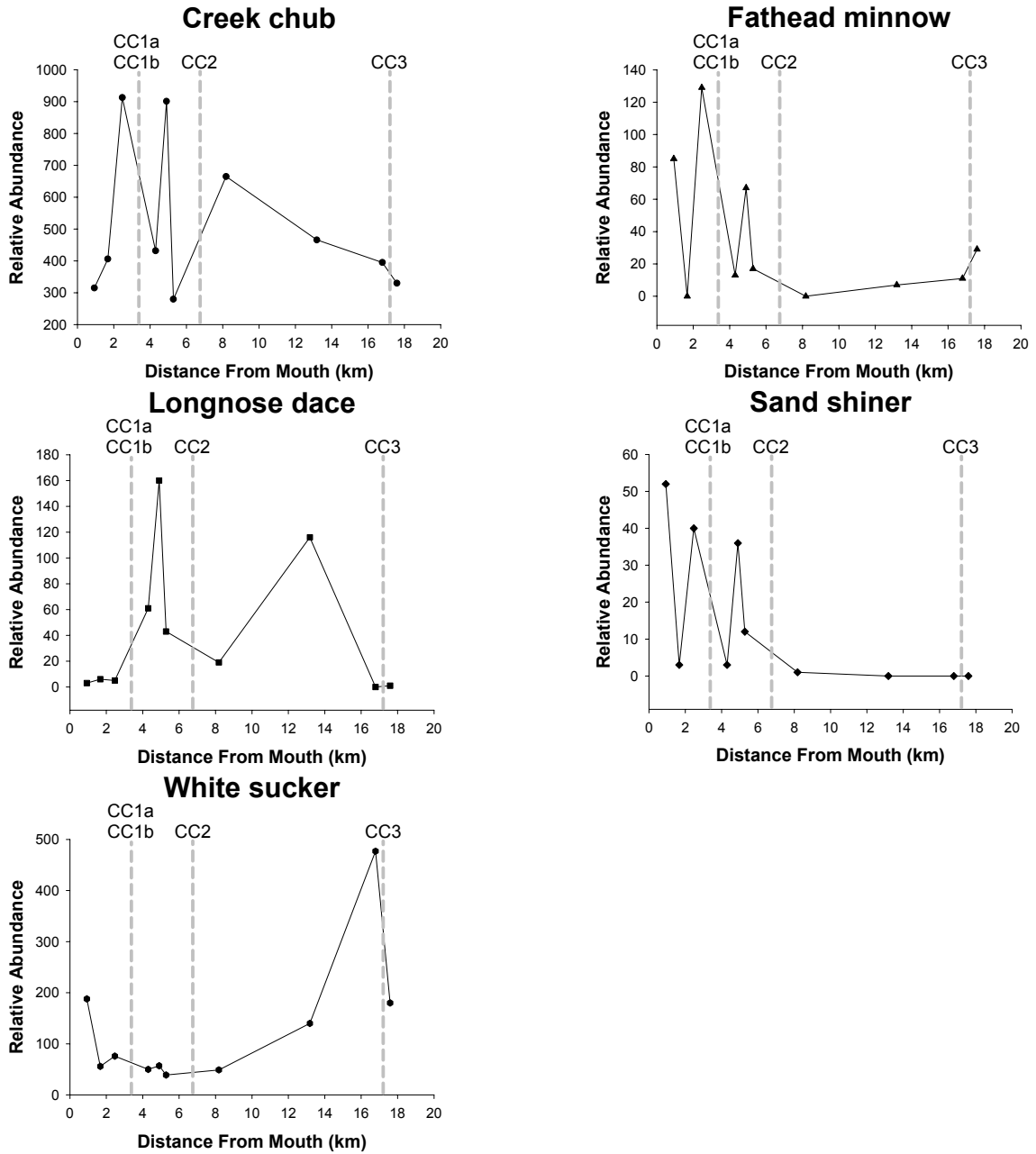


Figure 9: Relative abundance of creek chub, fathead minnow, longnose dace, sand shiner, and white sucker by distance from the stream mouth for sites above and below culvert crossings (Clear Creek 2006).

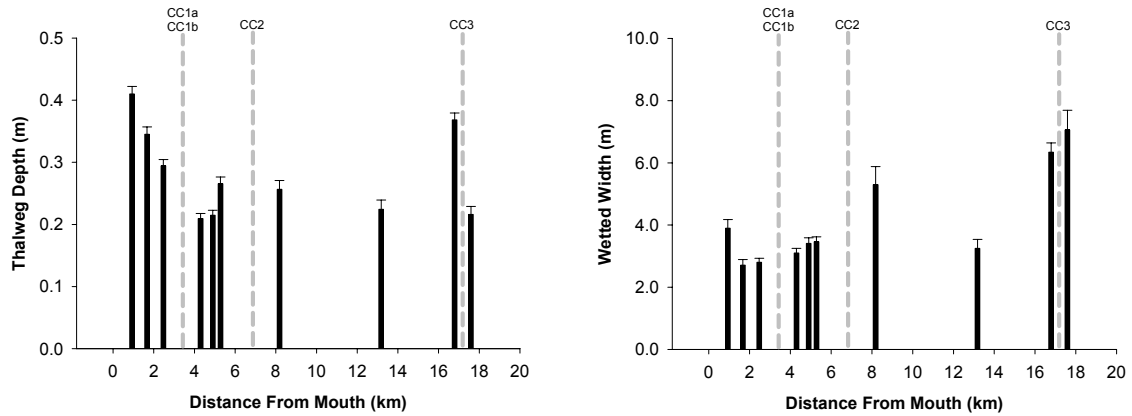


Figure 10: Mean thalweg depth and mean wetted width by distance from the stream mouth. Error bars represent standard error.

Fish Passage Experiments

Fish displacement experiments were conducted at all five crossings at two different water flows during the 2006 field season for a total of ten trials. A total of 1,109 fish dominated by four species were marked with VIE tags. These four species included creek chub ($n = 620$), flathead chub ($n = 63$), longnose dace ($n = 164$), and white sucker ($n = 200$). Several other species of fish were marked, but because of low sample size were excluded from this analysis. Only species in which at least 20 fish were captured during the initial sampling were used in each trial. Of the ten trials, creek chub were used in nine, flathead chub were used in two, longnose dace were used in five, and white sucker were used in four trials.

Overall fish movement was equal or greater through study culverts as through natural stream reaches (reference culverts) ($\chi^2 = 10.73$, OR = 1.81, $P = 0.001$). Of the four dominant species combined, 77 out of 491 (15.7%) fish were recaptured upstream of

reference culverts with an average fish length of 82.2 mm \pm 0.90 SE. Alternatively, 132 out of 556 (23.7%) fish marked in treatment reaches were recaptured above study culverts with an average fish length of 92.6 mm \pm 1.04 SE.

When evaluated by individual species, passage was not significantly restricted for creek chub, flathead chub, and white sucker (Table 5). White sucker movement was significantly greater through treatment culverts (31 of 62 recaptured fish, 50%) than through reference culverts (4 of 41 recaptured fish, 10%) ($\chi^2 = 17.82$, OR = 9.25, $P < 0.0001$). The odds ratio indicates that white suckers were 9.25 times more likely to move through study culverts than through natural reaches. In contrast, longnose dace showed restricted movement through study culverts compared to natural reaches ($\chi^2 = 4.17$, OR = 0.28, $P = 0.04$). The odds ratio value of 0.28 indicates that longnose dace were 3.57 times more likely to pass through reference culverts (11 of 23 recaptured fish, 48%) than through treatment culverts (5 of 25 fish recaptured, 20%).

Table 5: Results of Chi-square analysis and odds ratio tests for all fish displacement experiment trials.

Species	Chi-square value	Odds Ratio	95% CI	P-value
Creek chub	3.33	1.52	(0.97, 2.38)	0.07
Flathead chub	1.49	3.00	(0.49, 18.25)	0.22
Longnose dace	4.17	0.28	(0.08, 0.98)	0.04
White sucker	17.82	9.25	(2.94, 29.08)	<0.0001
All species	10.73	1.81	(1.27, 2.59)	0.001

Total length of fish did not appear to influence passability for any of the four species examined. Mean total length of fish that successfully passed treatment culverts was not significantly different from those that passed reference culverts for any of the species examined (Mann-Whitney $P = 0.07 - 0.57$).

The passage capability of creek chub and white sucker was further examined by grouping these two species as small fish (total length ≤ 80 mm) and large fish (total length > 80 mm). Of the marked creek chub, 341 (55%) were ≤ 80 mm, and 279 (45%) were > 80 mm. Similarly, 87 (43.5%) of marked white suckers were ≤ 80 mm, and 113 (56.5%) were > 80 mm (Figure 11). Passage was not significantly restricted for creek chub and white sucker in both length classes (≤ 80 mm and > 80 mm). For both species, the larger fish passed through the culverts at a higher rate than through the reference reaches, whereas smaller fish passed the culverts and the reference reaches at similar rates. (Table 6).

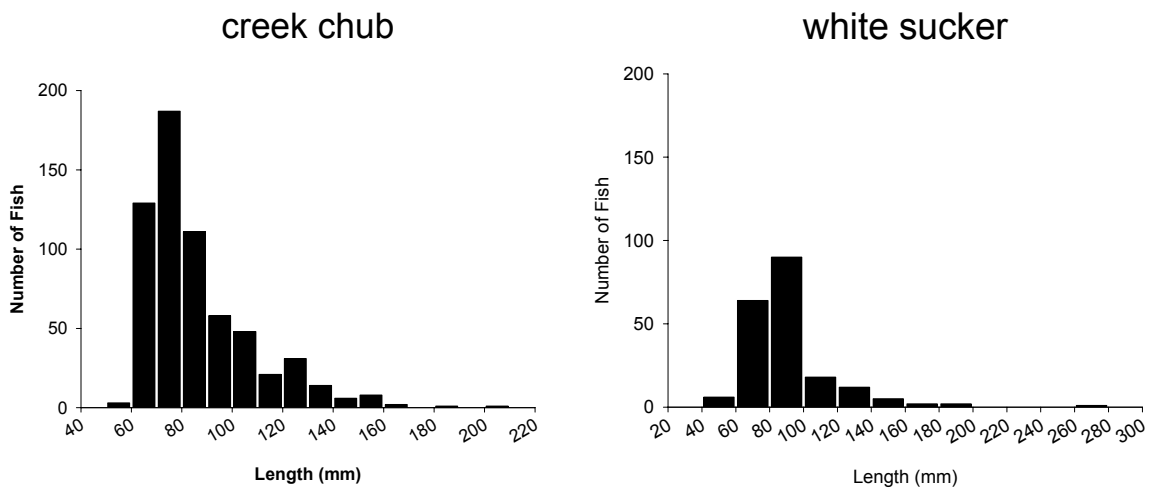


Figure 11: Length frequency histograms for creek chub and white sucker marked for fish passage experiments (Clear Creek 2006).

Table 6: Results of Chi-square odds ratio tests comparing the passage through reference and treatment culverts of creek chub and white sucker by length class.

Species	Total Length (mm)	Chi-square value	Odds Ratio	95% CI	P-value
Creek chub	≤ 80	0.09	1.10	(0.59, 2.05)	0.76
Creek chub	> 80	4.92	2.10	(1.09, 4.05)	0.03
White sucker	≤ 80	-- ^a	1.61	(0.23, 11.09)	1.00
White sucker	> 80	18.50	18.12	(3.78, 86.91)	< 0.0001

^a Dashes indicate situations where the sample size was insufficient for Chi-square analysis. Fisher's Exact test was used when two or more cells had counts of five or less.

Differences in the habitat characteristics of treatment and reference reaches varied according to individual crossings. Overall, mean water velocity did not differ significantly between treatment culverts and reference culverts (Mann-Whitney $P = 0.07 - 0.81$) (Table 7). Water depths between treatment and reference culverts tended to be the same or slightly deeper in reference culverts with the exception of crossing SC1. At this crossing, mean water depth was significantly lower in the reference culvert than in the treatment culvert (Mann-Whitney $P = 0.03$). Within the sampling reaches, treatment reaches were generally wider than reference reaches. This was likely a result of large, wide scour pools located immediately downstream of treatment culverts at crossings CC1b, CC3, and SC1. Mean thalweg depth was generally the same between treatment and reference reaches. However, mean thalweg depth was significantly higher in the reference reach at crossing CC2 (Mann-Whitney $P = 0.04$) as a result of a large pool habitat in the downstream segment, and significantly higher in the treatment reach at crossing SC1 (Mann-Whitney $P = 0.002$) as a result of a large, deep scour hole below the culvert.

Table 7: Mean values (\pm SE) of habitat variables measured in reference and treatment reaches for fish passage experiments. CC1a = Clear Creek rd 261 crossing #1, CC1b = Clear Creek rd 261 crossing #2, CC2 = Clear Creek I-94 crossing, CC3 = Clear Creek upper crossing, SC1 = Sand Creek rd 261 crossing. Asterisks indicate statistically significant ($\alpha = 0.05$) differences between reference and treatment reaches or culverts

Crossing	Reach	<u>Reach Measurements</u>		<u>Culvert Measurements</u>	
		Thalweg Depth (cm)	Wetted Width (m)	Water Depth (cm)	Water Velocity (m/s)
CC1a	Reference	29.4 (0.05)	3.2 (0.5)	11.8 (0.01) *	0.12 (0.02)
CC1a	Treatment	23.6 (0.04)	2.5 (0.6)	9.2 (0.01)	0.12 (0.02)
CC1b	Reference	30.5 (0.03)	1.5 (0.08)	28.5 (0.02)	0.07 (0.01)
CC1b	Treatment	27.0 (0.03)	2.1 (0.2) *	29.9 (0.03)	0.06 (0.01)
CC2	Reference	41.9 (0.08) *	3.3 (0.3)	15.0 (0.01) *	0.09 (0.01)
CC2	Treatment	18.3 (0.04)	5.0 (0.04) *	5.0 (0.01)	0.14 (0.02)
CC3	Reference	30.8 (0.02)	3.5 (0.5)	11.8 (0.01)	0.08 (0.01)
CC3	Treatment	49.4 (0.04)	9.2 (0.5) *	11.1 (0.01)	0.16 (0.03)
SC1	Reference	31.9 (0.05)	2.1 (0.2)	22.4 (0.02)	0.30 (0.03)
SC1	Treatment	57.1 (0.07) *	3.3 (0.3) *	31.9 (0.03) *	0.27 (0.04)

Because longnose dace were the only of the five most abundant species to exhibit significant culvert passage restriction, this species was further examined for relationships between passage and culvert characteristics. A passage index was created for each of the five experiments in which longnose dace were used. This passage index was calculated as the number of fish recaptured upstream of the study culvert divided by the number recaptured downstream. Index values less than 1.0 indicate restricted passage with less fish captured above than below the culvert, whereas index values greater than or equal to 1.0 indicate no restriction. Index values for longnose dace ranged from 0.00 to 2.00, with 3 of 5 trials showing restricted passage. No significant relationships were observed

between passage index values and any of the four culvert characteristics of culvert length, slope, water velocity, or outlet drop ($P = 0.48 - 0.97$). However, negative trends could be seen between passage and culvert length and outlet drop (Figure 12).

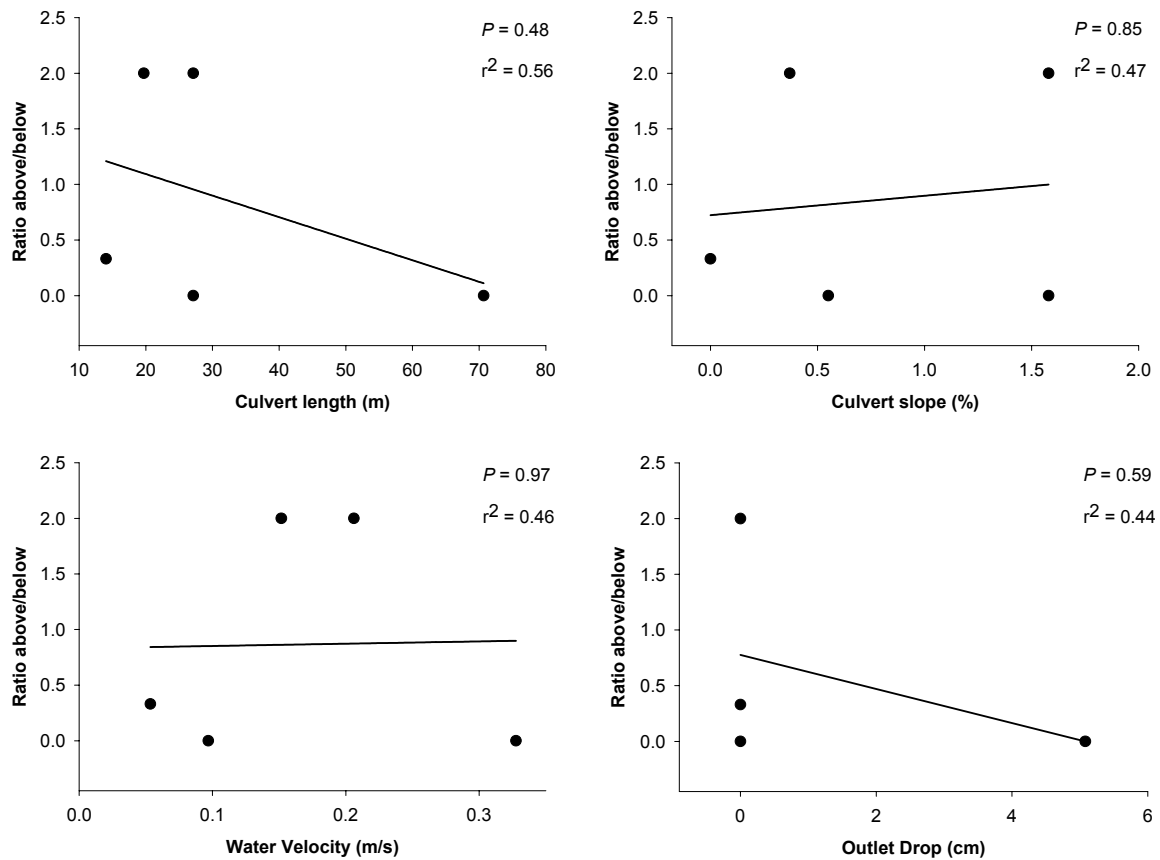


Figure 12: Regressions of longnose dace passage indices and culvert length, culvert slope, water velocity, and outlet drop.

Passage indices were also calculated for creek chub, a species that exhibited no significant passage restriction, to contrast results from the longnose dace regressions. Passage indices for creek chub ranged from 0.0 to 5.0 with five of the ten trials showing some restriction of passage. Creek chub exhibited some passage restriction at crossings

CC1b, CC2, and SC1. No significant relationships were observed between passage index values and any of the three culvert characteristics ($P = 0.37 - 0.99$). However, negative trends could be seen between passage and culvert length and outlet drop similar to the regressions of longnose dace (Figure 13).

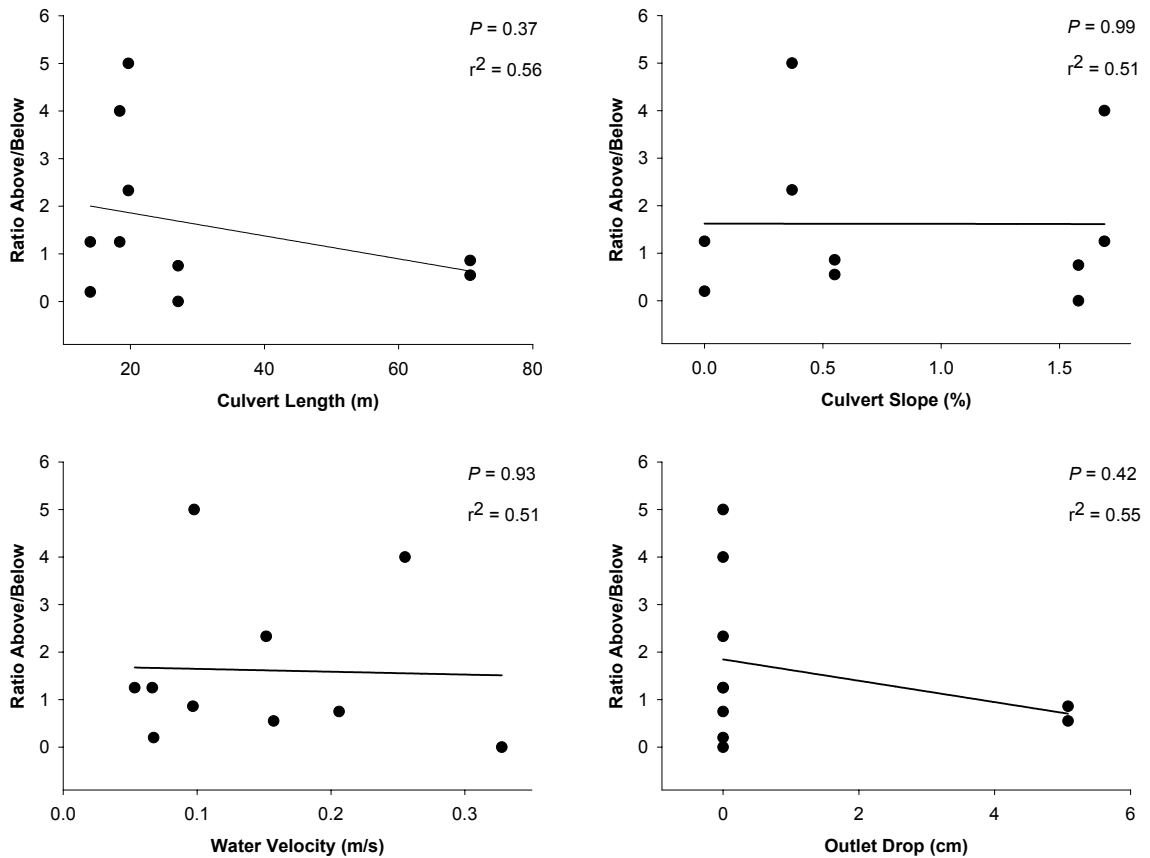


Figure 13: Regressions of creek chub passage indices and culvert length, culvert slope, water velocity, and outlet drop.

Modeling Using FishXing

Results from FishXing showed that all five crossings act as barriers to most of the four predominant species examined during the study period (March – October) (Table 8).

When FishXing predicts that a culvert is a barrier, it also indicates the hydraulic or

physical reason for being a barrier. At low flow rates, barrier status was the result of insufficient water depth in all five culverts, and sometimes insufficient water depth in the plunge pool (crossings CC1a and CC2). It is possible for more than one condition to contribute to barrier status. At high flows, all five culverts were predicted to have excessive water velocity and culvert CC2 also had excessive outlet drop.

Based on the high and low flows estimated from stream gauging equipment, passage windows were estimated for each culvert showing the range of low to high flows passable for each species (Table 8). Crossing CC2 was predicted a total barrier for all four species, whereas CC3 and SC1 were predicted to be a total barrier for flathead chub at all flows. Crossings CC3 and SC1 were also predicted to pass fish at low percentages of flows.

Table 8: Results of FishXing analysis showing the range of passable flows predicted from the high and low flows of the estimated hydrograph. CC = creek chub, FC = flathead chub, LND = longnose dace, WS = white sucker.

Crossing	Estimated		Range of predicted passable flows (cms) from FishXing Model			
	Low Flow (cms)	High Flow (cms)	CC	FC	LND	WS
CC1a	0.002	3.2	0.05 – 0.7	0.05 – 0.2	0.02 – 0.07	0.02 – 0.3
CC1b	0.0003	0.3	0.0 – 0.3	0.002 – 0.3	0.0 – 0.1	0.0001 – 0.3
CC2	0.0007	5.9	None	None	None	None
CC3	0.0000	7.8	0.001 – 0.2	None	0.001 – 0.03	0.01 – 0.05
SC1	0.0000	1.1	0.003 – 0.09	None	0.003 – 0.006	0.006 – 0.08

Percent of time passable, determined by overlaying upper and lower passage window boundaries on the hydrograph for each crossing, indicated that most culvert crossings provided passage for the four fish species at least a portion of the time

monitored in 2006 (March – October) (Table 9). Only one instance was observed in which FishXing predicted no passage, and no fish passed during displacement experiments. At the Interstate 94 stream crossing on Clear Creek (CC2), longnose dace were not recaptured upstream of the culvert during displacement experiments, and the culvert was predicted to be impassable by the model. FishXing results show this culvert to be a total barrier for all four species, however creek chub and white sucker successfully passed during experiments at low flows (0.01 – 0.02 cms). In contrast, stream crossing CC1b was passable for all four species for a majority of the study duration with percentages ranging from 83.8 - 99.9%. Hydrographs reveal the flashy nature of the two streams as well as the influence of irrigation, however passage windows show that these crossings provide passage for some species between and during some of these events (Figures 14 – 18).

Expanding passage windows to include experimental data increased the amount of time culverts were considered passable. There were 12 cases in which the FishXing window was not already essentially 100% of flows and the species in question was used at that culvert for a passage experiment (Table 9). In 6 of those 12 cases (50% of cases), including field data increased the percent of time passable predicted by FishXing by an average of 35%.

Table 9: Percent of time passable for each culvert crossing and all four species during the 2006 study period. Entries marked with an asterisk (*) indicate crossings in which species were not used in fish passage experiments. Bold entries are cases in which adding displacement experiment data increased the passage window flow range.

Stream Crossing	Species	% Passable FishXing	% Passable With Passage Experiments added
CC1a	Creek chub	73	73
CC1a	Flathead chub	50	--*
CC1a	Longnose dace	29	65
CC1a	White sucker	69	69
CC1b	Creek chub	100	100
CC1b	Flathead chub	97	--*
CC1b	Longnose dace	84	84
CC1b	White sucker	100	100
CC2	Creek chub	0	35
CC2	Flathead chub	0	--*
CC2	Longnose dace	0	0
CC2	White sucker	0	35
CC3	Creek chub	92	92
CC3	Flathead chub	0	--*
CC3	Longnose dace	30	--*
CC3	White sucker	51	82
SC1	Creek chub	38	38
SC1	Flathead chub	0	27
SC1	Longnose dace	3	48
SC1	White sucker	36	--*

Percent of time passable values were rounded to the nearest percent. No culvert was passable 100% of the time by either measure. The values in that appear as 100% are actually 99.97%, where a short duration of extreme high flow exceeded either passage window used.

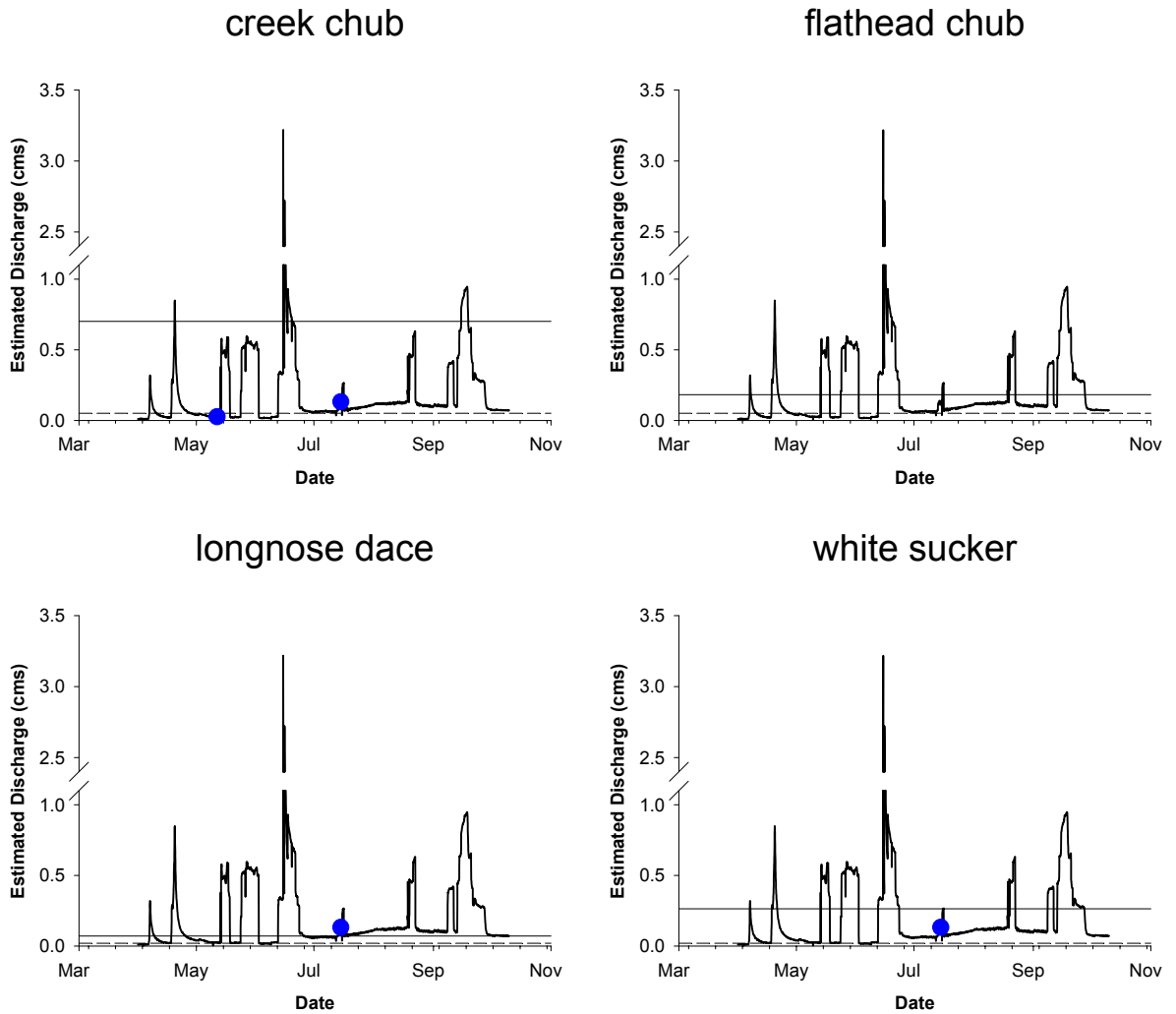


Figure 14: Passage windows for crossing CC1a. Solid horizontal reference lines represent the upper boundary of FishXing predicted passage (velocity barrier). Dashed horizontal reference lines represent the lower discharge boundaries of FishXing predicted passage (depth barrier). Blue dots indicate successful passage during displacement experiments.

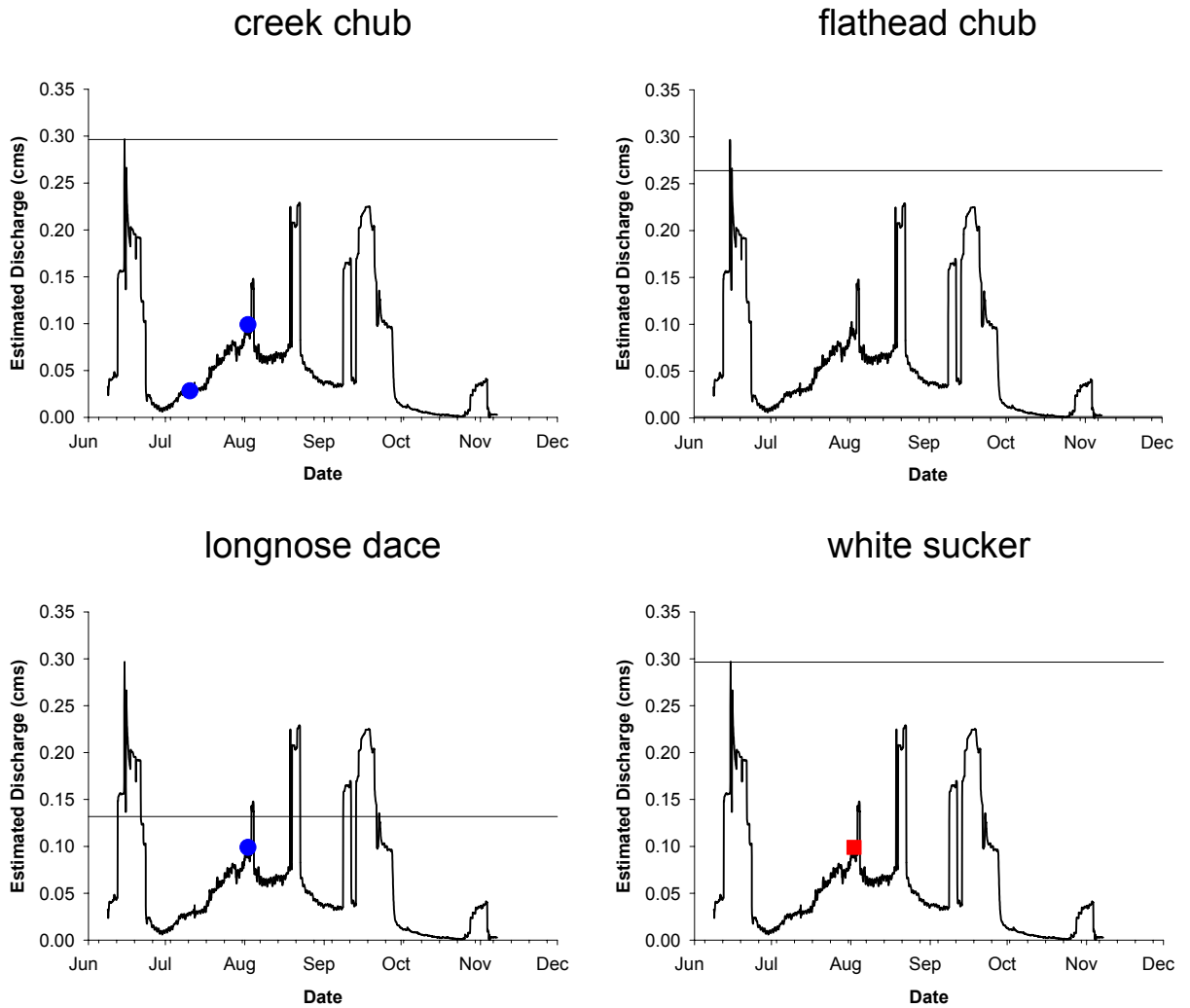


Figure 15: Passage windows for crossing CC1b. Solid horizontal reference lines represent the upper boundary of FishXing predicted passage (velocity barrier). Blue dots indicate successful passage during displacement experiments. Red squares indicate failed passage during displacement experiments.

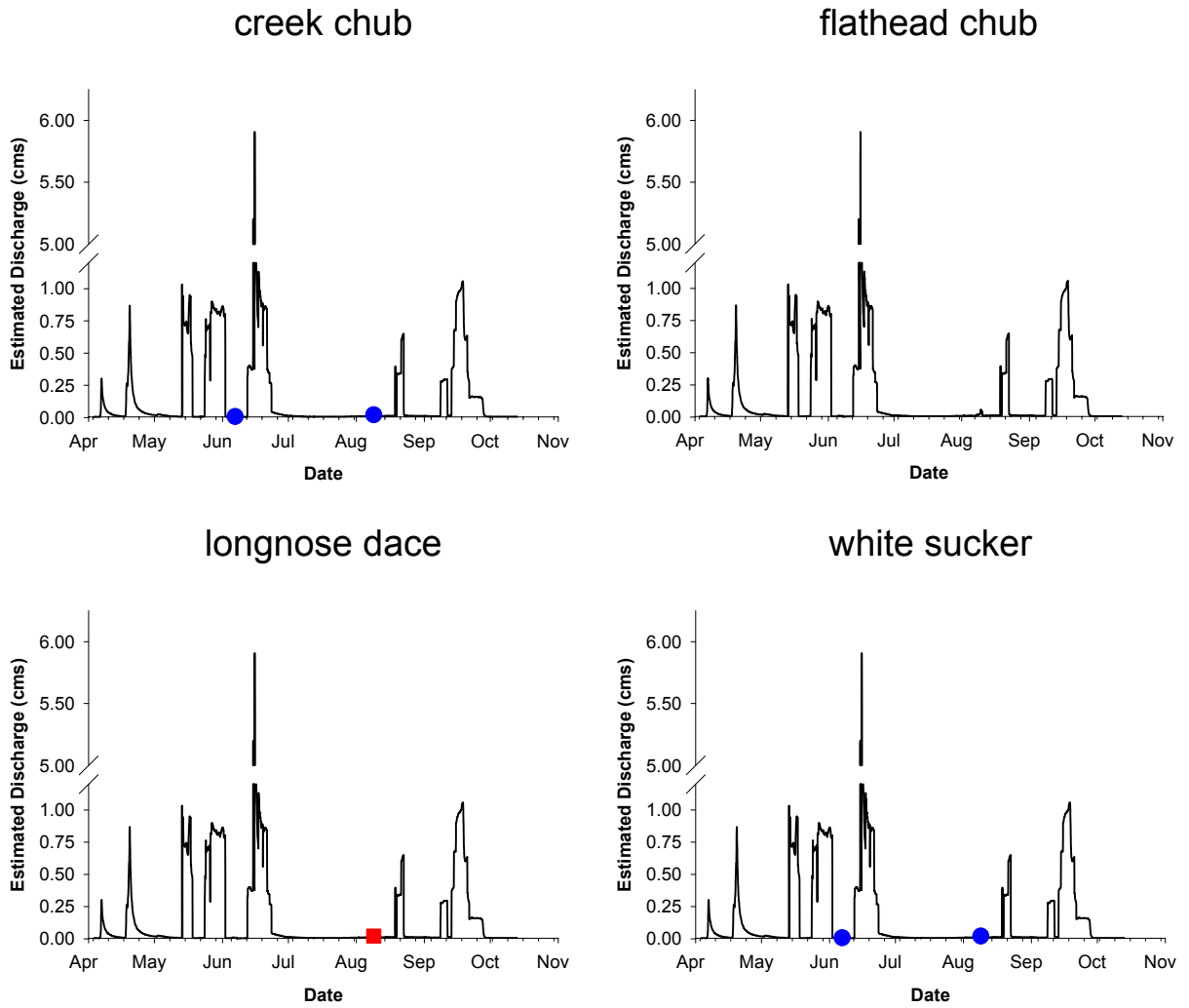


Figure 16: Passage windows for crossing CC2. FishXing predicted no passage of all four species at all flows. Blue dots indicate successful passage during displacement experiments. Red squares indicate failed passage during displacement experiments.

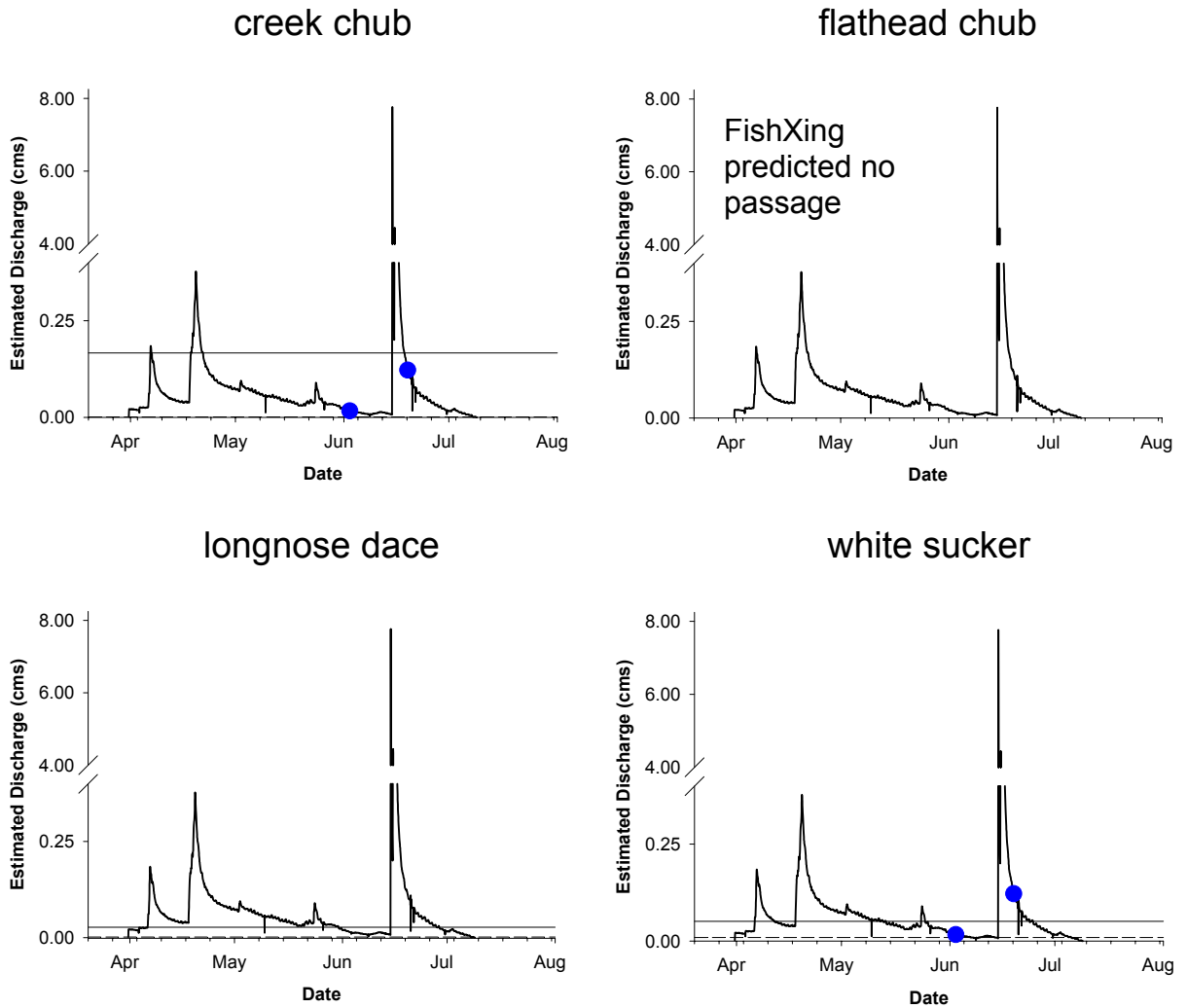


Figure 17: Passage windows for crossing CC3. Solid horizontal reference lines represent the upper boundary of FishXing predicted passage (velocity barrier). Dashed horizontal reference lines represent the lower discharge boundaries of FishXing predicted passage (depth barrier). Blue dots indicate successful passage during displacement experiments

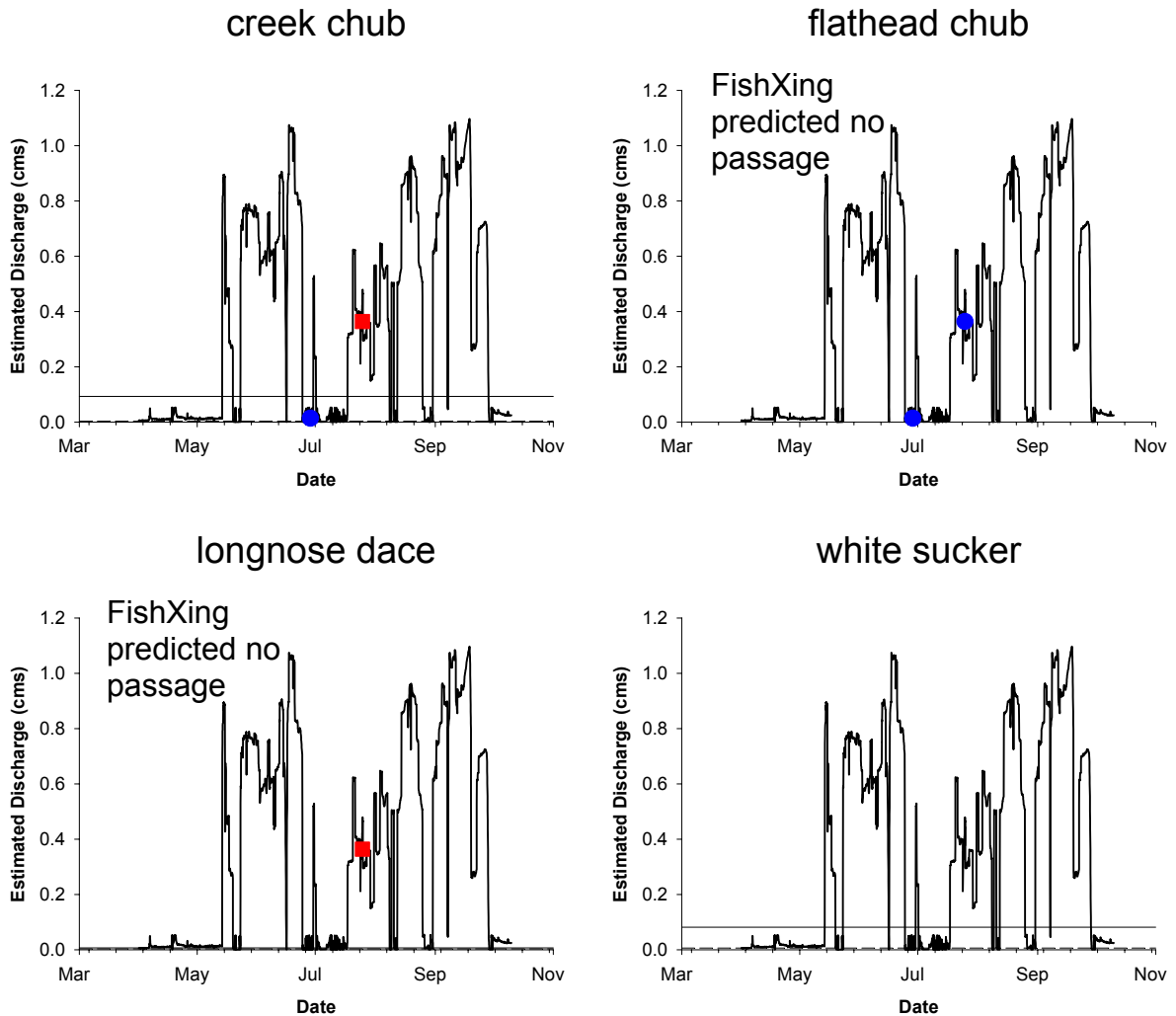


Figure 18: Passage windows for crossing SC1. Solid horizontal reference lines represent the upper boundary of FishXing predicted passage (velocity barrier). Dashed horizontal reference lines represent the lower discharge boundaries of FishXing predicted passage (depth barrier). Blue dots indicate successful passage during displacement experiments. Red squares indicate failed passage during displacement experiments.

Comparison of FishXing passage predictions with results from displacement experiments showed that FishXing results were consistent with experiment results in 55% (12 of 22) of the cases. By species, modeling results for creek chub were congruent with 70% (7 of 10) of the trials, and longnose dace were congruent with 75% (3 of 4) of trials.

In contrast, white sucker modeling results were congruent with only 33% (2 of 6) of trials, and the two trials with flathead chub were not congruent with modeling results (Table 10).

Table 10: Comparison of results from displacement experiments and FishXing modeling by culvert crossing.

Crossing	Experiment Date	Species	Experiment Fish Pass?	FishXing Fish Pass?	Congruent?
CC1a	5/10 to 5/12	CC	Yes	No	No
CC1a	7/13 to 7/15	CC	Yes	Yes	Yes
CC1a	7/13 to 7/15	LND	Yes	No	No
CC1a	7/13 to 7/15	WS	Yes	Yes	Yes
CC1b	7/9 to 7/11	CC	Yes	Yes	Yes
CC1b	8/1 to 8/3	CC	Yes	Yes	Yes
CC1b	8/1 to 8/3	LND	Yes	Yes	Yes
CC1b	8/1 to 8/3	WS	No	Yes	No
CC2	6/5 to 6/7	CC	Yes	No	No
CC2	6/5 to 6/7	WS	Yes	No	No
CC2	8/7 to 8/9	CC	Yes	No	No
CC2	8/7 to 8/9	LND	No	No	Yes
CC2	8/7 to 8/9	WS	Yes	No	No
CC3	6/1 to 6/3	CC	Yes	Yes	Yes
CC3	6/1 to 6/3	WS	Yes	Yes	Yes
CC3	6/18 to 6/20	CC	Yes	Yes	Yes
CC3	6/18 to 6/20	WS	Yes	No	No
SC1	6/26 to 6/28	CC	Yes	Yes	Yes
SC1	6/26 to 6/28	FC	Yes	No	No
SC1	7/24 to 7/26	CC	No	No	Yes
SC1	7/24 to 7/26	FC	Yes	No	No
SC1	7/24 to 7/26	LND	No	No	Yes

Simple linear regression was used to examine which culvert characteristics were influencing the amount of time a culvert was considered passable (Figures 19 and 20).

Outlet drop was not a characteristic used for this analysis because only one culvert had an outlet drop greater than 0.0 cm. No significant relationships were found between the percent of the study duration passable and culvert length ($P = 0.08 - 0.31$) and culvert slope ($P = 0.19 - 0.71$) for any of the species. However, flathead chub and longnose dace appeared to be most affected by culvert slope, and all species displayed negative relationships with culvert length though none of the relationships were statistically significant.

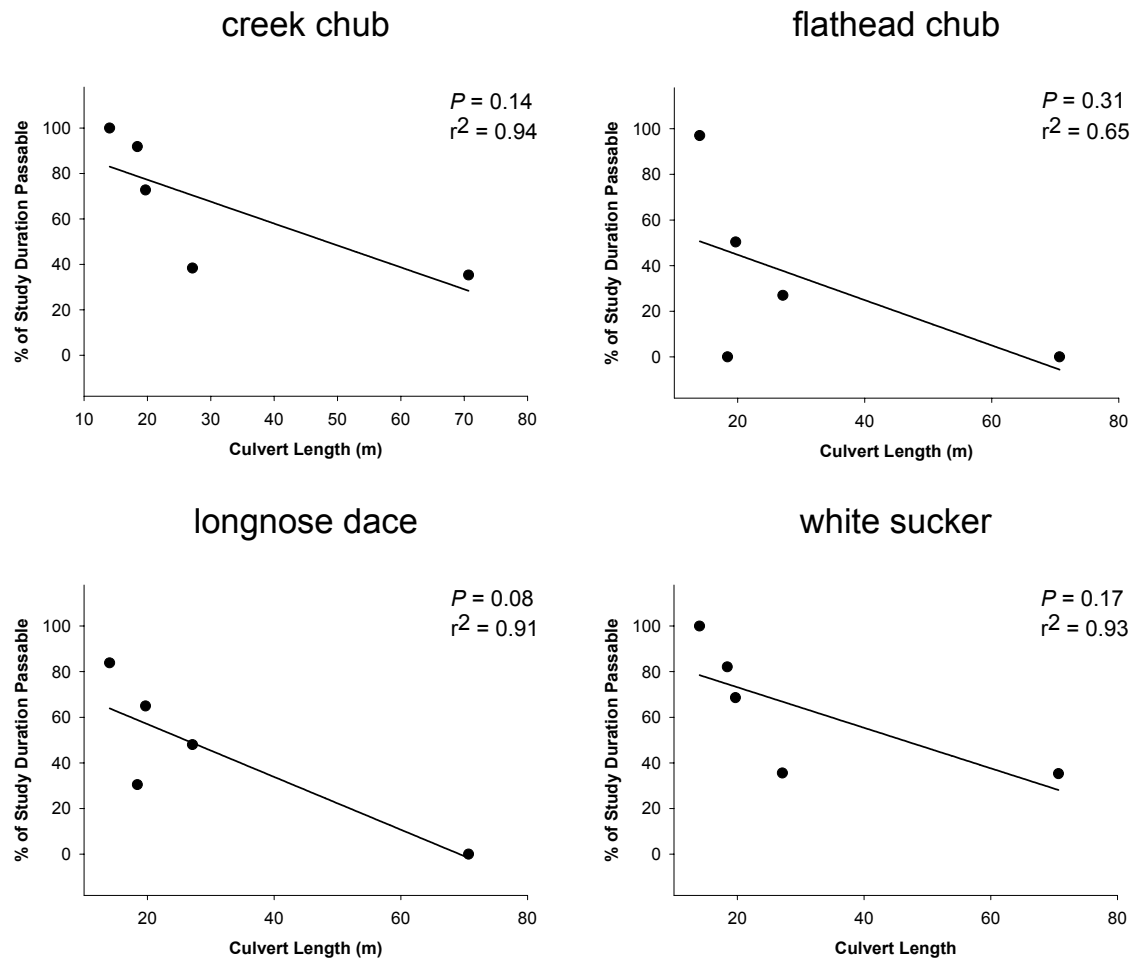


Figure 19: Regression plots of percent of study duration passable by culvert length for Clear and Sand creeks 2006. Percent of study duration passable includes fish displacement experiment data.

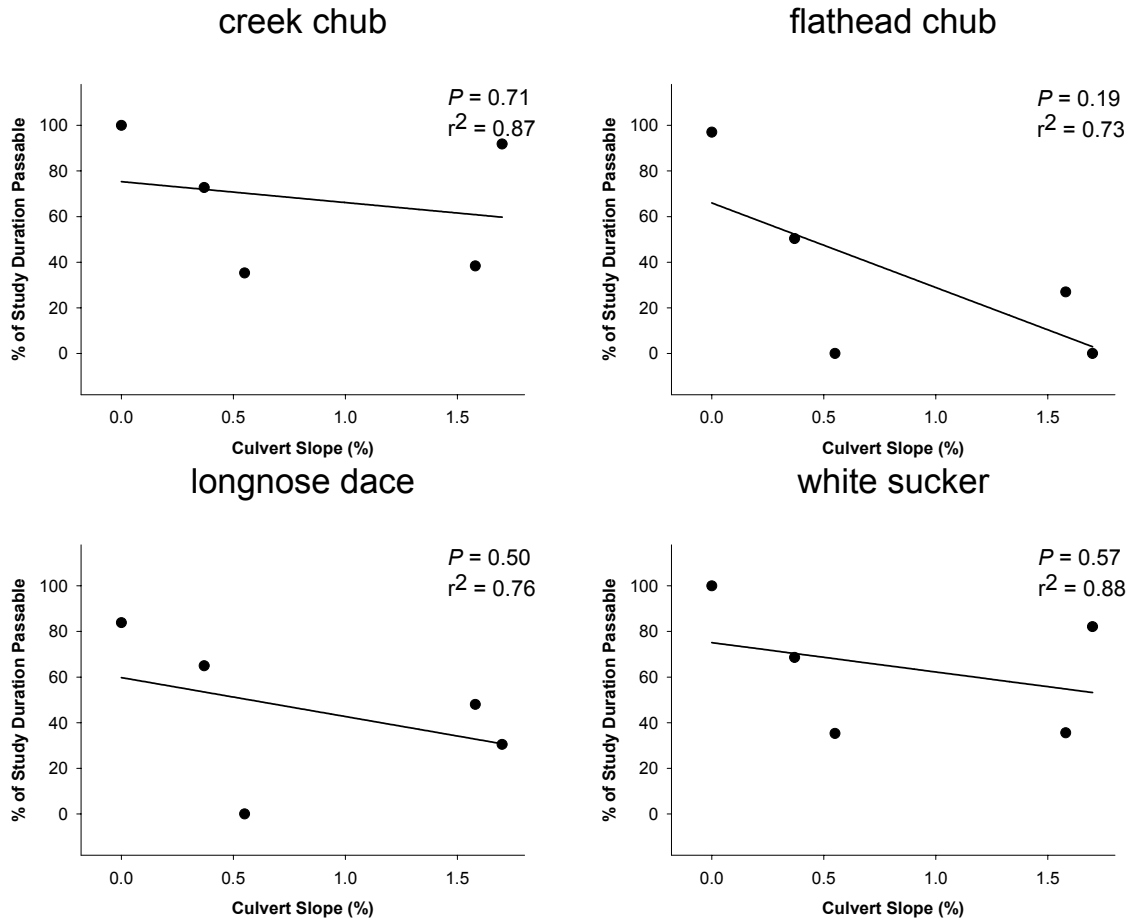


Figure 20: Regression plots of percent of study duration passable by culvert slope for Clear and Sand creeks 2006. Percent of study duration passable includes fish displacement experiment data.

DISCUSSION

A combination of three assessment techniques was used to examine fish passage at five culvert crossings in eastern Montana. Results from FishXing modeling revealed that all five culverts may be acting as barriers to common fish species for at least a portion of the year. However, fish distribution patterns indicated that if these crossings are affecting fish movement, the impacts on the overall assemblage may not be sufficiently large to cause local extirpation or significant declines in species abundance above crossings. Additionally, fish were observed passing culverts during displacement experiments in situations deemed impassable by application of current fish passage models.

The longitudinal distribution surveys showed widely varying species richness among study streams. Species richness on Clear Creek (21 species) was highest near the confluence with the Yellowstone River (18 species), dropping dramatically from there to the next upstream reach (9 species), only 0.74 km apart. In the uppermost 16 km, the nine sample reaches showed fairly consistent species richness ranging from a maximum of 12 species to a minimum of 6 species with an average of 9 species. Except for the difference between the most downstream reach and all the others, species richness on Clear Creek defies the trend of reduced richness with progression upstream from the mouth as reported by Schlosser (1987) and Ostrand and Wilde (2002). The limited data collected in Sand Creek also defies this trend, with more species being found in upper reaches than near the confluence with the Yellowstone River. However, this effect is likely due to the influence of irrigation canal returns which were quite substantial in this

stream. The lower level of species diversity found in Sand Creek overall is likely a result of limited natural flow during non-irrigation periods.

Although species richness in Clear Creek did not show significant declines, there did appear to be a change in species composition related to the possible injection of fish into the system through the irrigation canal. When compared to the site immediately downstream of the irrigation canal, the four sites upstream did not contain channel catfish, flathead chub, and goldeye, suggesting that these fish were artificially introduced into the system.

On Clear Creek, I found no significant differences in species richness and abundance above and below study culverts. This suggests that these crossings may not be affecting the overall distribution of fishes throughout the watershed, and that the absence of a species in upper reaches may be the result of natural changes in assemblage or else the result of injection of fish into lower reaches from irrigation practices. Winston et al. (1991) documented the extirpation of four prairie stream fish species upstream of the Altus Dam on the North Fork of the Red River in Oklahoma. This example describes how a total barrier to upstream fish movement can have long lasting consequences. Because no significant differences in species richness or abundance were detected above and below culverts in my study, it could be assumed that none of the culverts were acting as total barriers. However it is possible that a fish species could be found above a culvert if that species was injected into the system above that crossing. This could result in fish being found above the crossing even if the culvert is impassable to that species.

Although study culverts were not acting as total barriers to upstream fish movement, one crossing did appear to influence the abundance of both fathead minnow

and sand shiner. Both species had significantly lower abundances in sites upstream of crossing CC2, which had the highest outlet drop of all study culverts (5.2 cm) but were collected in upstream sites indicating that if this crossing is limiting their movement, individuals were either able to pass at certain times or else populations isolated upstream are reproducing.

Results from longitudinal distribution surveys were also contradictory to those of Rajput (2003) who found significantly lower species richness and species abundance upstream of culvert crossings. However, Rajput's analysis included several species from the sunfish (*Centrarchidae*) and catfish (*Ictaluridae*) families that were absent from my analyses. The absence of these families in my data could be a source of the discrepancy between studies. Additionally, culvert velocities measured in that study were substantially higher than those observed during my study. Mean culvert velocity measured in their study was 74 cm/s compared to 15 cm/s in my study. Additionally, over 85% of the water velocities measured in their culverts were greater than 25 cm/s indicating that my study culverts were fundamentally different from those that significantly affected species distribution in past studies.

Findings from fish displacement experiments revealed that all five study culverts, representative of culverts from our initial eastern Montana culvert survey, were capable of passing the common fish species, with the exception of longnose dace failing to pass at culvert CC2. However, experiments were limited to low flow conditions, and therefore the amount of passage during high flow events remains unknown. All of the species examined in my experiments spawn in the spring and early summer (Brown 1971), therefore successful passage during these times could be considered critical for access to

spawning sites in upper reaches (Dodds et al. 2004). Additionally, while the characteristics of the five culvert crossings examined in this study were representative of other crossings found in the culvert survey, extremes in outlet drop and culvert slope do exist in these types of crossings. Coffman (2005) described outlet drops greater than 22.9 cm as being impassable to the species similar to those in my study. My study culverts had either no outlet drop, or considerably lower outlet drops than Coffman's data suggest affect passage, therefore my results showing successful passage may not be unusual given the low outlet drops and velocities as compared to other passage studies.

Although creek chub, flathead chub, and white sucker did not show significant passage restriction during displacement experiments, there were instances in which longnose dace failed to pass study culverts. Crossing CC2 was a long culvert (71 m) that at low flow levels contained a 5.1 cm outlet drop. Both creek chub and white sucker traversed this culvert successfully at this flow, but no longnose dace were observed to pass. This finding supports the notion that different species and body types produce different swimming capabilities (Katapodis and Gervais 1991). The length of the culvert in this instance likely was the reason for the restricted passage of longnose dace. Three longnose dace without VIE tags were captured in the actual culvert during this experiment indicating that it may have been possible for these fish to overcome the small outlet drop. However, because of the limited information on the swimming and leaping capabilities of these fish, the actual cause for restriction remains unknown. It is interesting that longnose dace would be the only species that showed restricted passage because of their affinity for fast-moving, riffle habitats (Thompson et al. 2001). We might expect this species to have above normal swimming capabilities simply due to the

habitat they regularly occupy. Another interesting point here is not that longnose dace passage was restricted, but rather that the other species appeared to move freely through such a long culvert. Additionally, this movement occurred over a relatively short time span (48 hours) indicating that these species are not only capable of overcoming quite large obstacles but also capable of rapid recolonization of upstream reaches when passage is provided.

Total body length did not appear to influence passage abilities for both creek chub and white sucker. Both species showed successful passage of fish in both length classes (≤ 80 mm and > 80 mm), with more fish passing treatment culverts than reference culverts. These findings are consistent with those of Belford and Gould (1989), who found no relationship between body length and passability for several trout species in western Montana. The authors of that study noted that this finding may have been the result of smaller fish utilizing lower velocities along the bottom and sides of the culverts. This finding was also described in a study from Alaska examining the passage of juvenile salmonids (Kane et al. 2000). These authors observed juvenile fish utilizing lower velocity zones to traverse the length of culvert crossings. My study supports this idea as fish of both size classes were able to pass a 71 m culvert. I believe that these fish were able to use the depressions between culvert corrugations to rest as they passed the culvert. In this instance, corrugation widths were typically greater than the length of the fish examined. Additionally, a small amount of natural substrate has washed into this culvert adding areas of lower water velocity that these fish may have utilized. Visual observation and attempted sampling inside this culvert revealed that small fish were in fact residing in the culvert barrel.

When compared to other prairie fish passage studies, my displacement experiments indicate greater fish movement through culverts. However, this is likely due to my culvert crossings being similar to natural stream reaches (“stream simulation crossings”). Study culverts were typified by low gradients, no outlet drop, some natural substrate, and culvert diameters roughly equal to channel widths. As a result, mean water velocities measured inside study culverts were similar to those of natural reaches, and lower than those of previous studies. For example, Warren and Pardew (1998) found significantly lower fish movement through culverts with mean water velocities exceeding 40 cm/s than through all other reaches. Mean water velocities found in my study culverts were considerably lower, ranging from 6 to 27 cm/s (mean = 15).

Results from displacement experiments described greater fish movement through study culverts than through natural reaches. It is possible that fish in reference reaches failed to move through the reference culvert because they were able to find suitable habitat inside the reference culvert itself or in the downstream segment. During experiments at crossings CC1b and CC2, 33 and 21 fish respectively, were recaptured inside the reference culvert reach. A large pool habitat was present between the downstream, reference reach and the reference culvert reach at CC2. Of the 73 fish recaptured during both trials at this crossing, 57 (78%) were recaptured in these two segments. Additionally, high densities of non-tagged fish were also captured in these segments during these trials, suggesting that this pool habitat was preferred for the species present.

Congruency between displacement experiments and FishXing modeling varied by species, ranging from 0% for flathead chub, to 75% for longnose dace. Overall, FishXing

predictions were in agreement with my displacement experiment results in 55% of the cases. My results differ slightly with the findings of Rajput (2003) in which FishXing results were compared to field observations of species composition at 21 culverts on the Ouachita National Forest in Arkansas. The author found that FishXing results were congruent with species loss above culverts at 71% of sites. However, Rajput's study compared FishXing results to species composition and abundance data, whereas my study looked at actual fish movement versus modeling results. The lower percentage of congruence in my study may have also been the result of the swim speed information provided in FishXing. Congruence between FishXing and experimental results were lowest for flathead chub and white sucker. Of the flathead chub used in displacement experiments, mean body length was 82.6 mm (± 1.8 SE). However, swim speed data for flathead chub in FishXing relates to fish between 192 and 339 mm. Similarly, white sucker mean body length from experiments was 89.9 mm (± 1.9 SE) but FishXing swim speeds were for fish between 180 and 392 mm. At crossing CC2, white sucker passed during flows deemed impassable by FishXing as a result of low water depth. This is likely due to the minimum water depth required to pass fish in the size range provided in the software. Because my fish were smaller than the fish used in the model, it is likely that the water depth required for successful passage would be considerably less. Similarly, flathead chub were not predicted to pass crossing SC1 but readily passed during displacement experiments. FishXing deemed this culvert impassable to flathead chub as a result of a velocity barrier. However, because my fish were considerably smaller than the fish provided in the model, I suspect that these fish were able to utilize the lower velocities associated with the sides and bottom of the culvert to successfully

pass, similar to the findings of Kane et al. (2000). In contrast, congruency between displacement experiments and FishXing for longnose dace was relatively high (75%). These examples show how using surrogate species can produce results with varying precision, reinforcing the idea that more specific swim speed data is necessary, as well as testing the FishXing model predictions in the field.

The calculation of passage windows was an effective method of quantifying the amount of time a culvert was considered passable during a given time interval. Additionally, incorporating the displacement experiment results into the passage window calculations was effective in better defining passage probabilities with actual movement data. For example, FishXing predicted no passage for all four species modeled at crossing CC2. However, displacement experiments revealed that both creek chub and white sucker were capable of successful passage at some flows. Passage window calculations using the highest discharge in which fish were observed passing increased the amount of time considered passable from 0% to 35.3%. This increase in time considered passable supports the findings of Burford (2005) who demonstrated that in some cases, FishXing may give conservative results, and that field validation remains a necessary tool in passage assessment. It is important to note that raising the upper boundary flow to represent a known passable flow does not imply a threshold for that species, but rather a field validated correction for the model. In these situations, the actual upper threshold remains unknown.

Irrigation practices, while providing increased opportunities for experiments by supplying water later into the summer, affected the amount of time a culvert was considered passable. Crossings CC1a and SC1 were considered impassable by FishXing

(velocity barrier) most often during times in which large volumes of water were returned to the streams by irrigation return overflow. It is likely that a more natural (without irrigation) stream system would not produce these high discharge events as frequently, and may provide a greater amount of passage as a result. This is evident in the hydrograph for CC3 which is located upstream of any irrigation influence. At this crossing, high flows were limited to 3 or 4 events throughout the entire study period. In contrast, all other crossings in this study experienced more regular high flow events during the same time period. However, natural streams are more likely to dewater and thus water depth barriers could be more responsible for restricted passage. Further research addressing the effects of irrigation influences on fish passage is needed.

This study revealed that several of the small-bodied, prairie fish species are able to successfully traverse road culverts common to eastern Montana streams. However, the methods used in assessing the amount of passage at each crossing are not without their limitations. Comparing fish assemblages above and below road crossings can be an effective tool in examining the affects of barriers on a watershed scale. However, because prairie fish assemblages regularly display a longitudinal change in species composition, inferences about the consequences of culverts can be difficult. Fish passage experiments using active displacement were also successful in quantifying conditions that permitted passage for a variety of prairie fish species. However these experiments were labor intensive and were limited to flow conditions that allowed efficient fish sampling (seining and electrofishing) and maintenance of block nets. Additionally, recapture efficiency was not 100% and therefore inferences about fish that were not recaptured above culverts are limited. For example, if no marked fish were recaptured above a

culvert, it is unknown if the culvert was a true barrier, or because of inherent capture inefficiencies of sampling. FishXing modeling was effective in quantifying the amount of time culverts were passable for prairie fish over the course of a normal hydrograph. However, because congruency with actual fish movement data was only moderately accurate, these results must be interpreted with caution.

Conclusions and Management Implications

Conclusions that can be drawn from this study are limited to the conditions specific to the study area. Extrapolating these conclusions to settings having different fish species, hydrology or culvert characteristics should be done with that in mind.

Recent interest in the conservation of small stream prairie fishes requires fisheries biologists and managers to learn more about the movement and life history requirements of these lesser-studied species. This study documented that species diversity and fish abundance in small, irrigation-supplemented, prairie streams can be quite large, and that several of these fish species regularly traverse culverts and natural stream reaches during either seasonal or daily movement. The richness and abundance of the fish assemblages detected in the study streams reinforces the need to consider fish passage in the design of new stream crossings and the maintenance of existing stream crossings in settings having prairie fishes. Literature suggests that many of the fish species found in prairie streams are capable of rapid recolonization of upstream reaches after disturbances (Sheldon and Meffe 1994; Dodds et al. 2004). Therefore new culvert installations and culvert repairs should allow for recolonization of areas that may have become fishless over periods of isolation from source populations.

This study also revealed that inconsistencies in the methods typically used to assess fish passage do exist, and that methods for assessing prairie fish passage still need refinement. Comparisons of fish assemblages above and below crossings can be an effective tool in identifying total barriers, but its use in prairie settings appears to be limited. Natural changes in longitudinal distribution and the ability for fish that survive in isolated pools upstream of crossings to recolonize reaches can easily mask the effects of partial barriers. The use of hydraulic modeling software to assess fish passage at culverts was shown to be conservative in several instances as fish passed in situations deemed impassable. This study and others have shown that FishXing is a good indicator of passage success but not as strong an indicator of passage barriers. FishXing should still be used as a tool for assessing existing culverts, but only to separate culverts that need no further study (FishXing indicates passage) from culverts that should be subjected to more direct assessment of passage (FishXing indicates passage barrier). Combining the results of FishXing modeling and field observations yielded a wider view of the amount of passability occurring at a particular crossing. However, inefficiencies in sampling and the conservative nature of the model used, still limit the precision of this method.

Because the fish species found in study streams were observed moving through both natural and culvert crossed stream reaches, recommendations for culvert design should include this biological information. A combination of methods including obtaining a hydrograph, determining the fish species present, FishXing modeling, and field validation should be used to acquire a reliable picture of the local conditions fish may encounter. By including this information, culverts can then be designed or

retrofitted to safely convey water and debris, while meeting the life history requirements of the fish assemblage.

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APPENDIX A

FISH AND HABITAT SAMPLING PROTOCOL

Fish and Habitat Sampling Protocol for Prairie Streams

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1. ***Site location.***-Locate the sampling site using GPS for random sites, or by convenience for non-random sites. The GPS location will be the center of the reach, this is where you place the “F” flag (see Step 2). If the site is dry, shift the reach up or downstream to capture the most wetted channel possible on the parcel of land where you have permission for sampling.
2. ***Laying out the sample reach.***-Lay out a 300 m sample reach using a measuring tape and a set of 11 pin flags (labeled A-K). Follow the curves in the stream channel with the measuring tape; do not cut across curves. To avoid spooking fish, walk along the bank, not in the stream. Place a flag every 30 m. The “A” flag will be at the downstream end, the “K” flag will be at the upstream end of the reach. The “F” flag will go in the center of the reach.
3. ***Block nets.***-Place block nets (these can be old seines, 1/4” mesh) at the upstream (K flag) and downstream (A flag) ends of the sample reach if the water in the channel is continuous, deeper than 25 cm, and relatively clear. This prevents fish from leaving the sample reach.
4. ***Seining.***-Select the seine based on the size of the stream to be sampled. The seine length to be used should be approximately equal to or slightly greater than the stream width, and the seine height should be about 1.5 to 2 times greater than the depth of

the stream. Dip nets can be used in very shallow, small habitats. Seining begins at the upstream end (K flag) and proceeds downstream to the A flag. Seining is performed by two people, one on each end of the seine. In pools, the seine is pulled down the stream channel, using the shore and other natural habitat features as barriers. Begin with the seine rolled up on each seine braille. The seine is typically set perpendicular to shore and hauled downstream parallel to shore. As you proceed, let out enough seine so that the seine forms a “U” shape, but not so much that the net is hard to control. Adjust the length of the seine by rolling or un-rolling net on the seine braille. The speed of seining should be fast enough to maintain the “U” shape, but not so fast that the floats become submerged, or that the seine’s lead line come way up off the bottom of the stream. If rocks or other snags are on the bottom, the seine can be lifted off the bottom for a moment to avoid the snag, or one of the netters can bring the seine around the snag to avoid it, all the while maintaining the forward progress of the seine. Similarly, areas of dense aquatic vegetation can be avoided. It is important not to stop the forward progress, because fish will swim out of the seine. It is better to avoid a snag while keeping moving than to become snagged, which will allow fish to escape. In “snaggy” waters, keep more of your seine rolled up for better control.

Proceed downstream while seining. In narrow streams, the entire channel width is spanned with the seine. In wider streams, one person walks along the shore, while the other wades through the channel. The length of each seine haul will depend on the natural features of the stream channel and shoreline, but seine hauls should not

normally be more than 60 or 90 m long. Side channel bars or the end of a standing pool are good areas to haul out or “beach” the seine. Where a large bar or end of a standing pool is present both netters can simply run the net up on the shore. In streams with steep banks or lack of obvious seine beaching areas the “snap” technique can be used. At the end of the haul, the person near shore stops, while the person farthest out turns into shore, quickly, until the seine is up against the bank. The two netters then walk away from each other, taking the slack out of the seine, and keeping the seine’s lead line up against the bank.

In riffles, with moderate to fast current, the “kick seine” technique can be used. The seine is held stationary in a “U” shape, while the other team member disturbs the substrate immediately upstream of the net. Then the net is quickly “snapped” out of the water by both team members using an upstream scooping motion.

Seine the entire 300 m reach, covering the linear distance at least once. If part of the 300 m is dry, just skip it. If the stream is much wider than your seine, do extra seine hauls in the large pools to cover the extra width. Sample all habitat types (shoreline, thalweg, side channels, backwaters).

After each seine haul, place fish in a bucket. If the water is warm, or you have captured many fish, place fish in a fish bag to keep them alive until seining is completed, or use an aerator. If you have to work up fish before seining is completed, release processed fish in an area that has already been seined, as far away from the

area remaining to be seined as possible (or outside of the block nets). Large fish such as northern pike, common carp, white sucker, shorthead redhorse, or channel catfish, can be measured, given a small clip to the lower caudal fin and released immediately. Marking fish will prevent them from being counted more than once if they are captured again.

5. ***Processing captured fish.***-Record the species of each fish captured, and measure 20 “randomly” selected fish to the nearest millimeter, total length. If the species of fish is unknown, try to at least record it as Unknown type 1, Unknown type 2, etc. Keep track of and record the minimum and maximum length of each species.

For each species, preserve a subsample of at least 10 individuals per site to serve as voucher specimens. Record a small letter “v” next to the recorded length of the fish that is vouchered to allow for later validation. For *Hybognathus* spp., voucher up to 20 individuals per site. Kill the fish to be vouchered by placing them in a small bucket or 1000 ml nalgene jar with an overdose solution of MS-222. After fish processing is completed, drain the MS-222 solution and place the fish in a 1000 ml nalgene jar with a 10% solution of formalin (in clear water, if possible). For specimens longer than 150 mm, an incision should be made on the right ventral side of the abdomen after death, to allow fixative to enter the body cavity. The volume of formalin solution should be approximately equal to the twice the volume of fish tissue to be preserved, and the fish volume should be considered water when concentrations are determined. For example, if the fish take up 250 ml of the 1000 ml volume, you

need about 500 ml of 10% formalin solution (75 ml formalin and 425 ml water) in the 1000 ml nalgene jar. If necessary, use a second jar to accommodate all of the specimens. Use safety glasses and gloves when pouring formalin. Do not let the fish “cook” in the sun for a while and preserve them later, do it as soon as possible. Label all jars inside and out with Site, Site Number, Lat/Long, Date, Collectors names. Use pencil on Write-In-the-Rain or high rag paper for inside labels (just put the label right in with the fish), use a sticker label on the outside, cover it with clear (ScotchPad high performance packing tape pad 3750-P). Fish specimens should be left in formalin solution for at least 2-7 days. Fish specimens must have formalin solution soaked out before being handled extensively. Specimens should be soaked in water for at least 2 days, and water should be changed at least four times during this period. After soaking out the formalin, the fish specimens should be placed in either 70% ethanol or 40% isopropanol for long-term storage.

6. **Habitat survey.**-Channel width, depth of water, and substrate will be measured at 11 transects perpendicular to the stream channel (located at Flags A-K), and along the thalweg in 10 thalweg intervals between transects (deepest part of channel). Stream width is measured to the nearest 0.1 m, depth is measured to the nearest cm, and substrate sizes and codes are on the data sheet. One person will be in the stream taking measurements while the other records data. Record the Latitude and Longitude (in digital degrees) of the F flag, the stream name, site number, the date, the flow status (flowing, continuous standing water, or interrupted standing water) and the names of the crew members on the data sheet. Take photographs of the site,

capturing as much of the sampling reach as possible. Make sure the date feature on the camera is turned on, to allow for later identification of site photographs.

Transects.-Start on the left bank (facing downstream) at Flag A. Measure and record the wetted width of the channel to the nearest 0.1 m. Measure and record (separated by a comma on the data sheet) five equally spaced depth and substrate measurements across the wetted stream channel:

1. Left Bank-5 cm from the left bank;
2. Left Center-halfway between the Center and the Left Bank;
3. Center-center of the wetted stream;
4. Right Center-halfway between the Center and the Right Bank;
5. Right Bank-5 cm from the right bank

Thalweg.-Begin by recording the depth and substrate 3 m upstream of the transect, in the deepest part of the channel (thalweg). Proceed up the thalweg to Flag B, recording depth and substrate every 3 m along the thalweg. You will record a total of 10 depths and substrates between each pair of transects. If the stream channel is dry, record a 0 for depth, and record the substrate. The last thalweg measurement point should fall on the next upstream transect. The 3 m interval can be estimated, and it is helpful if the data recorder helps to keep the person in the stream from “squeezing” or “stretching” the thalweg measurements.

Repeat this procedure until all 11 transects and 10 thalweg intervals are completed.

Gear List

- 20', x 6' x ¼" heavy delta seines
- 15' x 4' x ¼" heavy delta
- 30' x 6' x ¼" heavy delta (or delta) with 6' x 6' x 6' bag
- Fish bags: nylon diver's bags, ¼" mesh 18" x 30"
- Mudders – 109.00 at Ben Meadows
- Block nets, Tent stakes
- Stream Conductivity meter
- Thermometer
- Turbidity meter (LaMotte, Ben Meadows 224805, \$795.00-might try the “transparency tube” Ben Meadows 224196, \$52.95)
- Waders (breathable waders are essential for this work-Cabelas has them for about \$100/pair), hip boots are usually too low
- Lug sole wading boots (Cabelas)
- Habitat pole (I make habitat poles out of 1.0" OD PVC pipe. 1.5 m long including caps. Score the pipe every 10 cm with a pipe cutter, then use a Sharpie to mark rings around the pole at the scores, and label the pole 10, 20, 30, etc. 5 cm marks are made between the 10 cm rings, you can visually estimate between the 5 cm marks to get to the nearest cm. Spray or brush a Urethane finish on the pole or your marks will come off fast with sunscreen and bug dope.)
- Metric 30 m tape (Ace Hardware actually carries a tape with metric on one side)

- Measuring boards, one short 300 mm (half a 6" PVC works well for *Hybognathus* "fin flotation", one long, ~0.5-1 m, or you can just use a meter stick for the odd big fish)
- Hand lens
- Small 1 gallon red bucket from Ace Hardware for doping fish
- 5 gallon buckets
- MS-222
- Labels and tape pads for fish samples
- 1000 ml Nalgene jars
- Formalin (buffered is great, but more expensive-I throw a Roloids in each jar of fish to neutralize the acidity)
- Clip board
- 11 Pin flags labeled A-F