Final Report: December 20, 2010 to December 31, 2012

Title: Fish Passage in Plains and Prairie Waterways

Grant Number: X-2-R-1

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**Summary** - This report provides a final update of work performed for the period beginning December 20, 2010 and ending December 31, 2012. The report describes two umbrella projects: (1) to improve fish passage and landscape connectivity for native species and 2) to determine the thermal effects on fish species sensitive to climate change. The work was performed through a partnership led by the Western Transportation Institute at Montana State University and the Bozeman Fish Technology Center (BFTC) of the United States Fish and Wildlife Service. The report is divided into five chapters that provide details on accomplishments to meet specific objectives outlined in our proposal during the period. Several of the projects that fall under the larger umbrella of project 1 and 2 involve multiple partners, additional funding, and are ongoing.

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# Agency Research Program Partners

Western Transport Institute (WTI) at Montana State University (MSU) and Montana State University Civil Engineering Department

The WTI-MSU is the nation's largest transportation institute focusing on rural transportation issues and is designated as a U.S. Department of Transportation University Transportation Center. The Institute was established in 1994 by the Montana and California Departments of Transportation, in cooperation with Montana State University. WTI-MSU has research and demonstration projects in 30 states, in such diverse fields as winter maintenance and effects, safety and operations, and systems engineering design and integration, and road ecology. The road ecology group has 11 research professionals focused on studying the interaction of roads and the environment. The Civil Engineering Department at MSU has a long history of successful research projects that focus on hydrology and hydraulics in a riparian setting.

Montana State University - Ecology Department

The Department of Ecology at MSU has a long-standing reputation as a leader in research on fish ecology and management. Areas of expertise at MSU include native fish species restoration and management, fish passage research, prairie fish ecology, and biological assessment of streams using fish assemblages.

Bozeman Fish Technology Center (BFTC) - United States Fish and Wildlife Service (USFWS)

The BFTC is a research center of the USFWS that focuses on conservation of imperiled fish and aquatic species. BFTC-USFWS research programs include reproductive physiology, immunology, water treatment and design, and the ecology of imperiled species. Research facilities include three indoor fish research buildings, outdoor concrete raceways including six replicated bays with an operational water recirculation and treatment system as well as diverse laboratory capabilities. BFTC-USFWS research facilities are ideal for conducting controlled, replicated studies in indoor or outdoor environments with flow and temperature control. BFTC-USFWS researcher programs combined with laboratory capabilities in stress response, spawning, fish health and immunology, histology and other areas readily complement studies of swimming stamina, leaping, and other physical attributes.

# **Chapter 1 – Development of a Test Bed Facility with Tools to Access Fish Swimming Capabilities**

## Background

The first objective met in this project was to develop a test bed to allow for rigorous, transparent, and replicable testing of scientific theories of fish swimming capabilities and passage requirements. Prior to our initiative no such facility existed within the Plains and Prairie Pot Hole region. The test bed facility developed at the BFTC consists of an open-channel flume system, a living stream (described in chapter 4), and two traditional swim tunnels. The facility and equipment are the products of partnerships involving the WTI at Montana State University, USFWS Region 6 Fish Passage Program, the Bozeman Fish Technology Center, USFWS Plains and Prairie Potholes LCC, U.S. Forest Service-Gallatin National Forest, Turner Enterprises, and Montana Chapter of the American Fisheries Society. With the addition of the research systems, the Bozeman Fish Technology Center expanded it science capabilities and is now a test-bed facility for addressing a broad range of questions related to habitat connectivity for aquatic species. The new capabilities are instrumental in delivering on the goals of an ongoing Fish Passage Research Program lead by WTI, MSU, and BFTC. This chapter satisfies parts of Objective 1, listed in the original grant.

## BFTC-WTI-MSU Fish Passage Program goals:

• Determine scientifically valid, volitional swimming or mobility capabilities of fish and other aquatic species that reside in the Northern Rockies Ecosystem, the Prairie Plains and Pothole Ecosystem, and threatened and endangered species of concern to the USFWS.

• Address a broad range of research needs related to aquatic connectivity, such as behavioral barriers, attractant flows, weir dynamics, and etc. The research results will allow fish passage practitioners to better to assess, design, retrofit and construct hydraulic structures within our streams and rivers.

• Promote interdisciplinary research and education. The facility and its research will help train future engineers, biologists, and ecologists, making them more interdisciplinary and better able to effectively solve aquatic passage problems.

## Construction of the Flume

The open-channel flume was "structurally" completed and water-tested in July 2010. In August of 2010, the flume was operational and an initial pilot study began (see chapter 2). Structural improvements continued throughout 2011 and the flume was fully completed and operational in 2012. The flume apparatus consists of a headwater and tailwater tank connected by a flume (see photos). The flume itself is 0.91 m wide (3 ft) and 17 m (56 Ft) long. The cross sectional area (width) of the flume is adjustable and decreasing the width allows for increased

water velocity. Water velocity is also created by tilting the flume at an angle (slopes of 6% or less are possible), increasing or decreasing flow volume, and use of flash boards.

The flume is equipped with a fish monitoring camera array system. Digital cameras, placed in boxes evenly distributed along the frame of flume so that the six views overlapped, record and generate the swimming data of fish in the flume. Typically the overhead video cameras monitor a 12.8 m section of the flume that is marked in 0.61 m increments (on the bottom). The marked increments on the bottom of the flume are used to determine the distance a fish traveled during a known period of time when the video is analyzed. Fish swims are analyzed using Sony PMB software (Sony Corporation, Tokyo, Japan). The flume is shielded from direct sunlight using cloth draped over a wooden frame above the flume. The shading provides cover for the fish and reduces glare that compromise video quality and make analyzing the video difficult.

The BFTC's unique warm and cold water springs allow researchers the ability to vary water temperatures between 8 and 22°C and allows work on both cold-water species important to the Great Northern LCC and warm-water species important to the Plains and Prairie Potholes LCC. Flow of up to 3,000 gallons per minute (gpm) can be attained. Additional equipment is required to effectively operate water flow and standardize flow to meet conditions of the hydraulic models. Velocity in the flume is measured with an acoustic Doppler velocimeter (ADV) to characterize three dimensional (3-D) flow patterns and 1-D velocity instruments (such as a Marsh McBirnery Flo-Mate).

Photographs of the flume during construction, completed flume, operational photos, and photos of the swim chambers follow.



Head-box. Water flows from the pipe, into the head-box, and down the channel. The Bozeman Fish Technology Center's unique warm and cold water springs allow researchers the ability to vary water temperatures between 8 and 22°C and perform work on cold water species important to the Great Northern LCC and warm-water species important to the Plains and Prairie Potholes LCC.

Photo show the flume structure. The flume is constructed of wood and rests on steel beams. The beams slope and flume are adjusted hydraulically to control slope and water velocity. The flume channel is 56 feet long and has an adjustable width that extends up to 3 feet. Water velocity is created by tilting the flume at an angle (slopes > 6% are possible). In a typical experiment a fish is placed in the tail-box and released. Overhead cameras and pit tag arrays record (not shown) the fish's movements as it swims through the channel.



Photo shows the interior of the flume with a center wall restriction inn place. The restriction is used to increase flow velocity or when working with small bodied fish species such as cyprinids.



Photo shows the completed flume. Overhead cameras are mounted on the frame and record fish movements swimming in the flume. The frame is draped with a shade cloth during a swim trial.



Photos show overhead views of the flume. The images are excerpts of video taken during the pilot study performed on westslope cutthroat trout. The arrows direct the viewer to the outline of a westslope cutthroat trout swimming in the flume. The video segments were used to estimate time-to-pass the segments of two feet in length sections of the flume (marked in orange on the flume floor). Flow velocity (water) and speed over ground (fish) are used to develop an endurance curve and estimate the westslope cutthroat trout swimming ability.



The photo shows Dr. Matt Blank measuring the water velocity at a road culvert. The endurance curves developed for a fish species can be used to determine if the structure acts as a barrier for fish passage. Endurance curves are calculated from determining the burst, prolonged, and sustained swim speeds of a fish species.

# Fish Swimming Cambers

Our primary design objective was to develop flexible systems and tools that would allow future investigations on the volitional and forced swimming abilities of large and small bodied fish species. In addition to the flume, additional tools were purchased or constructed including two swimming chambers capable of swimming both large and small bodied fish. The two swim clamberers were purchased from Loligo<sup>®</sup>, Denmark. Both tools have advantages under different circumstances and can perform best with different species. Swim chambers allow for quick data collection, easy replication, and relatively easy flow manipulation. One key difference between flume swimming trials and chamber swimming trials is that swimming chambers force fish to swim at a fixed point against flow, whereas volitional studies allow the fish to swim upstream through the flow, and arguably provide more realistic characterization of swimming abilities. Together, these tools provide a wide range of options for evaluating swimming abilities of different fish species over a range of flows, velocities, depths, and temperatures.



Photo shows the small fish swimming chamber used to test small bodied fish.

# Summary

Land transformation has altered the natural connectivity of fish communities that inhabit waterways. Our nation's waterways are obstructed by an estimated 2.5 million aquatic barriers, those present in the prairies of Montana, and North and South Dakota alone run into the thousands. Connectivity is essential for the long term viability of aquatic species. One of the most promising adaptive management strategies for addressing impacts to aquatic systems by climate change and other landscape stressors is increasing connectivity. In the short period that our research program has been operating it has assessed or is currently assessing the swimming capabilities of five fish species including sauger (*Sander Canadensis*), longnose dace (*Rhinichthys cataractae*), shovelnose sturgeon (*Scaphirhynchus platorynchus*), westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*Oncorhynchus mykiss*). The information gathered from those studies will help engineers to design effective passage ways for fish species. We have also provided opportunity for two graduate students, one in the MSU engineering department who is currently working on her PhD and a second in the MSU ecology department who is working toward his master's degree. The facility and its research have helped train a future engineer and biologist, making them both more interdisciplinary and better able to effectively solve future problems. In the following two chapters we describe the swimming capabilities of three of the species we have assessed.

# **Chapter 2 - Pilot Program for Testing Fish Swimming Capabilities - Rainbow and Westslope Cutthroat Trout Swimming Study**

#### Background

The following chapter describes a study performed to evaluate swimming performance of two trout species: westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*O. mykiss*) using an open channel flume at the BFTC. This chapter satisfies parts of Objective 1 and 2, listed in the original grant.

## Introduction

#### Fish Species

The West is renowned for pristine cold water fisheries - a resource that drives tourism, real estate value, agricultural productivity, and other economic engines. Mobility and connectivity in these systems is important to nearly all organisms, but in this project the focus was placed on the keystone ecological and economic components of the system - trout. For example, cold water streams in Montana provide habitat for native trout including bull trout (Salvelinus confluentus), westslope cutthroat trout and Yellowstone cutthroat trout (O.C. bouvieri), as well as nonnative trouts including brook trout (S. fontinalis), brown trout (S. trutta), and rainbow trout, and hybrids between native and nonnative species (e.g., cutthroat-rainbow hybrids). In this project, the trout species of focus was westslope cutthroat trout and rainbow trout. Cutthroat trout are often the impetus for connectivity concerns and restoration activities, could be used as a surrogate species for other trout, are available in enough abundance to collect specimens for research activities, and are sufficiently widespread to overcome concerns of geographic distribution. Rainbow trout are widely distributed in the region and throughout North America and bring a much larger audience to the results of the project. Rainbows have also been used as a surrogate species for native trouts in fish passage assessments and fish works. Additionally, fisheries professionals across the West involved with native species conservation are commonly involved with projects dealing with interactions between rainbow trout and cutthroat trout. Lastly, there are no studies to the author's knowledge that characterized the swimming performance of westslope cutthroat trout.

#### Descriptions of Swimming Capability

Barriers to fish mobility are often assessed, or designed, in terms of a fish's ability to overcome obstacles. One obstacle is related to swimming velocity – how well a fish can overcome fast flowing water through a culvert or other potential fish passage obstacle (e.g., irrigation diversions). Another potential obstacle is the height that a fish must jump to enter the structure or surpass it. A third potential obstacle is associated with very shallow water flow in the structure. This project addressed the velocity issue directly; however, leap heights can be inferred from burst speeds as described in more detail below.

### Swimming Modes

Fish swimming is often described in three forms - sustained swimming, prolonged swimming, and burst swimming (Katopodis and Gervais, 1991). Sustained swimming is the speed that the fish can maintain for an indefinite period of time (analogous with human walking). Prolonged swimming is a moderate speed that can be maintained for several minutes to a couple of hours (analogous to human jogging). Burst speed is the maximum speed that a fish can produce, usually maintainable for less than 15 seconds (a human sprinting). Fish will use different swimming modes and behaviors in response to a variety of factors including flow conditions, life history needs and interactions with other species (Hoar and Randall, 1978).

## Leaping Ability

As previously mentioned, excessive leap heights can be a barrier to fish passage. One method used to estimate the height a fish can leap (and whether a structure is a leap barrier to fish) is to predict the leap height using trajectory analysis with the fishes maximum burst velocity as the motive force (Furniss et al., 2008). Lauritzen (2002) developed a model that predicts the takeoff angle, minimum distance between takeoff and successful landing, and minimum takeoff velocity. This model was developed by combining field data of the physical parameters of two waterfalls studied, biological parameters of migrating fish (sockeye salmon) at the two waterfalls, and the assumption that fish jump as simple projectiles (Lauritzen, 2002). This study, which is the most comprehensive study of leaping behavior and trajectory motion to the author's knowledge, developed parameters for the model using data from field observations. Recent studies in laboratory settings in Colorado on brook trout (Kondratieff and Myrick, 2006) and Washington on juvenile coho salmon (Pearson et al., 2005) have produced good data on leap heights and the ratio of leap height to water depth at the location the leap was initiated.

### Swimming Abilities of Rainbow Trout and Cutthroat Trout

A review of swimming studies on rainbow trout and cutthroat trout swimming performance provides an interesting context regarding the purpose or intent of studies, the variety of experimental techniques used and the range of swimming information derived from them. Some studies characterized basic swimming performance and investigated effects of temperature, fish length, and experimental approach on swim performance of trout as measured using the U<sub>crit</sub> method (MacNutt et al., 2004; Jain et al., 1997; Keen and Ferrel, 1994; Webb et al., 1984). Others evaluated the effect of various contaminants on swimming performance (Jones And, more recently, studies to develop fish passage models using and Moffit, 2004). probabilistic tools were completed (Peterson et al., 2013; Cahoon et al., 2007). The literature is very deep in swimming studies that used a measure of swimming performance as a physiological response to environmental or biological conditions, with the classic example of studies of the physiological effect of swimming on white muscle in salmonids (e.g. Shulte et al. 1992 as cited in Burgetz et al., 1998; Milligan and Wood, 1986) Critical swimming is often used as a measure of fitness for studies evaluating the potential effects of hybridization between trout species (Seiler and Keeley, 2007; Hawkins and Quinn, 1996).

Studies completed to determine some measure of trout swimming performance for fish passage analysis and design are fewer in number than one might expect. Field studies in

Montana investigated passage of Yellowstone cutthroat trout, rainbow trout and their hybrids through culverts, and characterized the flow, depth and velocity conditions through which fish passed and were blocked (Burford et al., 2009; Blank, 2008; Solcz, 2007; Belford and Gould, 1989). Solcz used this data to develop a probabilistic model of culvert passage relative to average velocity within the culvert (Solcz, 2007). Hunter and Mayor summarized prolonged and burst swimming information for rainbow trout and other species (Hunter and Mayor, 1986).

Although there is some swimming information for rainbow trout and cutthroat trout, many researchers, engineers and biologists involved in practical fish passage projects suspect that we may be underestimating swimming performance of these and other trout. In addition to the need to further characterize swimming performance of rainbow trout, there is no swimming information for westslope cutthroat to the author's knowledge. Therefore, the objective of this project was to characterize the volitional swimming performance of rainbow trout and westslope cutthroat trout using an open channel flume.

## Methods

Flume construction began in May of 2010 and was operationally completed by August 2010. Once the flume was operational, a series of tests were conducted to evaluate the conditions within the flume. The first series of tests involved no fish and were primarily focused on measuring and confirming hydraulic conditions within the headwater tank, the flume and the tailwater tank. A series of initial swim trials with rainbow were performed during August and September 2010. The purpose of the initial trials was to validate and refine swimming methods prior to initiating the full swimming experimental program which began in 2011 and is described below. The remainder of this chapter describes the full-scale pilot swimming study.

### Fish Collection and Holding

Rainbow trout were collected from Hyalite Creek using a backpack-mounted electrofishing unit. Captured fish were placed in a bucket filled with creek water initially and transferred to live wells in a truck for transport to the Bozeman Fish Technology Center (BFTC) in Bozeman, Montana. The live well temperature and oxygen levels were controlled during transport to ensure minimal damage to fish during transport. Westslope cutthroat trout were initially collected from streams near Kalispell, Montana for broodstock and supplementation efforts. The fish were held in a hatchery prior to being transported to the BFTC.

Both rainbow trout and westslope cutthroat trout were kept in separate holding tanks near the flume. For each species, the fish were separated into size classes and each size class was placed in a separate holding tank. All fish were individually pit tagged, weighed and measured (FL) prior to initiating the swimming trials. Water temperature in the holding tanks was maintained at a constant temperature that closely matched the water temperature in the flume. The fish were fed daily with a commercial trout feed but food was removed 24 h in advance of a swim trial.

#### Swimming Experiments

In broad terms, there are two types of experimental devices that are used in various ways to characterize swimming performance. One is the use of a swimming tube or chamber where the fish are kept in a fixed area and forced to swim against different velocities of water for different amounts of time. One of the classic swim methods that use the stepped velocity type of approach is the U<sub>crit</sub> swimming experiment. It is argued that U<sub>crit</sub> is a measure of the maximum effort of swimming as the rate of work is proportional to the swimming speed (Webb, 1971). The second is the use of swim flumes to characterize fish swimming performance (Russon and Kemp, 2011; Vokoun and Watrous, 2009; Peake, 2008; and Castro-Santos, 2005). Flume studies set the flow, velocity and depth of water in the flume (referred to hereafter as a hydraulic challenge (HC)) using a combination of slope, area, and flow settings to achieve a predetermined hydraulic challenge (e.g. a combination of water depth and velocity). Fish are then placed either into a holding pool at the downstream end of the flume or directly into the current. The fish are allowed to swim upstream on their own volition, thus the name "volitional" experiment for this method. Fundamentally, these are very different experiments because one forces the fish to swim in a fixed location against a current, while the other allows the fish to swim upstream through the current.

For this study, swimming performance experiments to characterize rainbow trout and westslope cutthroat trout swimming abilities were completed during 2011. Two different swimming methods were used: (1) a volitional swimming experiment in the flume to characterize prolonged and sprint swimming behavior, and (2) a coerced experiment in the flume, referred to as a "spook test", to characterize sprint swimming behavior.

For both sprint and volitional experiments, fish movements in the flume were recorded by six high definition video cameras positioned above the flume. The cameras recorded fish movements at 30 frames per second. The bottom of the flume was marked every 0.15 m horizontally and every 0.60 m in the direction of flow. The markings were used to identify the position of each fish as it progressed upstream in the flume.

## Volitional Swimming Experiment

The volitional swimming experiments were performed following methods similar to those used at the Conte Anadromous Fish Lab in Turners Falls, Massachussetts (Castro-Santos, 2005). Both trout species were tested under two different HCs. As a reminder, a HC is a set of hydraulic conditions including flow, water depth, water velocity and temperature. The average hydraulic conditions for HC1 and HC2 are summarized in Table 1. Four different size classes were tested for both species. Size classes were as follows:

- Less than 17.8 centimeters (cm); referred to hereafter as 16.5 cm.
- 17.8 to 20.3 cm; referred to hereafter as 19.1 cm.
- 20.3 to 22.9 cm; referred to hereafter as 21.6 cm.
- Greater than 22.9 cm; referred to hereafter as 24.1 cm.

Rainbow Trout											
Trial	Experimental	Hydraulic	Number of		Water						
Number	Method	Challenge	Fish	Size Class	Temperature	Flow	Velocity	Depth			
				cm	°C	m <sup>3</sup> /s	m/s	m			
1	V	HC1	16	16.5	9.6	0.04	0.45	0.29			
2	V	HC1	20	19.1	9.8	0.05	0.51	0.27			
3	V	HC1	25	21.6	9.5	0.05	0.46	0.27			
4	V	HC1	12	24.1	9.5	0.04	0.49	0.28			
5	V	HC2	23	16.5	12	0.09	1.07	0.18			
6	V	HC2	20	19.1	11.5	0.07	1.06	0.19			
7	V	HC2	24	21.6	11.5	0.08	1.16	0.18			
8	V	HC2	14	24.1	11.5	0.08	1.11	0.20			
9	S	HC3	18	19.1	9.5	0.03	0.13	0.50			
10	S	HC3	12	21.6	9.5	0.02	0.13	0.49			
11	S	HC3	6	24.1	9.5	0.02	0.13	0.49			
Westelane Cutthmet Trout											
Trial	Experimental	Hydraulic	Number of	spe Cuttinoat	Water						
Number	Method	Challenge	Fish	Size Class	Temperature	Flow	Velocity	Denth			
Ttullioti	litethou	chunchge	TIM	om	°C	m <sup>3</sup> /s	m/s	m			
12	V	HC1	14	16.5	11	0.06	0.46	0.27			
12	V	HC1	14	10.5	11 5	0.00	0.40	0.27			
13	V		0	21.6	11.5	0.05	0.45	0.28			
14	V		<del>9</del> 7	21.0	11.5	0.05	0.49	0.27			
15	V	HC2	13	16.5	12	0.05	1.13	0.27			
17	V	HC2	0	10.5	12	0.07	1.13	0.19			
1/	V	HC2	<i>7</i> 8	21.6	0.5	0.07	1.05	0.20			
10	v V	HC2	7	21.0	9.5 12	0.00	1.12	0.10			
20	v S	HC3	7	24.1	12	0.07	0.14	0.19			
20	S	HC3	8	24.1	11	0.03	0.14	0.49			

Table 1: Summary of basic information for rainbow and westslope cutthroat trout experiments.

<u>Table Notes</u>: V – volitional, S – sprint, HC1 – hydraulic challenge 1, HC2 – hydraulic challenge 2, HC3 – hydraulic challenge 3.

Once the flume was set to the appropriate HC, an entire size class of trout of one species was randomly selected and placed in the tailwater pool at the downstream end of the flume. Each volitional trial lasted four hours. Based on pilot study experiments completed in 2010, four hours was determined to be an appropriate length of time for fish to attempt ascending the flume.

The speed at which a fish swam upstream through the flume (groundspeed) was determined through review of video images recorded by six overhead cameras that track fish progress. The cameras recorded the time that a fish took to swim each 0.6 m increment. The groundspeed for each increment was calculated by dividing 0.6 m by the time in seconds to ascend that distance. Fish swimming speed for that increment was calculated by adding the groundspeed to the average water velocity as measured in that section of the flume. Fish movement was tracked to the maximum distance of upstream ascent. If a fish swam the full

length of the monitored flume (12.8 m), the maximum distance of ascent was at least 12.8 m and the attempt was categorized as "fully ascended". Otherwise, maximum distance of ascent was the upstream-most point in the flume that a fish reached prior to backing down; this type of ascent was categorized as "partially ascended".

All attempts that progressed less than 1.5 m up the flume were removed from the data set prior to analysis. These attempts were removed because they were perceived as "exploratory" rather than a full attempt at passing through the velocity challenge.

## Sprint or "Spook" Swimming Experiment

Sprint swimming experiments were performed following methods similar to those used to characterize bull trout sprint swimming behavior (Mesa et al., 2008). For the sprint study, fish were only tested under one HC (referred to as HC3 in Table 1). The flume was set at a relatively low velocity to orient fish into the current, but not force them to swim excessively. Hydraulic conditions for the spook study are also summarized in Table 1. The speed at which fish swam upstream through the flume was determined using the cameras and the same method as described for the volitional swim experiment.

Fish were tested individually. A randomly chosen fish was removed from a holding tank and its PIT tag number was recorded. The fish was placed in a bucket and moved to the downstream end of the flume. A screen was placed between the tailwater pool and the flume to prevent fish from descending into the tailwater tank. The fish was placed in the flume and the time was recorded. The fish was observed for several minutes to determine if the fish could maintain its position in front of the screen. If the fish appeared to be able to hold its position it was left for 10 minutes to acclimate. If the fish was pinned to the screen or was struggling to stay off the screen it was removed and that was recorded. After the acclimation period, a soft nylon bristled broom was used to brush against the tail fin to startle or "spook" the fish upstream. This was an attempt to coerce the fish to begin ascending the flume. If the fish did not respond to the stimulation it was again given 10 min to acclimate. After the second 10 min the fish was stimulated again. If the fish did not ascend after a second stimulus it was removed and this was recorded. Attempts to coerce the fish were recorded as well as approximate times for the start of swimming if applicable. Once a fish had ascended the flume it was captured and placed back into the appropriate holding tank. The holding tank for each size class was divided into two areas: one for fish that had been swum and another for those that had not been swum.

### Hydraulic Methods

Prior to swimming a fish using either volitional or spook methods, the flume was turned on and allowed to equilibrate for 45-60 minutes. Once the flume had established equilibrium, the hydraulic environment was characterized. A combination of both hydraulic measurements and modeling were used to characterize the flow environment in the flume for each hydraulic challenge and swimming trial. Measurements included water flow, water depth and water velocity throughout the flume. All experimental data were recorded in a notebook and entered into Microsoft excel for storage and future data analyses. Hydraulic measurements were collected at the beginning and end of each swim trial. By collecting measurements pre- and post-swim, in combination with continuous stage measurements, any deviations or change in flow characteristics were identified and factored into the calculations for estimating swimming speeds.

Water depth measurements in the flume were collected using a graduated rod at every 0.6 m interval distributed evenly through the entire flume for a total of 21 measurements. Water depths in the headwater and tailwater tanks were measured continuously through each experiment using a TruTrack data logger. These measurements were used to verify that the flow environment was stable during the experiment and that it was within acceptable limits for each trial. Also, by continuously recording water depths during the experiment, we can determine if there were any flow surges that may have influenced the experiment and outcome.

The flow rate in the flume was measured using a continuous flow measurement device and checked using the USGS 0.6 x depth flow measurement method. Water velocities were collected using a Marsh McBirnery Flo-Mate flow meter at every 0.6 m interval distributed evenly through the entire flume for a total of 21 measurements. Velocity was collected at a depth equal to 0.6 times the water depth. This vertical location is typically representative of the average water velocity in a section of flume. Water velocities in the flume were also collected using an Acoustic Doppler Velocimeter (ADV) manufactured by Sontek to evaluate three dimensional patterns of velocities for a given HC. The ADV provides measurements of the x, y and z velocity at a point, representing a three dimensional characterization of flow. It also collects data at a high enough frequency (25 hertz) to estimate point turbulence. Measurements were collected for 90 seconds at each point in a grid. Velocity measurements were taken at flume station 42 in a 10 cm square grid pattern starting at 2.5 cm from each wall. The 2.5 cm boundary was used because of limitations of the ADV – basically the arms of the ADV prevent measurements close to boundaries and boundaries can interfere with the acoustics.

We created simple hydraulic models of the flume flow environment for each trial using gradually varied flow hydraulics. The model was created in Microsoft excel. The model was calibrated and validated against measurements recorded in the flume. The hydraulic model output provides water velocities at any station in the flume.

Upon completion of a swimming experiment, all video images were downloaded from the cameras onto a computer for future analysis. Hydraulic data was entered into a Microsoft Excel database.

## Data Analysis

Key swim parameters including maximum volitional swim speed, mean volitional swim speed, maximum distance of ascent, cumulative swim time, and maximum sprint swim speed were calculated from the volitional and sprint swimming experiments. Swim attempts were further evaluated by categorizing each attempt into one of two categories: (1) high sprint performers (HSP) and (2) low sprint performers (LSP) (McDonald et al., 2007).

The design of the volitional swimming experiments was done partly to simulate a natural setting as best possible. Past observational studies that evaluated attempts to pass upstream through culverts show that fish will execute multiple attempts at passing upstream through a velocity challenge (Blank, 2008). In experimental trials performed in a culvert test bed, similar behavior was exhibited by coastal cutthroat trout (*O. C. clarkii*) (Peterson et al., 2013). Therefore, the volitional study design and analysis considered each attempt at passing upstream through the flume as an independent observation. A fish may have learned something from a previous attempt, which could potentially influence how they chose to swim during subsequent

attempts. This "learning" type of behavior is believed to be important for fish passage analysis and design because trout will use multiple attempts to pass a velocity challenge. Other fish passage studies have viewed each attempt as an independent observation (Lauritzen, 2002; Vokoun and Watrous, 2009).

Statistical analyses of various swim speed data were performed to evaluate differences in measured swimming performance metrics within a species and between species. All statistical analyses were performed using Minitab V. 16 software with a significance level of 0.05. Data were checked for normality using an Anderson Darling test. For data that were not normally distributed a range of data transformations (ln, arcsine, square root, Log 10) on the response variables were attempted with little success. An F-test was used to check for equal variances for data that was identified as normally distributed. If variances were not equal, a t-test with unequal variances was used to evaluate differences in mean swim speeds for a particular comparison between data that were normally distributed. In most cases, data were not normally distributed and a non-parametric Mann-Whitney U-test was used to evaluate differences in median swim speeds or other response variables.

Statistical tests were done to evaluate differences between mean volitional swim speed, maximum volitional swim speed, and maximum distance of ascent between rainbow trout and westslope cutthroat trout for all size classes, all types of attempts and all volitional hydraulic challenges pooled together. As a reminder, each swim attempt was classified as either "fully ascended" if the attempt progressed upstream the entire monitored distance in the flume or "partially ascended" if the attempt was truncated prior to the end of the monitored distance. In addition, tests were done to evaluate differences between maximum sprint swim speeds and volitional sprint swim speeds between rainbow trout and westslope cutthroat trout.

Similar statistical analyses were done to evaluate differences between mean volitional swim speed, maximum volitional swim speed and maximum distance of ascent between rainbow trout and westslope cutthroat trout for each hydraulic challenge separately (HC-1 and HC-2).

Within a species, statistical tests were done to evaluate differences between mean volitional swim speed, maximum volitional swim speed, and maximum distance of ascent between different size classes. For these analyses, a Kruskal-Wallis test was used to evaluate differences in medians with the response variable being a measure of swim speed and the factor being size class. If a significant difference between the median of two data sets was identified, then pairwise Mann-Whitney U-tests for all possible paired combinations of data were carried out to determine which pair was different. Other analyses involved investigating differences between maximum volitional swim speed and maximum sprint swim speed within a species.

### Results

The results from the volitional swim study are first described, followed by results from the spook swim study. Comparisons between study methods are also described towards the end of this section. Figure 1 shows fish swim velocity, time and distance for one trout that swam upstream during a volitional study. This figure is included as an example of how the volitional trial is converted into fish swimming metrics. A similar figure was created for each individual swim attempt.



Figure 1: The figure shows fish swimming speed (solid line) and distance traversed (dashed line) as a function of time for one rainbow trout swimming in HC-2.

Figure 2 presents the mean swimming speed and time for all size classes of rainbow trout in both hydraulic challenges, including both "fully ascended" and "partially ascended" attempts.



Figure 2: The figure shows mean volitional swim time and swim speed for rainbow trout, all size classes and both HCs combined.

Figure 3 shows the maximum swimming speed, considered to be "sprint" behavior, for "HSP" and swim time for all size classes of rainbow trout in both hydraulic challenges. The points show a general trend of shorter swim times for higher swim speeds. The fastest rainbow trout swim speed recorded for the volitional study was 3.18 m/s by a 16.5 cm trout.



Figure 3: The figure shows maximum swim speed and time at maximum speed for rainbow trout, all size classes and both HCs combined.

Figure 4 presents the mean swimming speed and time for all size classes of westslope cutthroat trout in both hydraulic challenges.



Figure 4: The figure shows mean volitional swim time and mean swim speed for westslope cutthroat trout, all size classes and both HCs combined.

Figure 5 presents the maximum swimming speed for "HSP" of all size classes of westslope cutthroat trout in both hydraulic challenges. The fastest westslope cutthroat trout swim speed recorded for the volitional study was 2.51 m/s by a 16.5 cm trout.



Figure 5: The figure shows the maximum swim speed and time at maximum speed for westslope cutthroat trout, all size classes and both HCs combined.

Table 2 summarizes several key parameters from each volitional swim trial by size class including: mean of mean volitional swim speed, mean cumulative swim time, mean maximum distance of ascent and mean of maximum volitional swim speed. For example, mean of mean volitional swim speed is the mean for all swim attempts in a given size class and hydraulic challenge.

Table 3 summarizes key parameters from the sprint swim study (spook experiment). Key parameters include mean of maximum sprint swimming speed and mean sprint time. The right side of the table includes a subset with values for only swims greater than 1.5 m/s, which were considered "HSP".

			Volitional Swimming Experiments									
Species	Hydraulic Challenge	Size Class	Mean of Mean Volitional Swimming Speed	Standard Deviation	Mean Cumulative Swim Time	Standard Deviation	Mean Maximum Distance of Ascent	Standard Deviation	Mean of Maximum Volitional Swimming Speed	Standard Deviation	Sample Size	
		cm	m/s	m/s	S	S	m	m	m/s	m/s		
	HC1	16.5	0.85	0.20	31.74	24.22	8.75	4.51	1.16	0.56	10 (11)	
It	HC1	19.1	-	-	-	-	-	-	-	-	-	
Rainbow Trou	HC1	21.6	0.82	0.19	19.06	16.45	5.51	4.31	1.08	0.39	4 (13)	
	HC1	24.1	1.03	0.16	26.96	8.79	12.09	2.69	1.34	0.29	13 (1)	
	HC2	16.5	1.07	0.33	14.49	7.42	5.34	4.28	1.50	0.74	5 (17)	
	HC2	19.1	1.25	0.41	27.93	21.86	7.58	4.86	1.77	0.71	6 (9)	
	HC2	21.6	1.27	0.31	15.83	9.88	5.76	3.41	1.76	0.59	1 (10)	
	HC2	24.1	1.28	0.38	15.88	4.37	6.71	3.63	1.78	0.68	2 (10)	
ıt	HC1	16.5	0.77	0.19	21.87	10.76	6.10	4.63	1.04	0.32	4 (12)	
lino	HC1	19.1	1.02	0.19	21.07	14.98	8.81	5.02	1.39	0.25	12 (8)	
Westslope Cutth Trout	HC1	21.6	0.74	0.12	37.39	22.22	9.23	4.77	0.94	0.19	8 (6)	
	HC1	24.1	-	-	-	-	-	-	-	-	-	
	HC2	16.5	1.09	0.29	17.32	11.60	4.59	2.96	1.46	0.54	1 (16)	
	HC2	19.1	1.09	0.22	11.05	6.46	4.30	1.96	1.46	0.39	(18)	
	HC2	21.6	0.87	*	16.8	*	15	*	1.05	*	(1)	
	HC2	24.1	-	-	-	-	-	-	_	-	-	

Table 2: Summary of mean values of key swim parameters from volitional swim trials.

Table Notes:

(1) For sample size, the number without parentheses is for fully ascended, the number inside parentheses is partially ascended.

(2) The – indicates this size class did not swim out of the tailwater pool during the experiment.

(3) \* indicates there is not enough data to estimate the parameter.

		"Spook " Swimming Experiments										
Species	Size Class	Mean of Maximum Sprint Swimming Speed	Standard Deviation	Mean Sprint Swim Time	Standard Deviation	Sample Size	Mean of Sprint Swims Greater Than 1.5 m/s	Standard Deviation	Mean of Time of Sprint Swims Greater than 1.5 m/s	Standard Deviation	Sample Size	
	cm	m/s	m/s	S	S		m/s	m/s	S	S		
Rainbow Trout	16.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	19.1	1.64	0.31	0.45	0.11	13	1.59	0.25	0.41	0.07	10	
	21.6	1.56	0.30	0.45	0.09	8	1.71	0.26	0.40	0.06	5	
	24.1	1.92	0.28	0.36	0.05	5	1.92	0.28	0.36	0.05	5	
Westslope Cutthroat Trout	16.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	19.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	21.6	1.72	0.47	0.41	0.07	7	1.95	0.31	0.41	0.07	5	
	24.1	1.55	0.68	0.57	0.41	5	1.92	0.43	0.57	0.41	3	

Table 3: Summary of mean values of key swim parameters from "spook" swim trials.

Table Notes: ND means no data available.

A series of statistical analyses were done to evaluate differences in key swim parameters between rainbow trout and westslope cutthroat trout. Figures 6 to 8 present box plots of swim parameters as described in each Figures respective text. Figure 6 box plots show the maximum volitional swim speed with all size classes for both hydraulic challenges pooled together for rainbow trout (n = 112) and westslope cutthroat trout (n = 86). Mann-Whitney U-test results indicate no significant difference between the median value of the maximum volitional swim speed between species (p = 0.6953).



Figure 6: box plot of maximum volitional swim speed for rainbow trout and westslope cutthroat trout.

Figure 7 box plots show the mean volitional swim speed with all size classes for both hydraulic challenges pooled together for rainbow trout (n = 112) and westslope cutthroat trout (n = 86). Mann-Whitney U-test results indicate no significant difference between the median value of the mean volitional swim speed between species (p = 0.4947).



Figure 7: box plot of mean volitional swim speed for rainbow trout and westslope cutthroat trout.

Figure 8 box plots show the maximum sprint swim speed with all size classes for rainbow trout (n = 26) and westslope cutthroat trout (n = 12) from the "spook" swim trial. Mann-Whitney U-test results indicate no significant difference between the median value of the maximum "spook" swim speed for all fish swum during the spook trials (p = 0.913).



Figure 8: box plot of maximum "spook" swim speed for rainbow trout and westslope cutthroat trout.

Both species of trout swam faster against the higher flow hydraulic challenge (HC2). Figure 9 plots mean volitional swim speed by hydraulic challenge.



Figure 9: box plot of mean volitional swim speed for rainbow trout and westslope cutthroat trout by hydraulic challenge.

Although not necessarily the primary intent of this study, we did evaluate differences in swim metrics between size classes for rainbow trout and westslope cutthroat trout. Figure 10 box plots show the mean volitional swim speed for rainbow trout by size class. Kruskall-Wallis tests indicate that there are significant differences between the median values of mean volitional swim speed for different size classes of rainbow trout (p = 0.011). The 19.1 cm size class swam the fastest on average, with a general trend showing larger fish swimming at faster speed.



Figure 10: box plot of mean volitional swim speed by size class for rainbow trout.

Figure 11 plots mean volitional swim speeds for westslope cutthroat trout by size class. Kruskall-Wallis tests indicate that there are significant differences between the median values of mean volitional swim speed for different size classes of westslope cutthroat trout (p = <0.001). Similarly to rainbow trout, the 19.1 cm size class swam the fastest on average.





Interestingly, looking at only maximum swim speeds (see Figure 12) for HSP between species shows a slightly different story. Volitional sprint swimming speeds for HSP are speeds

greater than 1.5 m/s. Median value for rainbow trout sprint swimming speed was 2.32 m/s (n = 34). Median value for westslope cutthroat trout sprint swimming speed was 1.77 m/s (n = 22). Mann-Whitney U-test results comparing median values are significantly different and rainbow trout volitional sprint speeds were higher in the volitional trials than westslope cutthroat trout (p < 0.01). The highest recorded rainbow burst was 3.18 m/s as compared to 2.51 m/s for westslope cutthroat trout, which is 27% faster.



Figure 12: box plot of volitional sprint swimming speeds for all size classes of rainbow trout and westslope cutthroat trout.

Lastly, we investigated differences in key swim parameters between experimental methods by species. Figure 12 shows box plots of volitional sprint swim speed and "spook" sprint swim speed for rainbow trout. Median value for volitional sprint speed = 2.32 m/s (n = 34). Median value for sprint speed = 1.65 m/s (n = 26). Mann-Whitney U-test results indicate there is a significant difference between median values of sprint speeds for "spook" test compared to volitional test (p < 0.005).



Figure 12: box plot of sprint speed (from "spook" trial) and volitional sprint speed for rainbow trout.

Figure 13 shows box plots of volitional sprint swim speed and "spook" sprint swim speed for westslope cutthroat trout. Median value for volitional sprint speed = 1.77 m/s (n = 22) as compared to a median value for sprint speed = 1.67 m/s (n = 12). Mann-Whitney U-test results indicate there is no significant difference between median values of sprint speeds for "spook" test compared to volitional test (p = 0.46).



Figure 13: box plot of sprint speed (from "spook" trial) and volitional sprint speed for westslope cutthroat trout.

# Summary

This chapter presented a study designed to characterize the swimming performance of rainbow trout and westslope cutthroat trout using an open channel flume. Two methods were used including volitional swim challenges and "spook" swim trials. Some of the more interesting results show that rainbow trout swam at a maximum speed of approximately 3.18 m/s compared to a maximum speed of 2.51 m/s for westslope cutthroat trout. However, study results indicate mean swim speeds were similar between species. There was a significant difference in mean volitional swim speed between size classes for both species of trout, with the 19.5 cm size class swimming the fastest on average compared to the other size classes. For rainbow trout, the general trend was similar to other studies in that larger fish swam faster than smaller ones.

Rainbow trout swam significantly faster in the volitional swim trials as compared to the "spook" trials. Conversely, westslope cutthroat trout did not show any significant difference between swim speeds by test method. Interestingly, rainbow trout that were classified as HSP swam at significantly higher speeds in the volitional swim trial as compared to westslope trout that were classified as HSP. However, they swam at effectively the same speeds in the "spook" test.

The results of this study will provide additional data to support design and analysis of fish passage projects for these and other trout species. As an example, if a designer needs to size a culvert and ensure that it is passable to adults of these species, they should size the structure to create velocities around 1 m/s at the high fish passage design flow. This value was the mean value that trout chose to swim through the velocity challenges and is considered representative of a prolonged swim speed.

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# **Chapter 3 - Sprint Swimming Performance of a large bodied fish - Wild Shovelnose Sturgeon** (*Scaphirhynchus platorynchus*)

#### Background

The following chapter describes a study performed to evaluate swimming performance of shovelnose sturgeon using an open channel flume at the BFTC. This chapter satisfies parts of Objective 1 and 2, listed in the original grant.

## Introduction

Shovelnose sturgeon (*Scaphirhynchus platorynchus*) are native to the Mississippi and Missouri Rivers and inhabit many of their tributaries. Shovelnose sturgeon are one of the most abundant sturgeon species in North America (Keenlyne 1997). A recent status report of shovelnose sturgeon compared historic and current distribution and abundance and reported an overall decline in numbers but that stable, endangered, and extirpated populations existed within the species historic range (Koch and Quist 2010). The decline of shovelnose sturgeon population numbers is attributed to overharvest (Quist et al. 2002, Columbo et al. 2007, Koch et al. 2009) and habitat alterations (Keenlyn 1997, Koch and Quist, 2010). The habitat alterations responsible for decline include construction of dams, diversions, weirs, and road crossings on large rivers and tributaries. These changes have restricted shovelnose sturgeon migrations and movement patterns, and disrupted natural hydrological patterns (e.g. temperature, flow) throughout the rivers and tributaries of the Mississipi, Missouri, and Ohio River drainage basins (Keenlyne 1997).

The major rivers that support the Great Plains (GP) shovelnose sturgeon population have been impacted more than the major rivers of populations in the Central Lowlands, Interior Highlands, and the Coastal Plain (units described in Cross et al. 1986). Shovelnose sturgeon movements in the GP have been impacted by six mainstem dams on the Missouri River and two mainstem dam on the Yellowstone River. In addition to mainstem habitat structures, shovelnose sturgeon in the GP are inhibited by obstructions on smaller tributaries including dams on the Marias River, Milk River, and Tongue River.

Fish passage ways are constructed in order to allow migration of fish species in rivers obstructed by dams or other structures that alter the connectivity of a river system. In order to design effective fish passage ways, baseline swimming capability data needs to be collected for fish species of concern. One issue with using swimming performance data for designing fish passage structures is that different types of swimming behaviors are used by fishes to move through a passage structure. When characterizing fish swimming performance and behavior, Adams et al. (1999) classified swimming into three categories (sustained, prolonged, and burst) based on duration, muscle use, and fatigue. Sustained swims were determined to be greater than 200 min, prolonged swims were determined to be less than 30 s (Adams et al. 1999). By this definition sustained and burst swimming capabilities might be the most relevant to designing effective fish passage ways.

Critical swimming speed (U<sub>crit</sub>) is used to characterize the sustained swimming performance of fish at different velocities and is a measure of endurance. Sustained (e.g. critical) swimming information has been assessed in adult shovelnose sturgeon (Adams et al. 1997, Hoover et al. 2011) though other types of shovelnose sturgeon swim behavior (e.g. burst or sprint capability) have not been studied. Critical swimming speeds for adult shovelnose sturgeon in a laboratory study ranged from 65 to 116 cm/s (Adams et al. 1997) in 16°C water. Hoover et al. (2011) found that adult shovelnose sturgeon utilizing slower moving boundary water had higher critical swimming speeds (160 cm/s) than sturgeon swimming in faster rectilinear flow (102.7 cm/s). Additionally, Hoover et al. (2011) found and that shovelnose sturgeon swam faster in 22-25°C water than in 20°C water. The critical swimming speeds for sturgeon species in the slower moving boundary layer are relevant for fish passage because sturgeon spend most of their time in the boundary water near the bottom. Although critical swimming speeds have been used to determine swimming capabilities of shovelnose sturgeon, sprint swimming capability (e.g. burst) is also important for developing shovelnose sturgeon passage criteria. For example, Mesa et al. (2004) documented critical swimming speeds of bull trout, but later concluded that determining the maximum swimming speed (Vmax ; a burst or sprint swimming assessment) might also be useful for predicting the capabilities of fish to navigate through a fish passage structure (Mesa et al. 2008). An assessment of both V<sub>max</sub> and U<sub>crit</sub> swimming abilities will assist managers in planning structures that allow shovelnose sturgeon to navigate through varying currents (Beamish 1978) often found in fish passage structures. Sprint or burst swimming ability  $(V_{max})$  are especially ecologically relevant for species such as shovelnose sturgeon that have been shown to alternate between active sprint swimming and holding position in the current using large pectoral fins to "grab" onto substrate (Adams et al. 1997) rather than sustained swimming to navigate passage structures.

We conducted laboratory experiments in an outdoor experimental flume (described in Chapter 1 of this report) using Sony Handycam digital video cameras (Sony Corporation, Tokyo, Japan) to record the peak sprinting speeds of shovelnose sturgeon under four hydraulic conditions using two water velocities and two water temperatures. Our expected results were that shovelnose sturgeon would have a greater  $V_{max}$  in warmer water temperatures and in slower velocity water. The information that we collected during this study combined with previous knowledge on sustained swimming speeds will assist managers and engineers in designing fish passage structures.

#### Methods

#### Fish Collection

Adult shovelnose sturgeon were collected from the Yellowstone and Missouri Rivers in May 2011 with the assistance of Montana Fish Wildlife and Parks. The fish were captured and handled using techniques and protocols as described for pallid sturgeon (U.S. Fish and Wildlife Service, 2005). After capture the sturgeon were held in a flow through screened cage and remained in the river until sex and stage of reproductive
maturation could be determined. Once sorted, all selected shovelnose sturgeon were implanted with passive integrated transponders (PIT) and transported to Bozeman Fish Technology Center (BFTC) in Bozeman, Montana the day of capture using a hatchery transport truck with insulated and aerated tanks filled with BFTC source water that matched the source river temperature (e.g. 10-11°C). Upon arrival at BFTC the sturgeon were measured, weighed, and placed in covered 3 m circular outdoor tanks. Fish were fed a daily combination of earthworms (*Lumbricus terrestris*) and sub-yearling hatchery raised rainbow trout (*Oncorhynchus mykiss*), but food was removed 24 h in advance of a swim trial in order to ensure all fish were in a post absorptive state. The water temperature in the outdoor round tanks was held 11°C until May 10 when temperatures were increased to 12.3°C. Starting on May 21, temperatures were increased gradually ( $\leq$ 1° C/d) until the temperature reached 16°C. The water temperature was maintained at 16-18°C for the remainder of the study. The shovelnose sturgeon size ranged from 55.8 – 93.5 cm (fork length).

## Sprint Tests

We determined the sprint swimming capabilities of shovelnose sturgeon using a flume and a series of video cameras which recorded sturgeon as they swam up the flume. The flume apparatus consisted of a headwater and tailwater tank connected by a 0.91 m wide X 17 m long flume in which swim speeds could be calculated. The cross sectional area of all fish was less than 10% of the width of the flume, to ensure there was no blocking effect that might impede the fishes swimming ability. Six digital cameras, placed in boxes evenly distributed along the frame of flume so that the adjacent camera views overlapped, were used to record and generate the sprint swimming data. The overhead video cameras monitored a 12.8 m section of the flume that was marked in 0.61 m increments (on the flume bottom). The marked increments were used to determine the distance a shovelnose sturgeon traveled during a known period of time when the video was analyzed. Fish swims were analyzed using Sony PMB software (Sony Corporation, Tokyo, Japan). The flume was shielded from the sun using cloth draped over a wooden frame above the flume. The cover reduced any glare that might make analyzing the video difficult and provided a shaded environment for the shovelnose sturgeon. The sprint test consisted of four trials conducted during August 2011. Each trial was performed under one of four hydraulic conditions that consisted of a water temperature of either 12°C or 19°C paired with a low and high water velocity. Twenty five fish were swum in each of the four trials and some fish were used in more than one trial.

Prior to swimming a fish, the flume was turned on and allowed to equilibrate. After the flume established equilibrium the temperature and water level were continuously monitored and recorded using an AquaRod – Tru Track Digital Crest Gauge (Advanced Measurements and Controls, Inc., Camano Island, WA). The flume hydraulic configuration settings were recorded.

Shovelnose sturgeon were coerced to swim the length of the flume to characterize  $V_{max}$ . A shovelnose sturgeon was randomly selected from a holding tank, identified by PIT tag, placed in a tub, and transported to the flume. Each fish was placed with its head oriented into the oncoming current in the flume and released. A fish responded by maintaining its orientation facing into the current and holding a position using its pectoral

fins or turned and allowed itself to be swept by the current against the downstream screen. Fish that did not orientate into the current and were swept by the current to the downstream screen were immediately removed from the flume. A fish that held position for five min was coerced to move up the flume for a swim ability assessment. To force a swim, a technician gently touched or approached the fishes tail with a fish net frame (netting was removed). To ensure consistency the same technician performed each coerced swim. Once the fish begin to swim the technician (also inside the flume channel) continued to follow behind the fish the distance of the flume. If the fish stopped or slowed the technician gently prodded or approached the fish to encourage its upstream ascent. If a fish did not respond to a gentle touch the trial was immediately ended and the fish removed. In developing our technique and protocol we noted that a fish could use the pressure wave generated by the pursuing technician to facilitate its swimming ability and that the technician had the potential to influence the oncoming water flow. We selected swims that were determined to be unaided and uninfluenced by the methodology. In addition to the technician, we used an observer to rank and document the quality of each swim trial. A second examination of each swim trial was conducted using video to ensure that the swim protocol or technician had not affected the free swimming nature of the fish's ascent up the flume. Only sprints that were determined to be strong, vigorous swims unaffected by the techniques were used in the analyses.

#### Flume Flow Profiling

A velocity flow profile of the flume was conducted before each trial using a Flo-Mate model 2000 portable flowmeter (Marsh-McBirney Inc, Fredrick, Maryland, USA). Flow and depth were measured at each 0.61 m increment throughout the 12.8 m section of flume in which swimming was monitored. The flow data was collected so that swim speed could be calculated relative to the water velocity at each increment. Depths were recorded using a graduated rod (Marsh-McBirney Inc, Fredrick, Maryland, USA). When measuring velocity the center of the flow meter probe was located at 60% water depth. Water velocities for the low velocity trials ranged from 0.0s to 0.07 m/s. Water velocities for the high velocity trial ranged from 0.38 to 0.48 m/s.

#### Data Analysis

For each hydraulic condition we selected and analyzed the best 4-5 swims of the 25 attempted swims in each trial to represent the maximum swimming ability of shovelnose sturgeon at that hydraulic condition. We analyzed four swims at the 19°C and high velocity challenge, and analyzed 5 swims at the other three hydraulic challenges. Individual fish velocities and distance traveled were plotted against the amount of cumulative time spent swimming to provide a detailed summary of how each fish swam through the flume (Figures 2-5). The analyses provided an observation of peak swimming speed associated with time and distance, range of swimming speeds over time and distance, and duration of swimming speed over time and distance. We used one-way analysis of variance to detect differences in the  $V_{max}$  among four different hydraulic conditions to determine differences in maximum sprint speed of shovelnose sturgeon. All data were analyzed using R 2.14.0 software (R Development Core Team, 2010).

## Results

The mean  $V_{max}$  for shovelnose sturgeon among the four trials ranged from 2.13 to 3.21 m/s. No difference in mean  $V_{max}$  was detected between the 4 hydraulic conditions in which we tested shovelnose sturgeon sprint swimming abilities (Fig 1, P = 0.064). The mean  $V_{max}$  by trial was 2.94 m/s for the Low Velocity 12°C trial, 2.13 m/s for the Low Velocity 19°C trial, 3.01 m/s for the High Velocity 12°C trial, and 3.21 m/s for the High Velocity 19°C trial (Fig. 1). The  $V_{max}$  of individual shovelnose sturgeon among all trials ranged from 1.29 to 3.73 m/s (Figures 2-5). In general shovelnose sturgeon were able to ascend the flume in 10 to 15 sec and a fish typically reached  $V_{max}$  after swimming 5 sec or longer. The swim speed did not increase in a linear pattern but peaks in sprinting speed occurred at several points over the entire swim (Fig 2-5). Peak swimming velocities were only maintained for a fraction of a second before the fish returned to a slower swim speed. The  $V_{max}$  of shovelnose sturgeon in this study was independent of fish size (Figure 6).



Figure 1. The mean  $V_{max}$  (mean maximum swimming capabilities; ±SE) of shovelnose sturgeon (*Scaphirhynchus platorynchus*) at four different temperature and velocity treatment combinations. The four treatments consisted of 12°C or 19°C water paired with a high (H) or low (L) water velocity (e.g. 12L represents the 12°C and low velocity treatment). The water velocity in the low treatment ranged from 0.02-0.07 m/s and the high velocity treatment ranged from 0.38 – 0.48 m/s. One-way analysis of variance showed no differences in the mean  $V_{max}$  of shovelnose sturgeon among the four hydraulic conditions tested (P = 0.065).



Figure 2. Shovelnose sturgeon swimming velocities in m/s ( $\bullet$ ) and distance traveled in m ( $\bullet$ ) in an experimental open channel flume under low water velocities (0.02 - 0.07 m/s) and 12°C water temperature.



Figure 3. Shovelnose sturgeon swimming velocities ( $\bullet$ ) and distance traveled ( $\bullet$ ) in an experimental open channel flume under low water velocities (0.02 - 0.07 m/s) and  $19^{\circ}\text{C}$  water temperature.



Figure 4. Shovelnose sturgeon swimming velocities in m/s ( $\bullet$ ) and distance traveled in m ( $\bullet$ ) in an experimental open channel flume under high water velocities (0.38-0.48 m/s) and 12°C water temperatures.



Figure 5, Shovelnose sturgeon swimming velocities in m/s ( $\bullet$ ) and distance traveled in m ( $\bullet$ ) in an experimental open channel flume under high water velocities (0.38-0.48 m/s) and 19°C water temperatures.



Figure 6. Shovelnose sturgeon length and weight effect on sprint swimming capabilities.

### Summary

The  $V_{max}$  for adult shovelnose sturgeon obtained in the tests we performed are among the first efforts to characterize the burst swimming ability for any sturgeon species. Our data (3.73 m/s, the  $V_{max}$  for the fastest shovelnose in the trials) is the highest recorded burst speed ever reported for a sturgeon species to our knowledge. The  $V_{max}$  of shovelnose sturgeon was higher than the burst swimming reported for rainbow trout (Oncorhynchus mykiss, 2.77 m/s; Harper and Blake, 1990), nearly as great as cutthroat trout (Oncorhynchus clarkii, 4.05 m/s, Bell, 1986) and greater than that reported for Bull trout (Salvilinus confluentus, 2.3 m/s; Mesa et al 2008). It is widely described in laboratory studies that the swimming capabilities of sturgeon species is less than many modern teleosts (Peake et al. 1997, 2004; Deslauriers and Kieffer, 2011). Peake et al. (1997), in the only study we are aware of that has attempted to estimate the burst speed of a sturgeon species determined that adult lake sturgeon were capable of burst swimming speeds of 1.80 m/s. The techniques we used to assess burst swimming capabilities are different than previous studies have employed and may have allowed us to collect higher burst abilities than other techniques. Our observation and field observations of the leaping abilities of various sturgeon species s suggest that sturgeon species V<sub>max</sub> ability might be under rated. For example, reports of adult gulf sturgeon (*Acipenser oxyrinchus desotoi*) leaping 2 m or more above the water are common and adult white sturgeon (*Acipenser transmontanus*) is well known to show explosive leaping behaviour when hooked by fisherman. The leaping ability demonstrated by sturgeon species and our observations of shovelnose sturgeon (a relatively small species of sturgeon) suggests that sturgeon may be very capable of attaining high sprint swimming speeds when motivated.

Most fish demonstrate multiple peaks in swimming velocity during their swim. It is noteworthy that the peak swimming velocities of shovelnose sturgeon were only maintained for a fraction of a second before the fish returned to a slower swim speed. This pattern in quick burst swimming followed by slower swimming and gliding behaviour might reflect how sturgeon negotiate passage structures when highly motivated, illustrating the tendency to burst and then hold position on the substrate. The lack of difference in swimming capabilities at different temperatures is similar to that of lake sturgeon at burst swimming speeds (Peake et al. 1995). Temperature does not appear to have the same detectable effect on burst swimming as it has on the prolonged and sustained swims. The results of this study will provide additional data to support design and analysis of fish passage projects for shovelnose sturgeon and other sturgeon species.

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### **Chapter 4 - Construction and Design of the Artificial River**

#### Background

The artificial river is a result of a funded U.S. Geological Survey scientific support project (SSP), and partnerships involving funding from the Prairie Plains and Pothole LCC, the U.S. Geological Survey Northern Rocky Mountain Science Center, Montana Cooperative Fishery Research Unit, the Great Northern LCC, and direct funding from the U.S. Fish and Wildlife Service Mountain-Prairie Region. The system was installed and operational in spring 2011. The system was designed to be flexible and allow researchers to simulate varied stream conditions for addressing a wide variety of questions on fish ecology, behavior, and life-history requirements relative to selected environmental factors. This chapter satisfies parts of Objective 3, listed in the original grant.

To date the artificial river channel has provided a unique laboratory environment to perform a variety of studies (listed study titles below). The variety of studies shows the flexibility of the system. The system has performed well for studies that assess screening/barrier/passage issues, investigations of spawning behavior, and competition studies in a semi natural environment.

- Fitness Consequences of Hybridization between Native Westslope Cutthroat Trout and Nonnative Rainbow Trout
- Spawning of Pallid Sturgeon and Shovelnose Sturgeon in an Artificial Stream: Estimating the Effect of Temperature, Flow, Substrate, and Photoperiod on spawning behavior
- Testing the efficacy of the NEPTUN electric barrier on target and non-target fish species Electronic barrier

#### Design and Construction

The design specifications for the river were submitted to the federal bid process in the fall of 2010. Other significant materials also went through a federal bid process (submerged water pumps, electrical panels, water propulsion systems, etc.). The structural fabrication of the living river was awarded to Hydro Composites, L.L.C. (Stockdale, Texas). Delivery of living river sections occurred in December 2010. The river was reconstructed at BFTC in Jan-Feb 2011. Water testing of the river and water reuse system (pumps, sand filter, etc.) was completed in March 2011. The "endless" river is 20 m in circumference. The river channel is 1.5 m wide and 1.2 m deep, and can accommodate placement of rocky spawning substrates. Polycarbonate windows allow for underwater viewing. Water is supplied from cold and warm springs through a re-use system giving researchers the ability to vary water temperatures between 8 and 22°C. Water velocities of up to 1.5 m/sec can be achieved by propellers driven by electric motors. Lighting is adjustable to provide the required photoperiod.

Photographs of the artificial stream under construction and being installed at the Bozeman Fish Technology Center follow.



The artificial river under construction at Hydro Composites, L.L.C., in Stockdale, Texas. The constructed river sections were shipped to the Bozeman Fish Technology Center in January 2011.



Bozeman Fish Technology Center staff breaks ground in the hatchery building to make way for the artificial river, winter 2010-11.



The first sections are set into place with a forklift and many hands in January 2011. Each section weighs about 500 pounds. In this photo staff biologists Matt Toner (hatchery manager), Jason Illgen, Cal Fraser and center director Robert Muth discuss design elements of the river.



The artificial river begins to take shape.



The artificial river was water tested in February 2011.



A fish eye view of one channel of the river. A pilot study began in March 2011 to examine the artificial river under a biological load and observe fish health conditions.



The photo shows the artificial river in operation in June, 2011. The system capabilities are flexible and unique. The river has been used in several research studies to date including a competition trial involving westslope cutthroat trout, rainbow trout, and introgressed hybrid combinations of the two species. Note the motors that generate

current, the natural substrate bottom, natural and artificial lighting, and expansive windows for visual observation and camcorder placement.



Shovelnose sturgeon in the artificial river.

# Chapter 5 - Spawning of Pallid Sturgeon and Shovelnose Sturgeon in an Artificial River: Estimating the effect of temperature, substrate, and flow on the timing and duration of spawning

### Background

This chapter of the report provides an update of progress made to use an artificial river to examine the spawning requirements of plains and prairie fish species in a laboratory. The PPP LCC provided funding to develop the artificial river system now available to researchers and fish managers in the PPP-LCC region. The work detailed in this section is being performed by a partnership led by the BFTC of the USFWS and the USGS Montana Cooperative Fishery Research Unit, Department of Ecology, Fish and Wildlife Program, Montana State University, Bozeman, MT. The SSP research was funded as a 3 year project. The project was initiated in the spring of 2011 and year two and three will utilize the stream from April-July, in 2012 and 2013. The project was supported by a USGS Science Support grant in part because millions of dollars are currently being allocated to manage the Missouri River to meet pallid sturgeon biological opinion measures. The project objectives have potential important river management implications. Below we attach two annual reports submitted to meet the requirements of the Science Support Partnership grant. We also include an addendum that provides an example of recent data analyses and detail of the ongoing project. This chapter satisfies parts of Objective 3, listed in the original grant.

**2011 Annual Progress Report:** Spawning Pallid and Shovelnose Sturgeon in an Artificial River: Estimating the Effect of Temperature, Flow, and Photoperiod on the Timing and Duration of Spawning with Implications for Regulated River Management and Response to Climate Change

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Greg Watson, Regional Research Coordinator, Energy and Science Coordinator USFWS Mountain-Prairie Region Phone: 303-236-4514 Greg Watson@fws.gov Authors Note: The observations reported here are undergoing additional analyses and interpretation. Note that the initial interpretations provided in this preliminary report are subject to revision. Individuals requiring a specific reference are asked to contact the authors for a personnel communication.

# Summary

Understanding the spawning behavior and spawning habitat requirements of shovelnose sturgeon and pallid sturgeon affected by hydro-alteration is necessary to better manage shovelnose sturgeon and recover endangered pallid sturgeon. For the first time, research biologists were able to observe and characterize shovelnose sturgeon spawning behavior. Shovelnose sturgeon were observed volitionally selecting mates and spawning habitat in the constraints of a semi-natural environment (e.g., artificial river) created at the Bozeman Fish Technology Center. We performed concurrent trials with both hormonetreated shovelnose sturgeon and shovelnose sturgeon that received no hormone treatment under defined temperature, flow, and substrate conditions in the artificial river. We used luteinizing hormone releasing hormone (LHRHa) to initiate the hormonal cascade that led to spawning in shovelnose sturgeon in two test groups. In both trials, males and females that were treated with LHRHa responded to the hormone and selected multiple mates during spawning events that varied from 8 to 18 h (e.g., 8-18 h female spawning duration; defined as the shortest and longest periods from first oviposit to final oviposit for an individual female). In both trials, a single non-treated (no hormone) male participated in multiple spawning bouts with females treated with LHRHa. In addition to determining the spawning duration for female shovelnose sturgeon, we determined the duration of an individual spawning bout (a single male/female pairing; generally <5 sec). We observed many other previously undocumented behaviors including polyandrous and polygynous mating, nosing or bumping of the female abdomen by males prior to spawning (an apparent test of willingness/ability to spawn or mating courtship ritual), 'false spawns' (gametes released from an unaccompanied male or female), a raised activity level of spawning males and females (e.g. 'cruising behavior'), and ingestion of freshly spawned eggs by male and female shovelnose sturgeon.

Developing the conditions that provide the necessary environmental cues and habitat in which a female shovelnose sturgeon will ovulate without exogenous hormonal stimulation remains a critical unknown factor. Though we attempted to address this question in year one of the study, we were unable to provide conditions leading to an untreated female spawning in the artificial river. Though untreated male and female shovelnose sturgeon were held in the river for an extended period, no female from the natural treatment group spawned during the study. Research in 2012 will consist of additional field trips to collect wild sturgeon for the study and continued similar laboratory investigations. We will focus on developing techniques and providing conditions that promote spawning without the use of hormone, but will remain adaptive in our approach and use of hormones to facilitate spawning observations and hypothesis testing.

# Introduction

Pallid sturgeon (*Scaphirhynchus albus*) and shovelnose sturgeon (*Scaphirhynchus platorynchus*) are sympatric species native to the Missouri-Mississippi river basins in the United States. Shovelnose sturgeon populations have decreased throughout their historic range, but remain relatively abundant in the upper Missouri River in Montana, North Dakota, and South Dakota. The pallid sturgeon is listed as an endangered species throughout its range and is protected under the Endangered Species Act (Dryer and Sandoval 1993). Less than three hundred adult pallid sturgeon remain upstream of Gavins Point Dam (Yankton, South Dakota) in the Missouri River basin waters of South Dakota, North Dakota, and Montana (USFWS 2007). The decline of pallid sturgeon in this area has been correlated with a reduction in spawning habitat and recruitment failure; both of these factors have been associated with dam construction, reservoir development, river channelization, and changes to the river hydrograph and thermograph (USFWS 2000).

The reproductive cycle (gametogenesis) and spawning period (spawning migration to mate selection to gamete expression) of sturgeon are controlled by endocrine and environmental factors known as zeitgebers (from German for "time giver," or "synchronizer"; Dettlaff et al. 1993; Cech and Dorshov 2004). It is known that the endogenous cycle (internal, self-sustained rhythms), photoperiod, and temperature are key factors or ultimate (e.g., primary) cues that control the endocrine system which regulates an adult sturgeon's reproductive cycle (Webb and Dorshov 2011). Together these factors regulate the timing of an adult sturgeon's maturation from early gametogenesis to the stage of spawning readiness (Dettlaff et al. 1993; Webb and Dorshov 2011). In general, it is believed that photoperiod acts to 'set' the endogenous clock, while temperature acts to regulate gamete maturation or 'set the speed' of the endogenous clock. Once an adult sturgeon reaches the stage of spawning readiness, it is believed that a number of proximal (e.g. secondary) cues are necessary to elicit a spawning event. Those proximal cues are hypothesized to include, but are not limited to, the presence of a suitable mate, physical and chemical mating signals (e.g. display, pheromone release; Bayunova et al. 2011), temperature, photoperiod, lunar phase, seasonal discharge, water velocity and pattern, water chemistry, turbidity, and substrate (Papoulias et al. 2011). It is a challenge to determine what proximate cues are necessary and what factors may simply be associated with a necessary cue because of the interrelatedness of these proximal factors. While many factors are inherent and cannot be controlled, regulation on the upper Missouri River alters the timing and quantity of discharge, effects water temperature, effects spawning micro habitat (e.g. flow patterns, substrate, flooded vegetation, etc.), and thus can affect spawning behavior of shovelnose and pallid sturgeon. Determining the influence of discharge, temperature, and substrate on the spawning behavior (e.g., does a combination of these factors elicit a spawning response from a spawning ready adult?) of pallid sturgeon and shovelnose sturgeon is important if managers of regulated rivers are to provide discharge that supports the lifecycle needs of pallid and shovelnose sturgeon. Thus, improving pallid sturgeon spawning conditions through better management of regulated rivers has been identified as necessary in the Biological Opinion on what is needed to recover pallid sturgeon (USFWS 2000).

Pallid sturgeon and shovelnose sturgeon mating and egg deposition has not been directly observed in the wild (DeLonay et al. 2007, 2009; Wildhaber et al. 2007; Goodman 2009), but field observations have provided information on spawning behavior

and associated spawning habitat. Telemetry studies conducted on pallid sturgeon and shovelnose sturgeon have provided insight on habitat selection associated with spawning movements, water temperature associated with spawning, and discharge associated with spawning and embryo collection (DeLonay et al. 2007, 2009, Fuller et al. 2008, Goodman 2009). The proximal spawning cues (combination and magnitude) necessary to elicit and promote pallid sturgeon and shovelnose sturgeon spawning remain largely unknown, and field studies continue in an effort to collect the needed information. In 2011, we began a multiyear laboratory study designed to describe spawning behavioral characteristics and determine the relative effect and importance of discharge, temperature, and substrate used by spawning pallid and shovelnose sturgeon. The study was designed to provide information that is difficult to collect in field studies and test hypotheses that exist based on field observations. In this report, we describe the first visual observations of shovelnose sturgeon spawning and summarize progress to date on achieving the objective of determining the proximal cues that elicit a spawning event.

### **Methods and Preliminary Results**

#### Artificial River System Design and Use

We designed an artificial river at Bozeman Fish Technology Center (BFTC) to perform the study based on a design successfully used to study the spawning characteristics of shortnose sturgeon (Acipenser brevirostrum) (Kynard et al. in press; see Appendix A for design details and photos). The "endless" river used in the study was an oval tank fabricated by Hydro Composites, L.L.C. (Stockdale, Texas). The river was 20 m in circumference (3.7 m wide by 10 k long) with a center wall (island). The river design provided two "straight channels" (6.4 m long by 1.5 m wide) and two "river bends." Water was supplied to the river via a BFTC re-use system which used a mix of on-site cold and warm spring water to provide the temperature regime used during the study. The same source water that supplied the river was used to supply water to the circular tanks holding shovelnose sturgeon. Electric motors and submerged pumps provided the water velocity and produced varying linear and turbulent flow patterns. The water depth during the study was maintained at 1.2 m. Polycarbonate windows throughout the exterior of the tank allowed for underwater viewing. A series of high definition video recorders were used to monitor daily activity and support visual observations of spawning activities. The substrate used in the study was acquired at a local quarry.

#### Conditions in the Artificial River during the Spawning Trials

Developing the conditions that would provide the necessary environmental cues in which shovelnose sturgeon will spawn was the critical unknown factor we attempted to address in the first year. We attempted to provide environmental conditions that would promote spawning based on suspected and known information for preferred substrate, flow, and temperature of sturgeon species (Bruch and Binkowski 2002; Fuller et al. 2008; DeLonay et al. 2009; Goodman 2009; Kynard et al. in press). A natural photoperiod was maintained in the building housing the artificial river and natural light from the existing windows was present throughout the study. We supplemented the natural occurring light with an overhead lighting system using a timer set to mimic the existing photoperiod. Substrate in the river during our study was either a mix of gravel (2-64 mm) and cobbles (65–256 mm) or gravel only. Temperature was maintained at approximately 16-18°C. We attempted to provide a variety of water velocity habitat that shovelnose sturgeon could use for spawning or holding behavior. Water velocity in the river varied from approximately 20 to150 cm/s (60 cm depth) and 0 to 75 cm/s (5 cm above the bottom). The actual tank velocities during the trial period are currently being analyzed and a model of water velocities present in the river during spawning and non-spawning events is under development.

Substrate in the tank was manipulated to provide two different habitat scenarios during the trial period. Note that each test group was not presented with the same substrate conditions or a group was exposed to multiple substrate conditions during the study period. During the period from June 8 to July 12 the substrate in the river consisted of a homogenous, evenly distributed layer of gravel (2-64 mm) throughout the entire tank. Group 1 and Group 2 fish were presented these conditions. During the period from July 13 to July 29, the substrate in the tank consisted of cobble and gravel. Group 1 and Group 3 fish were presented these conditions. On July 13, we placed a layer of cobble (65–256 mm) over the existing gravel substrate throughout 50% of the tank. Once in place, the cobble layer covered an entire river bend and 50% of each of the two channels. The change in substrate was designed to provide insight into 1) the effect of substrate to elicit a spawning event and 2) determine if sturgeon exhibit a preference for one substrate over another. Because the trials were conducted with more than one group in the river at a time (concurrent), we describe the outcomes of each trial by group (e.g., Group 1, 2, and 3).

To determine the effect of velocity on spawning site choice, we collected water velocity metrics at the end of the trials using USGS standard methods (Rantz, 1982) and a Marsh McBirney 2000 current meter. We measured corresponding flow velocities under the two substrate conditions described above. Depth and velocity measurements were taken at multiple points across a channel, and these measurements were used to calculate the river hydrology. A relationship between flow and depth was used to develop a rating curve for the area of the tank. The data associated with shovelnose sturgeon locations (based on a previously determined grid pattern for subdividing the tank and observations) during non-spawning and spawning events is currently being analyzed and compared to the flow rating curve data.

### Shovelnose Sturgeon Collection and Holding

The shovelnose sturgeon used in the study were collected by Montana Fish, Wildlife and Parks (MTFWP) personnel. Shovelnose sturgeon originated from two river sources and were captured and handled using techniques and protocols similar to those described for pallid sturgeon (U.S. Fish and Wildlife Service, 2008). The majority of the shovelnose sturgeon used in the study were collected from the Yellowstone River near Miles City, MT on May 3, 2011. Four shovelnose sturgeon used in this study were collected from the Missouri River near the Coal Banks Landing Recreation Area on May

9, 2011. Yellowstone River temperatures during the fishing period varied from 10 to 11°C and Missouri River temperatures during the fishing period varied from 10 to 12°C. Once captured, shovelnose sturgeon were transferred to a live cage and held in the river. Individual shovelnose sturgeon were removed from the live cage and assessed to determine sex and stage of maturity using a biopsy technique (Conte et al. 1988). Females with Stage 5 ovarian follicles (large, fully pigmented post-vitellogenic follicles) and males of mature size with visibly developed testes were selected for the study. Shovelnose sturgeon not selected for the study were released immediately after the sexing and staging process. All fish selected for the study were weighed (nearest 0.1 kg), measured for fork length (nearest 0.5 cm), and PIT tagged. During the biopsy, approximately 40 ovarian follicles were removed from each female and a sample of testis was removed from each male and placed in 10% phosphate-buffered formalin for laboratory analyses to assess spawning readiness. The shovelnose sturgeon selected for the study were placed in an insulated holding tank on the BFTC hatchery transport truck. The holding tank had been previously filled with BFTC source water that was approximately  $(\pm 1^{\circ}C)$  the same temperature as the river source. A combination of oxygen supplied to an air stone and a mechanical aeration system provided a minimum 8 mg/L of oxygen during the transport from the river to the BFTC. Shovelnose sturgeon were transported to the BFTC on the day of capture, and once at BFTC, males and females were separated and placed in a 1.8 m or 3 m circular tank and held at 11°C. Male and female shovelnose sturgeon remained in the circular flow-through tanks and were monitored and fed daily when not in the artificial river.

On May 10, we increased the temperature in all tanks to  $12.3^{\circ}$ C and temperatures were maintained at  $12.3^{\circ}$ C thorough May 21. From May 21 to June 1, we increased temperature gradually ( $\leq 1^{\circ}$  C/d) until a temperature of  $16^{\circ}$ C was achieved on June 1. The gradual increase in temperature was designed to resemble a natural vernalization period and facilitate reproductive maturation (Webb et al. 1999). From June 1 to the end of the trials, temperature in all holding tanks and the artificial river were held at  $16-18^{\circ}$ C. All shovelnose sturgeon were exposed to the same water source and temperature regime.

## Assessment of sexual maturity

We monitored and assessed spawning readiness of shovelnose sturgeon throughout the study. To determine female maturity, 20 oocytes were bisected to measure oocyte polarization index (PI; a ratio of the distance of the germinal vesicle from the animal pole to the oocyte diameter) and 20 oocytes were measured to determine follicle diameter (Van Eenennaam et al. 2004). Measurements (nearest 0.005 mm) were made using a Lecia DM 2000 compound microscope (2.5x) equipped with a RT KE Spot camera and Spot Advanced imaging software. Along with the gonad sample collection previously described, we collected a blood sample and analyzed plasma samples to monitor sexual steroid concentrations (e.g., plasma testosterone and estradiol; Webb et al. 2002, 2008). Shovelnose sturgeon collected from the Yellowstone River and Missouri River, regardless of collection site, exhibited asynchronous patterns of spawning readiness (authors' data not included in this report). Ovarian follicle development patterns (diameter and PI) in females and patterns of steroid production in both male and female sturgeons allowed for determination of sex and stage of reproductive maturity. Sex steroid data and spawning readiness data are currently being analyzed and compared to spawning results. A description of physiological parameters we used to measure spawning readiness, individual progression of spawning readiness, and group comparisons will be produced in a future report or peer reviewed publication.

## Data Collection

We recorded observations and continue to review video to describe and characterize shovelnose sturgeon spawning behavior. In the trials performed, we were able to record: 1) number of males and females in a spawning aggregation, 2) positions of males and females during spawning, 3) timing and sequence of egg and milt release, 4) the relationship of males and females to water depth, substrate, and velocity during spawning, 5) female mate choice (how many males were selected, what size, etc.), 6) characterization of egg deposition and associated swimming behavior (looping or stationary behavior, size of depositional area, etc.), 7) individual male mating success vs. failure ratios, 8) female ovulatory intervals (number/min/h/d) and spawning duration, and 9) male number of spawning bouts (number/min/h/d) and spawning duration.

## Group 1 Shovelnose Sturgeon

Six male and four female shovelnose sturgeon were selected for the initial trial based on sexual maturity and spawning readiness (Table 1). The ten shovelnose sturgeon in Group 1 were moved from their respective holding tanks to the river on June 6, 2011. Temperature in the river was 16.4 °C, the same as the temperature in the holding tanks. Group 1 shovelnose sturgeon were allowed a short acclimation period to the tank before the motors were activated and river flow (velocity) was initiated. After initial placement in the river, the Group 1 sturgeon dispersed throughout tank in what appeared to be a random fashion. Once the motors were initiated and water velocity established the Group 1 shovelnose sturgeon orientated to face the current and began to congregate in what appeared to be specifically selected habitat.

We monitored the fish continuously and recorded fish position and activity multiple times each day. Though additional shovelnose sturgeon (Group 2 and Group 3) were placed in the river and river conditions were changed (e.g., substrate), the Group 1 fish remained in the artificial river from June 6 to July 29, 2011 (i.e., beginning to end of study period). During the period from June 6 to June 28, when only Group 1 sturgeon were in the artificial river, no spawning occurred. An analysis of fish locations and habitat selections is ongoing. The Group 1 fish received no hormone injections during the entire study period. No females in Group 1 spawned. One male in Group 1 (not hormonally induced to spermiate) spawned with hormonally treated shovelnose sturgeon during two hormonally induced spawning events after June 28 (details described below).

Table 1 - Group 1 shovelnose sturgeon remained in the river from June 6 to July 29. During the period they were exposed to a controlled flow regime and temperatures that varied from 16 to 18°C. Only one sturgeon from this group spawned naturally during the study. The Group 1 male spawned only with hormone treated females from Group 2 and Group 3 on June 29 and July 13.

Pit Tag	Sex	Date In River	Hormones applied	1 <sup>st</sup> Induced Spawning Event (June 29, 2011)	2 <sup>nd</sup> Induced Spawning Event (July 13, 2011
D4749	F	June 7	Ν	No	No
70C0A	F	June 7	Ν	No	No
4135B	F	June 7	Ν	No	No
D0575	F	June 7	Ν	No	No
77A0A	Μ	June 7	Ν	No	No
94F23	Μ	June 7	Ν	Spawned	Spawned
30A05	Μ	June 7	Ν	No	No
B230B	Μ	June 7	Ν	No	No
56A42	Μ	June 7	Ν	No	No
D0B26	Μ	June 7	Ν	No	No

## Group 2 Shovelnose Sturgeon

On June 28, we placed a second group (i.e., Group 2; Table 2) of shovelnose sturgeon into the river. The shovelnose sturgeon in Group 2 were injected with LHRHa prior to placement in the river following protocols described in the pallid sturgeon propagation plan (USFWS 2000). The LHRHa is a synthetic hormone that when injected into a sturgeon bypasses the hypothalamus (environmental cues act on the brain hypothalamus; Figure 1) and acts on the pituitary gland to initiate the hormonal cascade that leads to spawning in reproductively mature sturgeon. We hypothesized that shovelnose sturgeon would spawn volitionally 1) in a manner similar to wild shovelnose sturgeon when injected with LHRHa, 2) in the presence of suitable mates, and 3) in the semi natural river environment. Our goal was to gain information on the spawning behavior (duration, frequency, site selection, etc.). We selected Group 2 individuals that were likely to respond to a hormone based on their corresponding testosterone levels (males) and oocyte PI (females). The trial was performed concurrent with the ongoing trial involving Group 1 fish. The non-hormonally injected fish in Group1 remained in the artificial river and freely mixed with the Group 2 hormonally injected fish.

For this trial, we injected two males and two females with LHRHa on June 28 and placed them in the artificial river for observation for approximately 96 h. All shovelnose sturgeon in Group 2 (those injected with LHRHa) selected mates and spawned. In addition to those injected, a male from Group 1 participated in the Group 2 spawning aggregation and successfully mated with both females from Group 2. During the spawning period, both females had numerous confirmed oviposits accompanied by a milt release. The spawning period for both females was similar and lasted approximately 8-10

h from initiation of spawning beginning between 1-2 pm on June 28 to final oviposit between 9-11 pm on June 28. Males continued to court females after female ovipositing had ceased. On several occasions, we observed male quivering behavior accompanied by sperm release without an accompanied egg release from a female. The timing and duration of the male spawning periods were similar. Both males spawned for a period of 16-18 h (including false spawns or unreciprocated attempts) that ended between 7-9 am on June 30. Group 2 shovelnose sturgeon were removed from the artificial river on July 1, 2011.

Table 2 - Group 2 shovelnose sturgeon were placed in the river from June 28 to July 1. Group 2 shovelnose sturgeon were injected with LHRHa and exposed to controlled flow regime and a gravel substrate. Temperature in the river was relatively constant and varied from 16 to 18°C. During the three day trial, all hormone treated males and females in Group 2 spawned. The spawning aggregation formed by the Group 2 sturgeon elicited a spawning response from an untreated male from Group 1.

Pit Tag	Sex	Date In River	<b>Hormone Applied</b>	Results
4230	F	June 28	Y	Spawned
7 E31	F	June 28	Y	Spawned
6055B	М	June 28	Y	Spawned
B5621	Μ	June 28	Y	Spawned

Figure 1- The natural hormone cascade leading to ovulation. Environmental stimuli are relayed to the brain and act on the hypothalamus. An injection of hormone such as LHRHa bypasses the hypothalamus and acts on the pituitary gland to initiate the natural hormone cascade.

Figure 1 The Natural Hormone Cascade					
Strategies for Inducing Reproduction Hypothalamus					
Provision of appropriate spawning environment	Gonadotropin Releasing Hormones (GnRH) Pituitary Gland Gonadotropin				
Injection of LHRH or LHRH analogs plus dopamine antagonist Injection of HCG or Pitultary extract	<b>Ovary</b> Steroids Maturation Prostaglandins				
	Ovulation				

Figure Credit: Sea Grant Minnesota. Induced Reproduction in Fish; Fact Sheet — (A7) 1993.

#### Group 3 Shovelnose Sturgeon

On July 12 and July 13, we placed Group 3 shovelnose sturgeon into the artificial river with the Group 1 shovelnose sturgeon. Group 3 shovelnose sturgeon consisted of both male and female sturgeon that had been held in circular tanks, separated according to sex, and exposed to the same photoperiod and temperature regime as those fish in Group 1. The fish in Group 3 had no previous laboratory exposure to rock substrate or flow during the holding period at BFTC. We treated 4 females and 6 males from Group 3 with LHRHa prior to placing them in the river. Three females and 5 males in Group 3 were placed in the river and were not treated with hormone. In this trial, we hoped to gain additional insight into the spawning behavior of hormone treated shovelnose sturgeon while also testing to determine if any non-injected fish from Group 3 might respond to the cues present in the river and spawn without a hormone stimulant. Five males not treated with LHRHa were placed in the river on July 12. Six males and 4 females treated with LHRHa, along with 3 females not treated with hormone were placed in the river on July 13.

Non-hormone treated fish from Group 3 did not spawn during the trial. We are still analyzing data but are able to report that two of the four females from the hormone treated group spawned. The spawning period for the two females varied from 13 to18 h beginning on July 14 between 11 am and 1 pm and ending the next morning on July 15. All six males treated with hormone mated with at least one female. The spawning period for individual males varied from approximately 2 to 18 h. The same Group 1 male that participated in spawning with Group 2 sturgeon also spawned with the Group 3 sturgeon.

Table 3 - Group 3 shovelnose sturgeon were placed in the river on July 12 or July 13. Group 3 shovelnose sturgeon included non-treated shovelnose sturgeon and shovelnose sturgeon injected with LHRHa. Group 3 shovelnose sturgeon were exposed to a controlled flow regime and a 50% gravel and 50% cobble substrate. Temperature in the river was relatively constant and varied from 16 to 18°C. During the trial all hormone treated males spawned. We are still analyzing the female spawning data, but can report that at least two hormone treated females spawned. No untreated females spawned. The spawning aggregation formed by the Group 3 sturgeon elicited a spawning response from an untreated male from Group 1.

Pit Tag	Sex	Date In River	Hormone Applied	Results
75A03	F	July 13	Ν	No spawn
11817	F	July 13	Ν	No spawn
43362	F	July 13	Ν	No spawn
4C74	F	July 13	Y	Spawned
36358	F	July 13	Y	Under
		-		review
4B35	F	July 13	Y	Under
				review
7344	F	July 13	Y	Spawned
5621	М	July 12	Ν	No spawn
033A	Μ	July 12	Ν	No spawn
5C34	Μ	July 12	Ν	No spawn
6265	М	July 12	Ν	No spawn
7E4A	М	July 12	Ν	No spawn
220C	Μ	July 13	Y	Spawned
3236	Μ	July 13	Y	Spawned
055B	Μ	July 13	Y	Spawned
2110	Μ	July 13	Y	Spawned
5649	М	July 13	Y	Spawned
1E65	Μ	July 13	Y	Spawned

#### **General Observations and Future Plans**

Visual observations of sturgeon spawning behavior are rare for nearly all North American species except those described for lake sturgeon (*Acipenser fulvescens*) (Bruch and Binkowski, 2002) and shortnose sturgeon (Kynard et al. in press). We utilized behavioral descriptions and terms from those research efforts to describe and characterize the behavior of shovelnose sturgeon. Shovelnose sturgeon spawned close to or on the substrate (generally the female was less than a few inches off the bottom surface, e.g., rock substrate or vertical tank wall). We observed polyandrous and polygynous mating, individual male cruising behavior, brief spawning bouts (i.e., vibration; generally <5 sec), nosing or bumping of the female abdomen by males prior to spawning (an apparent test of willingness or ability to spawn, or mating courtship), 'false spawns', a raised activity level when spawning, and predation of recently spawned sturgeon eggs by male and

female shovelnose sturgeon. We did not observe an audible drumming sound or vocalization associated with spawning (we did not use hydro-acoustic equipment). Males were observed both successfully and unsuccessfully attempting to elicit a spawning response from a female. We observed multiple instances when a male released milt in a quivering fashion but the female did not reciprocate with an egg release. We noted that the number of eggs released during a spawning bout by a female, amount and concentration of milt released by a male (clear to cloudy), and total number of spawning bouts varied among individuals. We plan to review and summarize the behavioral data we observed in a short manuscript that will be submitted to a peer reviewed journal in the summer-fall of 2012.

Shovelnose sturgeon in our study maintained a state of spawning readiness for an extended period based on the physiological assessments we performed and the spawning observed in hormone treated individuals. We suspect that at least some sturgeon in Group 1 (exhibiting oocyte  $PI \le 1.0$  and high reproductive hormone levels in plasma) could have spawned in late May or early June. It would appear that females in our study were awaiting additional spawning cues for an extended period. It is possible that captivity conditions (e.g. facility noise, system maintenance, fish handling, interaction with observers) played a role in inhibiting spawning of untreated shovelnose sturgeon and we are planning methods to reduce possible stressors. Interestingly, a single male spawned in the presence of suitable mates indicating that for this fish the cues were present and stressors were not significant. It is also possible he had reached a stage in the wild previously that allowed him to spawn. A further examination of blood plasma levels may shed light on the differences between this male and other males in the study.

The challenge we undertook to develop conditions in the artificial river that provide the necessary environmental cues and habitat in which shovelnose sturgeon will spawn remain for us in year two of the study. We had hypothesized that spawning ready fish (both females and males), when provided optimal temperature, suitable flow and substrate, and access to mates would respond to the cues provided and spawn. As researchers, the lack of a female to spawn naturally makes the question of what proximal cues provide the tipping point for initiation of spawning even more intriguing.

Data analysis is ongoing and future analyses will provide a first look at the holding and spawning behavior patterns that may have been affected by conditions (velocity, substrate, etc.) in the artificial river. We hope to continue to develop tools to assess the reproductive endocrinology of shovelnose sturgeon during the spawning period. These tools when developed will allow field researchers a clearer understanding of what varying oocyte PI and blood chemistry parameters mean when associated with the timing of spawning and their relationship to environmental factors such as discharge, temperature, and photoperiod.

## Acknowledgements

The project described, the artificial river, infrastructure, equipment, electrical power, and personnel, were funded through a number of grants and partnerships. The shovelnose sturgeon project described in the report was funded through grants from the USGS Science Support Partnership Program, the USFWS Plains and Prairie Potholes Landscape Conservation Cooperative, and USFWS Bozeman Fish Technology Center. Additional

funding for the construction of the artificial river was provided by the USFWS Great Northern Landscape Cooperative. We are grateful to and thank the Montana Fish Wildlife and Parks (specifically Mike Backes, Jason Rhoton, and crews), USGS Montana Cooperative Fishery Research Unit, American Indian Research Opportunities (AIRO), staff at the BFTC, science support staff within the USFWS Region 6, and many others for their support.

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Appendix - Montana State undergraduate and graduate student researchers' involvement.



The photos show USFWS researchers Dr. Molly Webb (above) and Kevin Kappenman (following page) demonstrating blood collection techniques on shovelnose sturgeon in the study. Six undergraduate student researchers from MSU participated in the project. Students learned fisheries techniques including oocytes and blood sample collection methods and how to measure oocytes polarization and sex steroids. The physiological data collected were used to determine which fish were selected for trials and make comparisons between successful and unsuccessful male and female shovelnose sturgeon.



Sierra Alexander (above) transfers a shovelnose sturgeon blood sample to a hematocrit tube for separation of plasma. Plasma estradiol and testosterone levels were monitored throughout the spawning trials and examined as physiological indicators to predict spawning success.



MSU student researcher Michael Stein collects data on the first ever visual observations of spawning shovelnose sturgeon. Michael Stein is a non-traditional student at MSU and a military veteran who served as a black hawk helicopter pilot in the U.S. Army.



MSU student Sierra Alexander assists with data collection and observations. Ms. Alexander involvement in the project was funded by the American Indian Research Opportunities (AIRO), a program to recruit, retain, and graduate American Indians with associate, baccalaureate, master's and doctoral degrees in Science, Engineering and Mathematics (SEM).
**2012 Annual Progress Report:** Spawning Pallid and Shovelnose Sturgeon in an Artificial River: Estimating the Effect of Temperature, Flow, and Photoperiod on the Timing and Duration of Spawning with Implications for Regulated River Management and Response to Climate Change

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# Summary

As part of an ongoing study designed to promote better management techniques for the preservation of shovelnose sturgeon and the recovery/preservation of pallid sturgeon, the spawning behavior and spawning habitat requirements of shovelnose sturgeon were studied for a second year in a semi-natural environment at the Bozeman Fish Technology Center (BFTC). We performed two trials under predefined temperature, flow, and substrate conditions in an artificial river setting. We used luteinizing hormone releasing hormone (LHRHa) to initiate spawning in shovelnose sturgeon in two experimental trials. In both trials, male and female shovelnose sturgeon that were injected with LHRHa selected multiple mates during spawning activity that continued for approximately 10 hours (based on the longest female spawning duration in each trial; defined as the period from first oviposit to final oviposit). We were unable to create the conditions and cues in the artificial river to promote natural spawning (e.g. without the use of LHRHa). The parameters we manipulated to provide spawning cues included water velocity (e.g. high and low flows mimicking a spring freshet), a natural vernalization of increasing temperature, use of natural day light to provide natural photoperiod, and an interaction scenario with spermiating male shovelnose sturgeon (we hypothesized the interaction might provide cues such as naturally released hormones or courtship displays). The 2012 data is currently being analyzed to determine if shovelnose sturgeon have a preferred water velocity related to spawning site selection. We are comparing spawning habitat used (based on velocity at selected spawning site) to habitat available (total velocity habitat available in the artificial river). Research in 2013 will focus on the influence of environmental cues that promote natural spawning and attempt to determine if sturgeon substrate influences spawning site selection.

#### Introduction

Pallid sturgeon (*Scaphirhynchus albus*) and shovelnose sturgeon (*Scaphirhynchus platorynchus*) are sympatric species native to the Missouri-Mississippi river basins in the United States. Shovelnose sturgeon populations have decreased throughout their historic range, but remain relatively abundant in the upper Missouri River in Montana, North Dakota, and South Dakota. The pallid sturgeon is listed as an endangered species throughout its range and is protected under the Endangered Species Act (Dryer and Sandoval 1993). Less than three hundred adult pallid sturgeon remain upstream of Gavin's Point Dam (Yankton, South Dakota) in the Missouri River of South Dakota, North Dakota, and Montana (USFWS 2007). The decline of pallid sturgeon in this area has been correlated with a reduction in spawning habitat and recruitment failure; both of these factors have been associated with dam construction, reservoir development, river channelization, and changes to the river hydrograph and thermograph (USFWS 2000).

The reproductive cycle (gametogenesis) and spawning period (spawning migration to mate selection to gamete expression) of sturgeon are controlled by endocrine and environmental factors known as zeitgebers (from German for "time giver," or "synchronizer"; Dettlaff et al. 1993; Cech and Dorshov 2004). It is known that the endogenous cycle (internal, self-sustained rhythms), photoperiod, and temperature are key factors or ultimate (e.g., primary) cues that control the endocrine system which regulates an adult sturgeon's reproductive cycle (Webb and Dorshov 2011). Together these factors regulate the timing of an adult sturgeon's maturation from early gametogenesis to the stage of spawning readiness (Dettlaff et al. 1993; Webb and Dorshov 2011). In general, it is believed that photoperiod acts to 'set' the endogenous clock, while temperature acts to regulate gamete maturation or 'set the speed' of the endogenous clock. Once an adult sturgeon reaches the stage of spawning readiness, it is believed that a number of proximal (e.g. secondary) cues are necessary to elicit a spawning event. Those proximal cues are hypothesized to include, but are not limited to, the presence of a suitable mate, physical and chemical mating signals (e.g. display, pheromone release; Bayunova et al. 2011), temperature, photoperiod, lunar phase, seasonal discharge, water velocity and pattern, water chemistry, turbidity, and substrate (Papoulias et al. 2011). It is difficult to determine what proximate cues are necessary and what factors may simply be associated with a necessary cue because of the interrelatedness of these proximal factors. In a natural river system, the factors are inherent and cannot be controlled. In regulated rivers, factors such as flow and temperature can be The upper Missouri River is regulated by dams, and these dams can be controlled. operated to alter the timing and quantity of discharge and water temperature. These changes effect spawning macro habitat and micro habitat (e.g. flow patterns, substrate, flooded vegetation, etc.) and thus can affect spawning behavior of shovelnose sturgeon and pallid sturgeon. Determining the influence of discharge, temperature, and substrate on the spawning behavior (e.g., does a combination of these factors elicit a spawning response from a spawning ready adult?) of pallid sturgeon and shovelnose sturgeon is important if managers of regulated rivers are to provide discharge that supports the lifecycle needs of pallid and shovelnose sturgeon. Thus, improving pallid sturgeon spawning conditions through better management of regulated rivers has been identified as necessary in the Biological Opinion on what is needed to recover pallid sturgeon (USFWS 2000).

# **Study Objectives**

In 2011 (year 1), we began a multi-year laboratory study designed to describe spawning behavioral characteristics and determine the relative effect and importance of discharge, temperature, and substrate used by spawning pallid and shovelnose sturgeon. In 2011, we observed and provided the first ever description of shovelnose sturgeon spawning behavior (see Kappenman et al 2011). Our goals for 2012 were to 1) validate spawning observations described in Kappenman et al. (2011), 2) test the use of flow, temperature, substrate, and presence of naturally spermiating males to determine if we could promote natural spawning, and 3) describe micro habitat flow (water velocity m/s) used for spawning site selection.

## **Methods and Preliminary Results**

#### Artificial River System Design

The artificial river described in (Kappenman et al. 2011) was used to perform the 2012 trials. In the 2012 trials, water velocity was generated using two Sulzer electric motors (Sulzer/ABS RW3022 A17/6 Mixers; Sulzer Inc. Switzerland) in place of the four minn kota motors (Johnson Outdoors Inc. Racine, WI. U.S.A) described in 2011. The modification was made in an effort to provide uninterrupted flow velocity for extended periods of time.

#### Conditions in the Artificial River during the Spawning Trials

We attempted to create the environmental conditions (e.g. photoperiod, flow, substrate, temperature) within the artificial river environment that might provide the necessary cues to promote natural shovelnose sturgeon spawning. The conditions we implemented were based on the results of 2011 trials (Kappenman et al. 2011) and suspected and known information for preferred substrate, flow, and temperature of various sturgeon species (Bruch and Binkowski 2002; DeLonay et al. 2007, 2009; Fuller et al. 2008; Goodman 2009; Kynard et al. 2012). The substrate in the artificial river consisted of homogeneous gravel (2-64 mm) evenly distributed in the tank. A natural photo period was supplied using lighting from north-facing windows along the entire east-west wall of the room housing the artificial river. Water temperatures were maintained between 16-22°C and water was supplied from BFTC warm and cold water springs. Temperature in the artificial river was regulated by increasing the amount of incoming warm spring water while decreasing the amount of incoming cold spring water or vise versa. Flows were manipulated by adjusting a rheostat that controlled the revolutions per minute of the electric mixer propellers. The water velocity and temperature profiles used in each trial were designed to promote spawning and assess spawning site selection and are described below for the individual trials. Water velocity measured at 5 cm above the substrate with a Marsh-McBirney flow meter (Hach Company, Loveland, CO) varied from approximately 0 to 75 cm/s. The artificial river velocities during the trial periods are currently being analyzed and a model of water velocities present in the river during spawning is under development. Flow velocity, generated by the mixers and designed to mimic natural river conditions, was adjusted using a rheostat with a variable range electrical setting from 0 to 100%. Relative flow data (velocity m/s at various settings from 0 to 100%) will be presented in a future report.

#### Shovelnose Sturgeon Collection and Holding

Shovelnose sturgeon were collected from two locations using techniques and protocols similar to those described for pallid sturgeon (U.S. Fish and Wildlife Service, 2008). The first group of shovelnose sturgeon were collected from the Missouri River near the Coal Banks Landing Recreation Area on May 9, 2012. Missouri River temperatures during the fishing period varied between 10 and 12°C. Once captured,

shovelnose sturgeon were transferred to a live cage and held in the river. Individual shovelnose sturgeon were removed from the live cage and assessed to determine sex and stage of maturity using a biopsy technique (Conte et al. 1988). Females with stage 5 ovarian follicles (large, fully pigmented post-vitellogenic follicles) and males of mature size with visibly developed testes were selected for the study and placed in an insulated tank on a fish transport truck. Shovelnose sturgeon not selected were released immediately after the sexing and staging process. All fish selected for the study were weighed (nearest 0.1 kg), measured for fork length (nearest 0.5 cm), and PIT tagged. During the biopsy, approximately 40 ovarian follicles were removed from each female, and a sample of testis was removed from each male and placed in 10% phosphate buffered formalin for laboratory analyses to assess spawning readiness. The fish transport holding tank had been previously filled with BFTC source water that was within 1-2°C of the rivers temperature. A combination of oxygen supplied to an air stone and a mechanical aeration system provided a minimum 8 mg/L of oxygen during the transport from the river to the BFTC. Shovelnose sturgeon were transported to the BFTC on the day of capture, and once at the BFTC, placed into 3 m circular tanks (males and females together) and held at 10°C. On May 14, the holding tank temperatures were gradually increased (~1°C per day) to 15°C over a five day period. The gradual increase in temperature was designed to resemble a natural vernalization period and facilitate reproductive maturation (Webb et al. 1999). Fish were held at 15°C in the 3 m circular tanks throughout the study except when involved in a trial.

A second group of shovelnose sturgeon were collected from the Yellowstone River near its confluence of the Powder River on June 15, 2012. Yellowstone River temperatures during the fishing period varied between 16 and 18°C. Fish from the Yellowstone River were processed and transported in a similar manner as described above, except for a difference in the technique used to determine sex and reproductive stage. Males were selected for the study based on the collection of milt using a syringe fitted with tubing and inserted into the urogenital pore. Once at the BFTC, the additional shovelnose sturgeon were placed in the 3 m circular holding tanks with the shovelnose sturgeon that had previously been collected or placed directly into the artificial river.

## Assessment of Sexual Maturity

We monitored and assessed the spawning readiness of each shovelnose sturgeon throughout the study. We determined the stage of reproductive readiness in male sturgeon using steroid profiles and in female sturgeon by assessing ovarian follicle development patterns and steroid profiles. To determine female spawning readiness, approximately 20 ovarian follicles were bisected to measure the oocyte polarization index (PI; a ratio of the distance of the germinal vesicle from the animal pole to the oocyte diameter), and approximately 20 ovarian follicles were measured to determine follicle diameter (Van Eenennaam et al. 2004). Measurements (nearest 0.005 mm) were made using a Leica DM 2000 compound microscope (2.5x; Leica Microsystems Inc. Buffalo Grove, IL, USA) equipped with a RT KE Spot camera and Spot Advanced imaging software (SPOT Imaging Solutions, Sterling Heights, MI, USA). We examined blood plasma sex steroid concentrations according to protocols described in Webb et al. (2002, 2008).

## Data Collection

Fish were visually monitored periodically throughout each day. Once a spawning event began, we monitored behavior using constant direct observation and recorded video. We observed and recorded: 1) number of males and females in a spawning aggregation, 2) positions of males and females during spawning, 3) timing and sequence of egg and milt release, 4) the relationship of males and females to water depth and site location during spawning, 5) female mate choice (how many males were selected, what size, etc.), 6) characterization of egg deposition and associated swimming behavior (looping or stationary behavior, size of depositional area, etc.), 7) individual male mating success vs. failure ratios, 8) female ovulatory intervals (number/min/h/d) and spawning duration, and 9) male number of spawning bouts (number/min/h/d) and spawning duration.

To facilitate spawning site selection observations, the artificial river was divided into 22 sections (labeled 1 through 22; see Figures 1 and 2). Each of the 22 sections was further divided into 4 cells (A, B, C, and D). The resulting diagram (Figure 2) allowed each observed spawning event to be assigned to an individual cell. Our goal was to determine and compare the mean water velocity (MWV) available and the MWV used for spawning based on site selection. In effort to characterize the water column velocity of each cell, we measured water velocities at 5% and 20% of total depth (e.g. 5 and 18 cm above the substrate respectively) a using a Marsh-McBirney flow meter. We visually estimated that all spawning occurred from 0 -18cm from the bottom. The MWV of a cell was estimated by collecting 20 velocity measurements for each cell. Velocity was measured at 5 points along a longitudinal line (every 6 cm) at the upstream and downstream edge of each cell. At each point, we recorded the water velocity at 5% and 20% of the water depth. Thus, the mean water velocity for each cell included 5 upstream and 5 downstream points at 5%, and 5 upstream and downstream points at 20% for a total of 20 measurements.

# Spawning Trial 1 (June 11 – June 26)

In the first 11 d of trial 1, we attempted to provide the conditions in the artificial stream that would promote natural spawning (e.g. spawning without the use of hormonal stimulants). In an effort to simulate spring-like river conditions (e.g. a spring freshet) that might provide natural spawning cues, flow velocity and temperatures in the artificial river were gradually increased from June 11 to June 15. From June 16 to June 22 flow was decreased (e.g. simulating a declining hydrograph) while temperature remained unchanged. We also attempted to provide a hormonal and behavioral cue in the form of an introduction of naturally spermiating male shovelnose sturgeon. We hypothesized that a hormonal release or a male courtship gesture from a naturally spermiating male might provide an additional spawning cue to the female sturgeon.

Based on sexual maturity and spawning readiness, three female and four male shovelnose sturgeon were selected for the trial. The trial fish were moved from the 3 m circular holding tanks to the artificial river on June 11, 2012 (day 1 of trial) and allowed to acclimate in the artificial river for 1 d. On day 1, temperature in the artificial river matched the holding tank temperature of 15°C, a natural photoperiod was present, no

flow velocity was present (motor setting was zero), and a gravel substrate was present. On day 2, we initiated flow at 20% of the motor setting<sup>1</sup> and began a gradual increase in warm water inflow. On day 3, the motor setting was increased to 45% and temperature in the tank was increased to  $16.5^{\circ}$ C. On day 4, motor setting was increased to 70% and temperature was increased to 18°C. On day 5, the motor setting remained at 70% and temperature was increased to 19°C. On June 15, at 10:30 pm five spermiating male shovelnose sturgeon (collected from a spawning area in the Yellowstone River) were introduced into the artificial river. On day 6, conditions in the artificial river remained unchanged, and no spawning had occurred. On June 17, flow was reduced to 45% while temperature was maintained at an average of 20.2°C. The scenario of introduced spermiating males coupled with a 45 % flow was maintained from June 17 to June 22 while temperatures varied from 20.2 to 21.9°C. No spawning occurred during the 11 d trial. In general, the spermiating males exhibited cruising behavior and actively investigated the female sturgeon, but no additional courtship signs were observed and no spawning occurred. As time progressed (approximately June 17-20), the spermiating male's activity level decreased and their cruising behavior ceased.

# Use of hormones to facilitate spawning

On June 23 and 24, the original seven shovelnose sturgeon placed in the artificial river were injected with LHRHa. We followed an injection regime that allowed female and male sturgeon to be in spermiating and ovulatory condition during the same time period. The previously spermiating male shovelnose sturgeon (e.g. Yellowstone River sturgeon) were not injected, but remained in the artificial river. We observed the first spawning event approximately 22 hrs after the resolving dose of LHRHa was administered to the females. Two of the three female shovelnose sturgeon injected with LHRHa spawned and one did not spawn. Three of the four male shovelnose sturgeon injected with hormones spawned. Males that were not injected (e.g. the previously spermiating Yellowstone River males) did not spawn. Thirteen individual spawning events took place during a 10 hour period. The individual males varied in their spawning duration from 3-10 h. A preliminary analysis of water velocities at spawning sites ranged from 0.18 m/s to 0.40 m/s (measurements taken at 5 cm above the substrate). An additional analysis comparing the water velocity at a selected spawning site to the velocity habitat that was available throughout the artificial river is being performed to determine if shovelnose sturgeon select particular velocities in association with spawning site selection.

### Spawning Trial 2 (June 26 – June 29)

Trial 2 was designed to collect additional data on shovelnose sturgeon spawning behavior, specifically spawning site selection relative to water velocity. We used the hormone LHRHa to induce spawning. The sturgeon used in the study were selected based on sexual maturity and spawning readiness as determined by testosterone levels (males)

<sup>&</sup>lt;sup>1</sup> Note that the 20% motor setting, as are all rheostat settings from 0 to 100%, is a relative flow setting and can be replicated. Additional data is being collected to provide a true velocity model in the artificial river relative to the various rheostat settings that are discussed in the report.

and oocyte PI (females). On June 26 and June 27, 2 female and 6 male shovelnose sturgeon were injected with LHRHa. A motor setting of 70% and 45% were used to create the water velocity scenarios in the artificial river and to allow an assessment of site selection at two different water velocity regimes. We recorded spawning observations for 5 h at the 70% setting flow setting and the remaining observations at a 45% setting. Water temperature during the trial was maintained at 16.3°C, and substrate and lighting remained as previously described for trial 1. During the trial, two female shovelnose sturgeon and a single male shovelnose sturgeon spawned. Fifty one unique spawning were recorded during an eleven hour period. Water velocities at selected spawning sites (measured 2 inches above the substrate) were between 0.07 m/s and 0.67 m/s. An analysis of the velocity habitat used for spawning site selection to the velocity habitat available throughout the artificial river is being performed to determine if shovelnose sturgeon select particular velocities for spawning.

#### **Future Plans and Products**

For a second year, we were able to make visual observations of shovelnose sturgeon spawning behavior in an artificial river. The data collected in year 2 will provide new insight into the microhabitat used by shovelnose sturgeon during spawning. The information will be published in a final report. We plan to present a manuscript that describes shovelnose sturgeon spawning site selection based on velocity and substrate preference. The substrate preference trials will be performed in 2013.

Year 2 also provided a second year of data on the spawning behavior characteristics of shovelnose sturgeon. The courtship characteristics observed in 2012 were similar to our previous observations (e.g. year 1). A manuscript describing the spawning behaviors is in draft. We hope to submit a draft to a journal in 2013. Again in 2012, we observed polyandrous and polygynous mating, individual male and female cruising behavior, brief spawning bouts (i.e., vibration; generally <5 seconds), nosing or bumping of the female abdomen by males prior to spawning (an apparent test of willingness or ability to spawn, or mating courtship), false spawns', a raised activity level when spawning, and predation of recently spawned sturgeon eggs by male and female shovelnose sturgeon. On multiple occasions, we observed a male quivering and releasing milt alongside a non-participating female. The courtship behavior of shovelnose sturgeon appears to be characterized by a male actively seeking out and selecting a more stationary female followed by a male mating attempt (nosing, quiver and milt release) in which the female may or may not release eggs. In all observations, shovelnose sturgeon spawned close to or on the substrate (generally, the female was less than a few cm off the bottom surface).

Sex steroid data and spawning readiness data collected in 2011 and 2012 are currently being analyzed and compared to spawning results. A description of physiological parameters we used to measure spawning readiness, individual progression of spawning readiness, and group comparisons will be produced in a final report and peer reviewed publication. This information, when developed, will allow field researchers a clearer understanding of what varying oocyte PI and blood chemistry parameters mean when associated with the timing of spawning and their relationship to environmental factors such as discharge, temperature, and photoperiod.

## Acknowledgements

The project described, the artificial river, infrastructure, equipment, electrical power, and personnel, were funded through a number of grants and partnerships. The shovelnose sturgeon project described in the report was funded through grants from the USGS Science Support Partnership Program, the USFWS Plains and Prairie Potholes Landscape Conservation Cooperative, and USFWS Bozeman Fish Technology Center. Additional funding for the construction of the artificial river was provided by the USFWS Great Northern Landscape Cooperative. We are grateful to and thank the Montana Fish Wildlife and Parks (specifically Anne Tews, Mike Backes, Jason Rhoton, and crews), USGS Montana Cooperative Fishery Research Unit, American Indian Research Opportunities (AIRO), staff at the BFTC, science support staff within the USFWS Region 6, and many others for their support.

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Addendum – This section shows an example of how data for a trial designed to explore the relationship of shovelnose sturgeon spawning site selection to water velocity (m/s) is being analyzed. The figures below provide some details not present in the 2011 and 2012 annual reports. We provide this as an example of how future analyses will be performed to look at substrate and velocity preferences of shovelnose sturgeon spawning sites. This study will continue in 2013-14 and products will be finalized under the SSP grant supporting the research described.



Shown is a hand drawn diagram depicting the living stream. The oval tank was divided into 22 sectors numbered 1-22, and the sectors were divided into 88 individual cells labeled a, b, c, or d. The cells allowed each spawning event to be partitioned into a known velocity. For example if a sturgeon spawned in the sector indicated by the arrow it was recorded as a sturgeon spawn in 7A. The water velocity in each cell was measured at 5 and 18 cm above the substrate respectively using a Marsh-McBirney flow meter. The mean water velocity of a cell was estimated by collecting 20 velocity measurements for each cell.



Shown is a contour plot of water velocities in the living stream depicted in a linear fashion during a spawning trial. Note there are 22 sectors and 88 cells. The color scale depicting velocity is on the right. The red represents fast water and blue represents slow water. Each X represents one spawning observation in a particular cell. The Letter B indicates a section is in a bend of the stream and letter L indicates a section is in a linear portion of the stream.



The figure shows a percentage used to percentage available analyses of water velocity and spawning sites in the living stream during a trial. The X axis shows velocity and Y axis shows percent. Mean water velocity data was categorized into 3 different bins or groups. In this scenario (trial) the categories were 0–14 cm/s, 15–29 cm/s, and 30–44 cm/s. The percent available is depicted by gray bars. The percent of area selected for spawning is depicted by the black bars. Mean water velocities of 30–44 cm/s were the most frequently used velocity category for spawning shovelnose sturgeon. Mean water velocities of 15–29 cm/s were the most available velocity category. One-way chi-square log-likelihood tests were used to determine if MWV categories were used by shovelnose sturgeon for spawning in proportion to availability for each flow regime. Shovelnose sturgeon did not select velocities in proportion to availability ( $\chi 2 = 18.3$ , P < 0.0001), selecting against slow water velocity (0-14 cm/s) and selecting for high water velocities (30-44 cm/s).



This figure shows the Manly selection ratio analyses for a spawning trial exploring water velocity selected for a spawning site by shovelnose sturgeon. The X axis shows mean water velocities in three categories and the Y axis is the selection ratio. Positive selection is indicated by values greater than one. Negative selection is a value less than one. Values equal to one indicate selection in proportion to availability. Shovelnose sturgeon positively selected for high velocity areas (30-44 cm/s) and negatively selected for low velocity areas (0-14 cm/s).