Effects of the Reservoir Headwater Environment on Survival and Behavior of Larval Sturgeon: Are Reservoirs Ecological Sinks for Recruitment of Sturgeon?

Final Report

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Executive Summary

Adult pallid sturgeon *Scaphirhynchus albus* are spawning and produce viable larvae in the upper Missouri River; however, no naturally produced pallid sturgeon have been documented recruiting to the population since the species was listed as endangered in 1990. The most plausible contemporary hypothesis regarding recruitment failure relates to the limited drift distance for larvae. Drifting pallid sturgeon larvae require an estimated ~ 200-500 km of lotic habitat before they initiate the benthic life stage (Braaten et al. 2012), and the estimated lotic habitat is not available between putative pallid sturgeon spawning locations and reservoir headwaters (Braaten et al. 2012).

The fragmentation of the Missouri River has negatively influenced pallid sturgeon, but the specific mechanism responsible for larval pallid sturgeon mortality was unclear and the subject of this research. While much of the research and recovery efforts associated with pallid sturgeon have focused on downriver effects of dams in relation to spawning habitat and cues needed to facilitate spawning and recruitment, this is the first long-term study to examine the effects of upriver headwater habitat on pallid sturgeon recruitment.

In order to identify abiotic factors that may be influencing larval mortality, we began by characterizing the habitat upriver of Ft. Peck Reservoir and determining where larval pallid sturgeon drift in the available habitat upriver of the reservoir. This first entailed systematic sampling water-quality parameters – dissolved oxygen (DO), pH, water temperature, velocity, substrate, and presence of contaminants – in river, reservoir, and headwater (also termed transition zone) habitat. We then conducted drift trials with immediate post-hatch shovelnose sturgeon *Scaphirhynchus platorynchus* and pallid sturgeon larvae. The data collected from our field sampling and larval drift trials revealed:

1. The location of the Ft. Peck Reservoir headwater habitat is dynamic and a function of inflow and discharge from Ft. Peck Dam (Chapter 1).

2. Ft. Peck Reservoir headwater habitat (also termed transition zone) is characterized by reduced water velocities relative to the river, highly variable

dissolved-oxygen concentrations (hypoxic and anoxic conditions are present), and high sedimentation rates (Chapter 1).

3. Shovelnose sturgeon and pallid sturgeon larvae drift mainly in the thalweg in the headwater habitat, which is consistent with previous studies (Chapter 2). Shovelnose sturgeon and pallid sturgeon do not appear to drift into flooded terrestrial vegetation that can be inundated in the headwater area (Chapter 2). Interestingly, three larvae where captured a day after the drift experiments in the headwater habitat and all were dead, which corroborates our findings in Chapter 6.

The field data collected during the first two years of the study provided us with important information that we used to guide the laboratory experiments for the remaining three years of research. We focused our efforts on designing experiments that evaluated the effects of specific abiotic factors on larval mortality. Laboratory experiments in 2010 addressed the effects of pH, dissolved oxygen (DO), and sedimentation rate. All treatment levels were based on the field data collected in the Ft. Peck Reservoir headwater, as previously stated. The data obtained from the DO and sedimentation rate experiments were likely influenced by system design flaws, and ultimately we decided to attempt these experiments again in 2011. However, we were able to draw the following conclusions from the pH experiments:

4. We determined that pH varying from 6.5 to 8.0 had no effect on mortality of shovelnose sturgeon and pallid sturgeon larvae. Thus, given the empirical pH levels observed and those tested in the laboratory, pH does not appear to be a mechanism for recruitment failure (Chapter 3).

In 2011, we made modifications to system design and conducted DO and sedimentation rate experiments with shovelnose sturgeon and pallid sturgeon larvae. While the sedimentation rate experiments were again plagued with system-related problems, the DO experiments demonstrated:

5. Dissolved oxygen concentrations of 1.5 mg/L caused nearly 100% mortality in shovelnose sturgeon larvae and 100% mortality in pallid sturgeon larvae within 5 days or less (Chapter 4).

In 2011, we also conducted experiments addressing the effects of substrate type on larval mortality. Over the course of these experiments, we observed high levels of unionized

ammonia (UIA) in tanks containing fine particulate organic material (FPOM) from the Ft. Peck Reservoir headwater habitat. There was high mortality associated with the FPOM substrate, and we thought it possible that UIA released from the FPOM might be causing the mortality. Because this substrate predominates in headwater habitat, we wanted to examine whether UIA is a mechanism for larval pallid sturgeon mortality. After sampling UIA in the Ft. Peck Reservoir headwater in the summer of 2012 and conducting dose response experiments in 2012 we concluded:

> **6. Unionized ammonia at 0.22 mg/L influenced mortality of immediate post-hatch shovelnose sturgeon, but not 40-DPH shovelnose sturgeon. Field observations of unionized ammonia collected in 2012 were well below levels shown to negatively influence survival. Thus, unionized ammonia is likely not a factor influencing recruitment failure (Chapter 5).**

Hypoxic conditions similar to those that caused 100% mortality in pallid sturgeon larvae in our laboratory experiments were consistently observed in water samples collected from the lower 0.5 m of the water column during the unionized ammonia sampling in 2012. These conditions had not been documented during the field sampling in 2008 and 2009 because data were collected higher in the water column. Based on the results of the dissolved oxygen laboratory experiments, we decided to investigate further the presence of hypoxic/anoxic conditions near the substrate throughout the headwater area (i.e., transition zone—see Chapter 6). The sampling was conducted in 2012 and 2013 and revealed:

> **7. Anoxic conditions exist in the thalweg and outside the thalweg in the headwater habitat. The locations of anoxic conditions correspond with sites where shovelnose sturgeon and pallid sturgeon larvae drift. Thus, we surmise that anoxic conditions in the headwater habitat are the mechanism for recruitment failure (Chapter 6), and reservoirs are an ecological sink for pallid sturgeon. These data were published in the journal Fisheries (Guy et al. 2015; Fisheries 40: 6-14) and was the major significant finding from this research project.**

In 2012, we were able to develop a suitable experimental system to determine if the settling of sediment caused mortality in larval pallid sturgeon as the current breakdown of fluvial transport as a function of impoundments causes sediment to settle from suspension because of reduced water velocity. We found:

8. Sedimentation rates used in this study did not cause mortality of shovelnose sturgeon and pallid sturgeon at immediate post hatch and 40-day post hatch. Shovelnose sturgeon and pallid sturgeon in varying sedimentation treatments experienced mortality rates similar to the control. Despite the fact that sedimentation rates in the headwater habitat are unnatural, it appears that it is not a factor in influencing survival (Chapter 7).

Because of the potentially confounding effects of unionized ammonia in the 2011 substrate experiments, we conducted substrate experiments again in 2012 and we were able to maintain water-quality conditions suitable for larvae. These experiments demonstrated:

9. Substrate type did have an influence on mortality of immediate post-hatch shovelnose sturgeon and pallid sturgeon. Mortality of pallid sturgeon and shovelnose sturgeon was lowest in treatments with sand substrate and highest in treatments with artificial sediment (Fuller's Earth) and Missouri River fine particulate organic matter. These experiments were in a static environment (i.e., no flow), which likely mimics the headwater habitat more than the sedimentation rate experiments. Thus, substrate type may play a role in recruitment failure. The exact mechanism for the differences in mortality among substrate treatments needs further research (Chapter 8).

Our research provided a great deal of information on the effects of multiple factors on larval pallid sturgeon and shovelnose sturgeon mortality in the upper Missouri River. Additionally, our experiments allowed us to draw general conclusions about potential species-related differences, such as:

10. In general, immediate post-hatch and 40-day post-hatch pallid sturgeon grew slower and had high mortality rates than shovelnose sturgeon for most of the laboratory studies. The difference in growth rates and sensitivity to treatments may partially explain why shovelnose sturgeon recruit whereas pallid sturgeon exhibit recruitment failure. This general observation suggests that there are slight physiological differences between the species that needs additional study.

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Chapter 1
Habitat Sampling in the Ft. Peck Reservoir Headwater

Preface

From 2008 to 2013, we conducted investigations to attempt to unravel the recruitment failure mystery of the endangered pallid sturgeon. The major premise of our investigations was based on the two hypotheses that pallid sturgeon drift into the headwater environment and that habitat is an ecological sink. The ecological sink hypothesis is discussed throughout the report. Details of sturgeon spp. larval drift behavior can be found in Kynard et al. (2007), Braaten et al. (2008), and Braaten et al. (2012). Succinctly, it is believed that pallid sturgeon embryos hatch, immediately begin a fluvial drift life stage, are deposited into the reservoir, and mortality occurs because the reservoir habitat is an ecological sink. From 2008 to 2013, we examined habitat conditions in the reservoir headwater habitat to determine if changes to river habitat conditions because of the reservoir might be the mechanism for recruitment failure. Our investigations lead us down several research trajectories of which some were difficult to implement given the complexities of trying to recreate river conditions in the laboratory. The observations of habitat conditions in the reservoir headwater habitat lead us to attempt to test specific conditions in the laboratory and to investigate and test specific hypotheses that we thought might negatively influence larval sturgeon survival. In general, we attempted to examine a specific variable among a “cocktail” of associated habitat changes that have occurred since Fort Peck dam was built. Results from our laboratory studies and reservoir habitat sampling lead us to additional hypotheses that we addressed with added laboratory studies and reservoir habitat sampling. We begin our report with a description of the reservoir habitat and our efforts to characterize the habitat in 2008, 2009, 2012, and 2013.

Introduction

Dams influence upriver and downriver habitat through alterations to habitat such as sediment dynamics, water depth, water velocity, and water quality. Additionally, modifications made through channelization and dam construction can reduce connectivity and, ultimately, biodiversity within affected river segments (Wohl 2012). These changes to river hydrology and

habitat quality are important for migratory species, like salmon spp., and species that evolved in large turbid rivers, such as sturgeon spp. Specifically, pallid sturgeon in the upper Missouri River experience recruitment failure such that no naturally recruited pallid sturgeon have been sampled since the species was listed as endangered in 1990.

Pallid sturgeon embryos incubate for 3 – 18 days after fertilization, depending on water temperature (Kappenman et al. 2013). After hatching, pallid sturgeon larvae drift from their hatch locations as ichthyoplankton (Kynard et al. 2007; Braaten et al. 2008; Braaten et al. 2012). Recent research indicates that, depending on water temperature and water velocity, larval pallid sturgeon require ~200-500 km and larval shovelnose sturgeon require slightly less distance of riverine habitat (Braaten et al. 2008; Braaten et al. 2012). The contemporary available riverine habitat between pallid sturgeon spawning locations and reservoir headwater habitat is less than that needed for larval drift (Braaten et al. 2012). Therefore, it is hypothesized that larval pallid sturgeon are deposited into a reservoir headwater habitat, characterized by conditions not suitable for larval survival. We designed this study to evaluate how water quality in the Ft. Peck Reservoir headwater differs from conditions found in the Missouri River and Ft. Peck Reservoir. Specifically, our objective was to collect data on habitat parameters that may be causing mortality in larval pallid sturgeon. This information was used to inform the 2010-2012 laboratory studies.

Study Area

The Ft. Peck Reservoir is located in northeastern Montana (Figure 1.1), and is the first (i.e., farthest upstream) of six US Army Corps of Engineers' reservoirs on the Missouri River within the historical range of pallid sturgeon (Keenlyne 1989). Ft. Peck Reservoir is 214 km (USACOE 2001) at full pool (685 m), making it the fifth largest reservoir in the United States (USACOE 2004).

Water levels vary among years because of water-management practices and changes in annual snow and rainfall (Figure 1.2). The maximum annual difference recorded in pool elevation was a decrease of 7.5 m, which occurred between 1955 and 1956. A loss of pool

elevation can ultimately decrease total reservoir length, thereby increasing available lotic habitat and allowing for colonization of sandbars by terrestrial vegetation [e.g., grasses (Poaceae, Cyperaceae, and Juncaceae), willows (*Salix* spp.), and cottonwoods (*Populus* spp.)] in areas once inundated by the reservoir. Conversely, increases in pool elevation lengthen the extent of the reservoir; the effects of this vary from extending areas of sedimentation further upstream to increases in fluctuations of dissolved oxygen and carbon dioxide levels as result of flooded vegetation (Ruttner 1963; Horne and Goldman 1994).

The headwater habitat of a reservoir occurs at the point of transition from lotic to lentic habitat, called the transition zone (Thorton 1990). The Ft. Peck Reservoir headwater habitat has been located near the UL Bend (Figure 1.1) since 2004 because of current water-level conditions. Water velocity is low, though a main channel still exists. The transition zone is a dynamic environment because it moves depending on inflow and outflow and its location is relative to reservoir elevation.

Methods

2008 & 2009. – We collected field data from the Ft. Peck Reservoir headwater area in an effort to characterize the habitat. In each year, we *a priori* stratified the area upstream of the reservoir into three zones: river (similar to the historic Missouri River), transition zone (slowing water velocities – headwater habitat), and side channels (slower water but connected to the transition zone). We also collected data from a randomly selected, unaltered stretch of the Missouri River (i.e., not in the reservoir flood pool; reference) and in the reservoir (reservoir).

We estimated the sample size needed to adequately represent each strata of the Ft. Peck Reservoir transition zone using the equation:

$$n = \frac{4 \sum (W_h \times s^2{}_h)}{d^2}$$

where n is the total sample size required, the number four defines the equation for 95% confidence intervals, W_h is the weight of stratum h, $s^2{}_h$ is the observed variance of stratum h,

and *d* is the desired absolute precision of the stratified mean (Krebs 1999). We then distributed the total estimated sample size among strata by multiplying the total estimated sample size by the weight of each stratum (Krebs 1999). Our criteria for the absolute precision of the stratified mean velocity (m/s) resulted in the highest total sample size. We did not include the reference sites in this sample size estimator because those locations are not descriptive of upper Ft. Peck Reservoir.

We collected data on velocity (m/s), dissolved oxygen level (DO; mg/L), and water temperature (T; °C) in a stratified, systematic sampling design. We sampled each habitat type with a minimum of 15 transects (McMahon et al. 1996). We sampled each transect at 25%, 50%, and 75% across the main channel. We divided the number of samples needed for a given stratum by three to determine the number of transects to sample for that stratum. We divided the length of the stratum by the number of transects to determine the distance between each transect.

We sampled water velocity with a Marsh-McBirney Flowmate 2000 portable flow meter (Hach Company, Loveland, Colorado) in the lower 0.5 m of the water column by attaching the sensor on the end of an extendable 5-m long probe. Temperature (T) and DO data were collected with a YSI Model 52 Dissolved Oxygen Meter (Yellow Springs International, Yellow Springs, Ohio). We attached the DO and T probe immediately above the flowmeter sensor on the 5-m extendable probe to ensure that we were sampling in the lower 0.5 m of the water column (this sampling location was higher in the water column than the sampling locations reported in Chapter 6). We collected substrate data using the “feel pole” method (Bramblett and White 2001). In addition to the transect sampling, we conducted continuous habitat data collection by deploying two programmable, unattended data loggers in the lower 0.5 m of the water column to capture long-term habitat data on T, DO, pH, conductivity (µS/cm), and turbidity (NTU).

Additionally, in 2008 we collected water (n = 20) and substrate (n = 6) samples to test for environmental contaminants. Samples were sent to Energy Laboratories, Inc. (Billings, MT) to be tested for sulfides (mg/L), total Kjeldahl nitrogen (TKN; mg/L), copper (Cu; µg/L), mercury

(Hg; µg/L), total phosphorous (TP; µg/L), and selenium (Se; µg/L). Substrate was tested for Cu (mg/kg), Hg (mg/kg), and Se (mg/kg). The minimum detectable limits for each of the water contaminants above were 0.04 mg/L, 0.5, mg/L, 1.0 µg/L, 0.5 µg/L, 100 µg/L, and 5.0 µg/L, respectively. The minimum detectable limits for the soil contaminants were 5.0 mg/kg, 1.0 mg/kg, and 5.0 mg/kg, respectively. The concentration of contaminants detected in this study were compared to the benchmarks defined in the Basin-Wide Contaminants Assessment for Pallid Sturgeon (Assessment; Webb et al., unpublished), except for sulfides which were not considered in the Assessment and TNK which is only a fraction of the total nitrogen analyzed in the Assessment. The benchmark is the concentration of a chemical that produces a predetermined change in the response rate of an adverse effect compared to background. The benchmarks for water analytes were: 0.23 µg/L for TP, 17.3 µg/L for Cu, 0.7 µg/L for Hg, and 2 µg/L for Se. As Cu is affected by water hardness, we converted the benchmark value using average hardness data for the area. Hardness data from three Missouri River locations in the region (NWIS sites 6185500, 617700, and 613200) varied from 140 to 230 mg/L. Using the average hardness value of 205.68 mg/L, the copper benchmark is 17.3 µg/L (D. Rouse, pers. comm.). The benchmarks for sediment analytes were 0.32 mg/kg for Cu, 0.18 mg/kg for Hg, and 4 mg/kg for Se.

All analyses were conducted using the statistical software R (Version 2.13.1). Data were tested to determine if the assumptions of normality were met, and, when data did not meet these assumptions, a transformation of the data was performed and analyses conducted on the transformed data. We used a post-hoc Tukey Test to determine if any significant differences ($\alpha = 0.05$) existed among water-quality parameters collected in the different habitat zones in 2008 and 2009. Additionally, measures of location and spread (i.e., average ($\pm$ SD) were calculated for the water-quality parameters collected as part of the habitat sampling.

2012 & 2013. - In 2012, water samples were collected at three depths (surface, 50% maximum depth, and 100% maximum depth) using a Van Dorn sampler in the river and transition zone above Fort Peck Reservoir, Montana, USA. Samples were collected on 19 and 20 June 2012 when water temperatures in the river were optimal for pallid sturgeon spawning. Samples were emptied into an 18.9-L plastic container and dissolved oxygen (DO), temperature, pH,

and unionized ammonia were measured using an YSI Professional Plus meter. We were concerned about the dissolved oxygen measurements in 2012 because the meter would not stabilize for samples near the substrate (i.e., at low dissolved oxygen).

Thus, in 2013, we used the YSI ProODO, which uses an optical sensor to measure dissolved oxygen and reduced uncertainty in our measurements. Dissolved oxygen, water temperature, and velocity were systematically measured along transects in the river and transition zone above Fort Peck Reservoir, Montana. Water velocity, substrate, and channel characteristics were used to delineate the river and transition zone. River was defined as having surface water velocity ≥ 0.5 m s^{-1}, sand substrate, and a river channel was defined within the riverbanks. The transition zone was defined as having a surface water velocity ≥ 0.1 m s^{-1} and ≤ 0.5 m s^{-1}, silt substrate, river channel not well defined, and the reach resembled a lentic environment. The transition zone habitat has been previously describe in reservoirs by Thorton (1990). Measurements were collected on 18 June when water temperatures in the river were optimal for pallid sturgeon spawning. Transects within each habitat type were spaced approximately 1 kilometer apart. Measurements at 50, 75, and 100% of the maximum depth were collected in the thalweg and immediately outside the thalweg on river left and right along each transect. In addition, vertical profile measurements were collected in the thalweg, and dissolved oxygen, water temperature, and velocity were measured at 0.25-m increments. All dissolved oxygen and water temperature measurements were measured using an YSI (Yellow Springs Instrument, Inc.) ProODO meter, and velocity was measured using a Marsh McBirney Flo-Mate 2000. Unlike 2012, all measurements in 2013 we collected in situ because meter sensors were attached to a sounding weight attached to the boat via cable. Measurements at the maximum depth were 14 cm above the substrate because meter sensors were attached to the hanger bar for the sounding weight. Kruskal-Wallis test was used to compare dissolved oxygen concentration between habitat types and among depths because these data were not normally distributed.

Results

2008 & 2009. - We collected habitat data from 213 locations within the headwater habitat. Velocity (m/s) decreased from the river to the reservoir (Figure 1.3). The transition zone was characterized by an average velocity (± SD) of 0.32 m/s (± 0.22), which was roughly one-third of the recorded velocity in the sampled section of the Missouri River [0.98 m/s (± 0.27)], but greater than the velocity of Ft. Peck Reservoir [0.04 m/s (± 0.03)]. There were no significant differences between velocities in the reference and river zones, nor were there significant differences between the side channel and transition zone (all *P*-values > 0.05; Figure 1.3). Velocities in the reservoir were significantly lower than those in the side channel, transition, river, and the reference zone (all *P*-values ≤ 0.05; Figure 1.3).

Dissolved oxygen decreased from the river to the reservoir, but there were no significant differences in DO between the reference and rivers zones or between the side channel and transition zone (Figure 1.4; all *P*-values > 0.05). Dissolved oxygen measurements in the reservoir were significantly lower than those measured in the side channel, the headwater zone, the river, and the reference zone (Figure 1.4; all *P*-values < 0.05).

In 20 water samples tested for contaminants, TNK occurred at or above minimum detection limits only once and did not exceed the benchmark. Copper occurred above minimum detection limits in all 20 samples. Using the average hardness value to determine the Cu benchmark in this region, it is reasonable to assume that 2 of the 20 samples contained Cu concentrations that were elevated relative to the benchmark value. Mercury exceeded the benchmark value in all samples in which it was detected (5 of 20). The TP exceeded the benchmark in all samples in which it was detected (16 of 20) (Table 1.1). Sulfides and selenium were not detected above minimum reporting limits in any samples. In six soil samples tested for contaminants, Cu was detected in all samples but did not exceed the benchmark (Table 1.2). Mercury and Se were not detected.

2012 & 2013. - Unionized ammonia was well below lethal levels (see Chapter 5) at all locations in the transition zone of Ft. Peck Reservoir (Table 1.3). However, the transition zone was hypoxic or anoxic near the substrate in and outside the thalweg in the transition zone

(Table 1.4). Conversely, in the river (i.e., representing natural conditions) dissolved oxygen concentrations were > 7 mg L^{-1} at all depths and lateral locations (Table 1.4). Dissolved oxygen differed significantly near the substrate (i.e., 100% of maximum depth) between the river and transition zone (2013; Kruskal-Wallis $\chi^2 = 24.9$, $P < 0.0001$, $df = 1$), but dissolved oxygen did not differ between the river and transition zone at shallower depths (2013; Kruskal-Wallis $\chi^2 = 0.33$, $P = 0.56$, $df = 1$ for 75% of maximum depth; Kruskal-Wallis $\chi^2 = 1.93$, $P = 0.16$, $df = 1$ at 50% of maximum depth).

Discussion

In all sampling years, DO was lower in the transition zone than the river. Additionally, we consistently documented anoxic or hypoxic conditions in the portion of the water column inhabited by drifting sturgeon larvae (see Chapter 2 and Braaten et al. 2012 for larval drift). Hypoxic events may also negatively affect food availability for sturgeon larvae as they become benthically oriented and begin to feed exogenously in the headwaters and reservoir. It is important to continue to study DO levels in the transition zone to better understand the spatial and temporal variation in hypoxic conditions. It is interesting to note that when we first measured DO at ~ 0.5 m above the substrate we did not observe anoxic conditions. It was not until we measured DO very close to the substrate that we discovered anoxic conditions. It is clear that we were not measuring metrics at the location and scale experienced by larval sturgeon in the early stages of the study.

Given that free embryo pallid sturgeon drift into reservoirs (see Braaten et al. 2012); we have provided the data necessary to explain the mechanism for pallid sturgeon recruitment failure in the upper Missouri River. Prior to the fragmentation of the Missouri River by dams, pallid sturgeon free embryos would drift for hundreds of kilometers near the thalweg and settle out of the drift as they aged and could negotiate the flow. Patches of suitable habitat (low velocity with high dissolved oxygen) existed within the thalweg, mostly likely behind velocity breaks such as woody debris or underwater sand dunes. Under natural conditions, it is believed that drifting near the thalweg substrate was a mechanism to avoid predation, which evolved over millions of years (Braaten et al. 2012). In the current human-altered ecosystem, the river

enters the transition zone, velocity slows, and FPOM settles to form a flocculent that is anoxic (i.e., dead zone) because of high microbial respiration. This is also the area where free embryo pallid sturgeon prematurely settle because the needed drift distance is hindered by river fragmentation from dams (Braaten et al. 2012). Thorton (1990) previously describe the transition zone in the book title *Reservoir Limnology: Ecological Perspectives.*

Several contaminants were detected in water samples above the benchmarks defined for pallid sturgeon indicating that these analytes (Cu, Hg, TP) exist at levels high enough to affect survival of larval pallid sturgeon in certain areas of the Missouri River above Ft. Peck Reservoir. Likely sources of Cu in this region include mineral extraction and smelting. The Hg concentrations detected in the water were 1.28 to 3 times higher than the national recommended water quality criteria. The largest contributor of Hg in Montana is coal-fired power plants. The TP was detected at concentrations 4.5 to 23.7 times higher than EPA criteria (http://www.epa.gov/waterscience/criteria/nutrient/ecoregions/rivers/index.html), and likely sources of TP are from soils, application of fertilizer for agriculture, and wastewater treatment plants. These data are included in the Pallid Sturgeon Basin-Wide Contaminants Assessment. Further sampling efforts should be undertaken to assess the spatial extent at which Cu, Hg, and TP exceed the benchmarks defined for pallid sturgeon; this could be used to better determine the exposure time of pallid sturgeon to elevated contaminants and how it influences survival.

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Table 1.1. Results of contaminant levels in water samples collected in 2008 in the headwater habitat of Ft. Peck Reservoir (ND=not detected).

Sample	Sulfide (mg/L)	Nitrogen (mg/L)	Copper (ug/L)	Mercury (ug/L)	Phosphorus (ug/L)	Selenium (ug/L)
1	ND	ND	9	1	191	ND
2	ND	ND	11	ND	239	ND
3	ND	ND	16	2.1	389	ND
4	ND	0.5	11	ND	243	ND
5	ND	ND	11	ND	208	ND
6	ND	ND	11	1.8	227	ND
7	ND	ND	8	0.9	207	ND
8	ND	ND	7	ND	135	ND
9	ND	ND	18	ND	422	ND
10	ND	ND	6	ND	120	ND
11	ND	ND	5	ND	ND	ND
12	ND	ND	3	1	546	ND
13	ND	ND	5	ND	ND	ND
14	ND	ND	7	ND	151	ND
15	ND	ND	5	ND	ND	ND
16	ND	ND	5	ND	ND	ND
17	ND	ND	5	ND	114	ND
18	ND	ND	5	ND	103	ND
19	ND	ND	6	ND	116	ND
20	ND	ND	6	ND	120	ND

Table 1.2. Results of contaminant levels in benthic samples collected in 2008 in the headwater habitat of Ft. Peck Reservoir (ND=not detected).

Sample	Copper (ug/L)	Mercury (ug/L)	Selenium (ug/L)
1	10	ND	ND
2	11	ND	ND
3	14	ND	ND
4	10	ND	ND
5	10	ND	ND
6	21	ND	ND

Table 1.3. Unionized ammonia (mg/L) in the river and transition zone by depth (values in parentheses are 95% confidence interval). Samples were collected in 2012 in the Missouri River above Ft. Peck Reservoir and in the transition zone of Ft. Peck Reservoir.

Habitat	Depth (m)	Unionized Ammonia
River	0.15	0.020 (0.000)
River	1.7-2.2	0.017 (0.005)
River	3.1-4.3	0.010 (0.007)
Transition	0.15	0.010 (0.011)
Transition	1.7-2.2	0.000 (0.000)
Transition	3.1-4.3	0.010 (0.010)

Table 1.4. Results of dissolved oxygen, temperature, and velocity field measurements by year, location, and depth (values in parentheses are 95% confidence intervals). Samples were collected in the Missouri River above Ft. Peck Reservoir and in the transition zone of Ft. Peck Reservoir. (Results are repeated in Chapter 6).

			Percent of maximum depth		
Year	Location[a]	Variable	50	75[a]	100[b]
2012	Transition zone				
	Thalweg				
		DO	8.55 (0.17)		1.32 (1.49)
		Temperature	18.30 (0.00)		17.83 (0.32)
	River				
	Thalweg	DO	8.25 (0.37)		7.61 (1.02)
		Temperature	18.15 (0.08)		18.03 (0.11)
2013	Transition zone				
	Thalweg				
		DO	7.95 (0.07)	7.93 (0.07)	0.00 (0.00)
		Temperature	20.6 (0.3)	20.6 (0.2)	19.9 (0.2)
		Velocity	0.34 (0.04)	0.30 (0.05)	0.08 (0.06)
	Outside thalweg				
		DO	8.02 (0.06)	7.94 (0.12)	0.00 (0.00)
		Temperature	20.9 (0.6)	20.6 (0.7)	20.5 (0.7)
		Velocity	0.25 (0.12)	0.19 (0.08)	0.04 (0.07)
	River				
	Thalweg				
		DO	7.94 (0.01)	7.91 (0.05)	7.92 (0.01)
		Temperature	21.9 (0.3)	21.9 (0.3)	21.9 (0.3)
		Velocity	0.68 (0.09)	0.59 (0.07)	0.38 (0.14)
	Outside thalweg				
		DO	7.96 (0.02)	7.96 (0.02)	7.96 (0.02)
		Temperature	22.0 (0.2)	22.0 (0.2)	22.0 (0.2)
		Velocity	0.57 (0.10)	0.52 (0.10)	0.27 (0.15)

[a] No measurements were made outside the thalweg and at 75% of maximum depth in 2012.
[b] In 2013, measurements were 14 cm above the substrate given where the meter sensors were attached to the sounding weight.

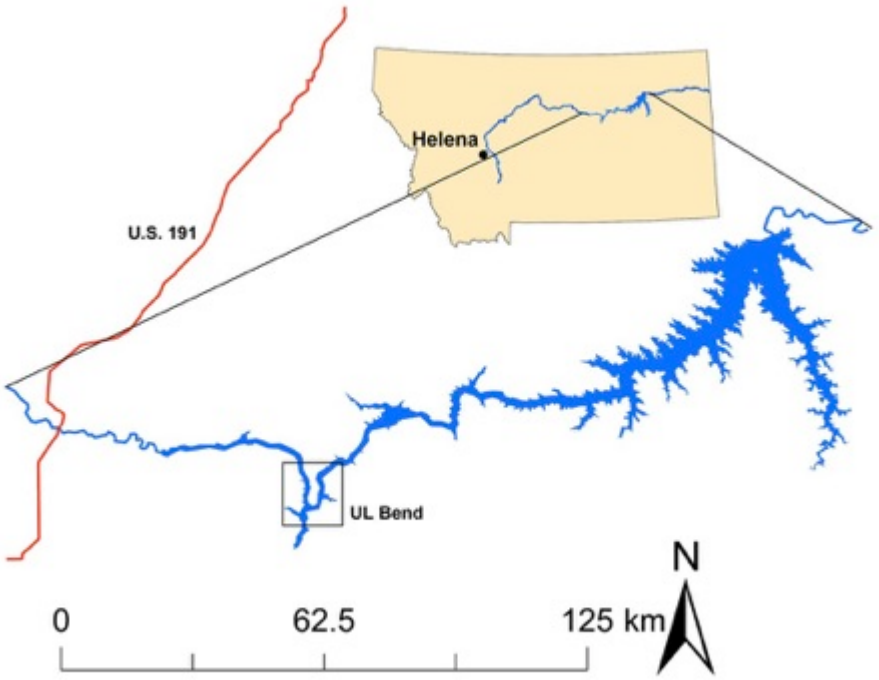

Figure 1.1. Image of Ft. Peck Reservoir in eastern Montana. For approximately 10 years the headwater habitat (or transition zone, as indicated by the box) of Ft. Peck Reservoir has been located near the UL Bend.

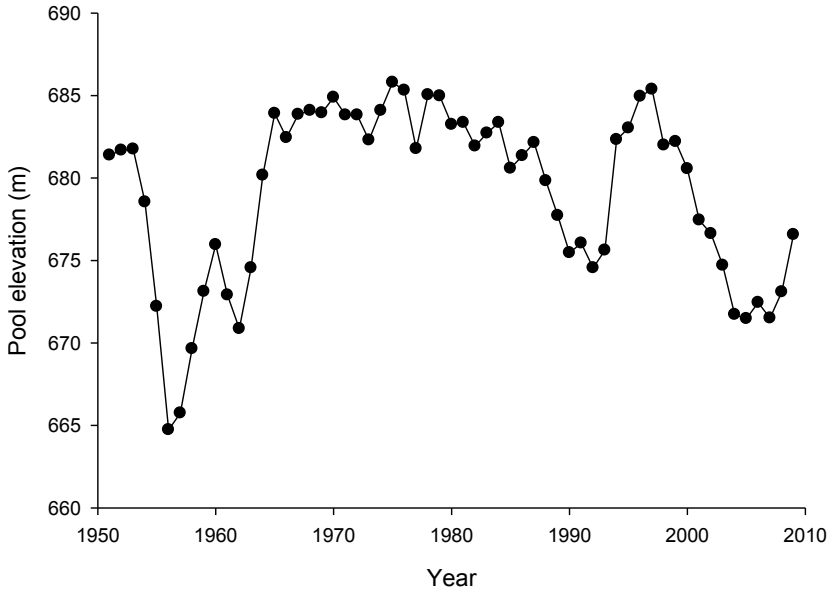

Figure 1.2. Ft. Peck Reservoir pool elevation measured annually on 30 June. Annual fluctuations have varied from 7 meters (1955 – 1956) to 0.5 meters (2004 – 2005).

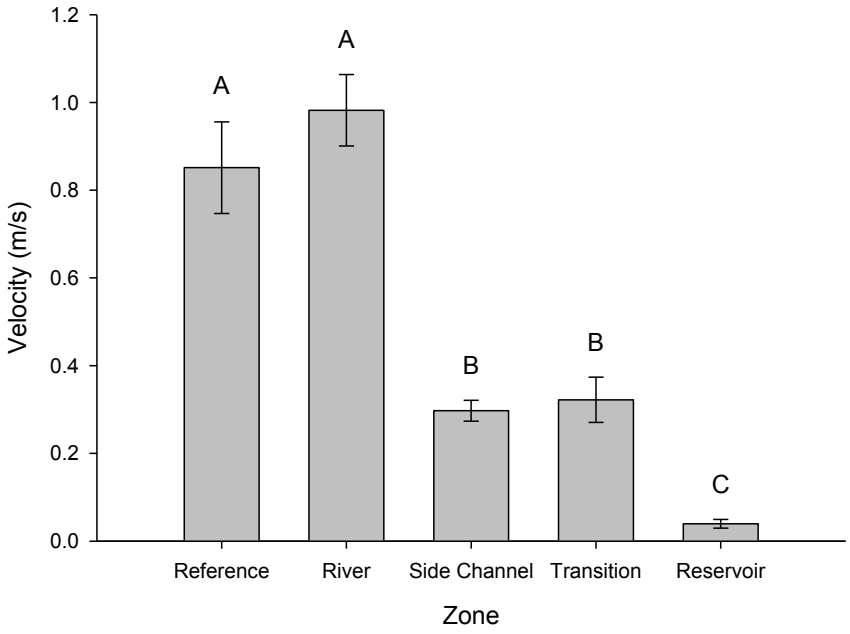

Figure 1.3. Mean velocity (m/s) by zone in the Missouri River and Ft. Peck Reservoir [unaltered Missouri River ("Reference")]. Data were collected in 2008 and 2009. Bars with different letters are significantly different.

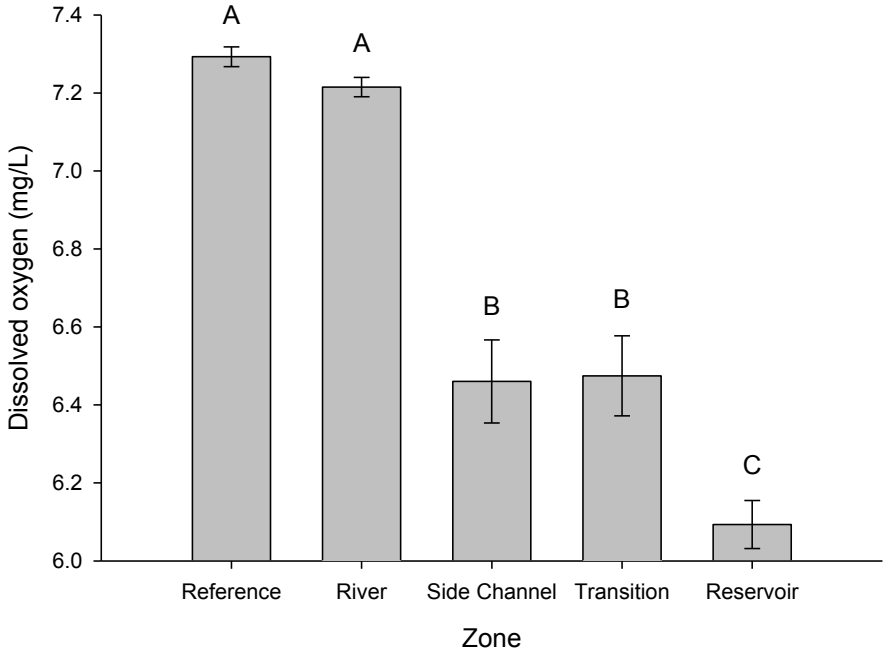

Figure 1.4. Mean dissolved oxygen (mg/L) measured at 0.5 m above the substrate by zone (samples collected in 2008 and 2009; different sampling location compared to results presented in Chapter 6). Bars with different letters are significantly different.

Chapter 2
Larval Drift in Ft. Peck Reservoir Headwater

Preface

We began our initial efforts in 2008 (see Chapter 1) with an examination of the headwater habitat, and after this initial effort we determined that a better understanding of the drift dynamics of pallid sturgeon as they enter the headwater habitat was necessary to guide our future sampling efforts. The dynamics of the headwater environment and the overall scope of the project lead us to believe we needed to refine our approach based on where pallid sturgeon drift. In order to inform our habitat assessment and sampling strategy, in 2009, prior to habitat sampling, we conducted larval drift trials with immediate post-hatch shovelnose sturgeon and pallid sturgeon larvae. We hoped the data collected as a result of our field sampling and larval drift trials would reveal where larval sturgeon drift in the headwater environment. Specifically we were interested in whether larval sturgeon remained in the thalweg as they approached the slow velocity headwater habitat or if larval sturgeon might drift into the flooded terrestrial vegetation that can be inundated. We used the information collected in the drift studies to guide the 2009, 2012, and 2013 abiotic sampling effort.

Introduction

Larval drift is an important component of the life history of many fish species. The success or failure of this initial drift phase can exert influence on future population structure and stability (Pavlov et al. 1995). A variety of factors – time of day, time of year, and habitat quality – can influence larval drift (Hay 2008; Reeves 2010), and differences in drift patterns among species and habitats can make it difficult to develop general predictions of larval drift behavior.

Knowledge of larval drift in the world's sturgeon species is important to their conservation. Lake sturgeon *Acipenser fulvescens* larvae tend to drift largely at night close to the substrate. A laboratory study on larval Sacramento River white sturgeon *Acipenser transmontanus* found that drift was brief and occurred primarily near or at the substrate (Kynard and Parker 2005).

Recent research indicates that pallid sturgeon larvae undergo an extended drift and dispersal period where downstream dispersal may persist for 8–14 days, depending on water temperature and development (Braaten et al. 2012). Braaten et al. (2012) determined that larval pallid sturgeon *Scaphirhynchus albus* require approximately ~ 200-500 km, while shovelnose sturgeon *Scaphirhynchus platorynchu*s require slightly less distance (Braaten et al. 2008). However, in the absence of these required drift distances, it is important to understand drift dynamics, especially as they enter headwater habitat (Kynard et al. 2002; Kynard et al. 2007). Our objectives were to evaluate larval drift dynamics in pallid sturgeon and shovelnose sturgeon as the entered the headwater habitat.

Methods

Shovelnose sturgeon broodstock collection and spawning. – On 4 May 2009, we collected 23 ripe female and four ripe male shovelnose sturgeon from the Missouri River downstream of Coal Banks Recreation Area, Montana (N 48.032004 W 110.235293) using 45.72-m long by 1.83-m deep drifting trammel nets. The outer mesh panel was 25.4 cm (bar measure) and the inner-mesh panel was 5.1 cm (bar measure). We drifted nets in 10-minute intervals over known spawning grounds to target spawning adults.

Fish were transported in a 1,893 L oxygenated tank to the Bozeman Fish Technology Center (BFTC) in Bozeman, Montana. Once at the BFTC, fish were held at 14° C (river temperature at capture) until we were able to sample ovarian follicles from mature females and calculate individual oocyte polarization index (PI). Polarization index is used as an indicator of spawning readiness and is the ratio of the distance of the germinal vesicle from the animal pole to the oocyte diameter (Dettlaff et al. 1993). Females that are ready to spawn will have a PI ≤ 0.07 (Dettlaff et al. 1993). Based on the calculated PIs, water temperature was used to synchronize maturation cycles so that we could spawn all fish on the same day. Nineteen females were hormonally injected to induce spawning, and, on 24 June 2009 we spawned 17 of those females via cesarean section (individuals were euthanized with an overdose of MS-222 immediately prior to spawning). We collected and fertilized 399,578 eggs, which were incubated in McDonald hatching jars at 16° C for a period of four days until hatched.

Shovelnose sturgeon drift study. – Immediate post-hatch shovelnose sturgeon larvae were packed into 3.75-L plastic bags at a density of 10,000 larvae/bag and transported in 153-L coolers to Devils Creek Recreation Area, Charles M. Russell National Wildlife Refuge, Montana. Water temperature during transport was 16° C and oxygen was injected into each water bag at 4-h intervals to supplement DO levels. Once at the release point, transport water was tempered with Missouri River water and increased to 18° C over a period of 1 h. After this acclimation period, we released an estimated 220,000 2-day post-hatch (DPH) shovelnose sturgeon at the surface of the water in the thalweg on 1 July 2009.

Approximately 1-km downstream from the release point, three boats were anchored in varying positions across the main channel: one near a section of flooded vegetation, one in the thalweg, and one between the thalweg and shoreline. Each boat deployed paired, conical larval drift nets (750-µm mesh, 1.5-m long by 0.5-m mouth tapering to 0.9 m at the cod end). The cod end of each net contained a partially-screened, 0.754-L removable sample cup. The paired drift nets were lowered to the bottom of the water column and then pulled up ~0.3 m to increase the probability of capturing the released larvae (as per Braaten et al. 2008). The boat positioned in the thalweg also deployed larval drift nets in the middle of the water column to determine if larvae drifted above the sediment-water mixing zone.

Larval drift nets were deployed for 5-minute intervals. This amount of time prevented nets from becoming filled with river detritus and allowed for adequate larval sampling. After each 5-minute set, nets were retrieved, sample cups were removed from the nets, and the contents of the sample cup transferred to secondary container. The sample cups were then reattached to the drift nets and the nets were redeployed in the same manner as described above. The transferred contents were inspected to determine if they contained larval shovelnose sturgeon that had drifted downriver. All collected shovelnose sturgeon larvae were preserved in a 10% formalin solution. Field counts of shovelnose sturgeon larvae were confirmed in the laboratory.

After the “peak” (i.e., highest number of larval shovelnose sturgeon observed in the drift nets) had moved through the first transect, all three boats moved 1 km downstream. At the second transect (2 km below the release point), an additional two boats joined the first three for a total

of five boats sampling with paired, conical drift nets (n = 10 nets). Sampling continued similarly to the first transect with boats anchored across the channel (Figure 2.1). Sampling continued at the second transect until the peak was identified and all five boats moved an additional 1 km downstream. Sampling continued at the third transect until the peak was identified and then all five boats moved into the headwaters (~ 0.02 m/s water velocity) of Ft. Peck Reservoir. Because reservoir headwater environments have low water velocity, active collection methods are needed instead of passive collection methods. Therefore, we standardized our active towing efforts to five minutes of sampling with each boat engine operating at 1,000 revolutions/min.

Volume of water sampled was estimated using General Oceanics Digital Flowmeters (General Oceanics, Inc., Miami, Florida). Flowmeters were suspended in the mouth of each drift net, and readings were recorded prior to deploying each net and after net retrieval. The volume of water sampled (m^3) was calculated using the following equation:

$$\text{Volume}\,(\text{m}^3) = \frac{3.14 \times \text{d}^2}{4} \times \text{distance}$$

where *d* is the net diameter and distance is calculated by

$$\text{Distance}\,(\text{m}) = \frac{(\text{R}_\text{a} - \text{R}_\text{p}) \times \text{C}}{999999}$$

where R_a is the count on the flowmeter after retrieval, R_p is the count on the flowmeter prior to deployment, and *C* is the rotor constant (i.e., 26,873; General Oceanics, Inc., Miami, Florida).

Pallid sturgeon drift study. – We collected 135,000 1-DPH pallid sturgeon larvae from Garrison Dam National Fish Hatchery (Riverdale, North Dakota) on 1 July 2009 and transported them to the Devils Creek State Recreation Area in 3.78-L oxygenated bags (water temperature = 12° C). The oxygenated bags were packed in insulated 40.6-cm x 40.6-cm x 20.3-cm Styrofoam boxes. Pallid sturgeon larvae were packed at a density of 5,000 larvae/3.78 L.

We tempered the transport water with river water to a temperature of 18° C over a period of 45 min before releasing the larvae. Similar to the shovelnose sturgeon larval release, pallid sturgeon larvae were released at the surface of the Ft. Peck Reservoir headwaters (N 47.465.21 W 107.858163) on 2 July 2009. Immediately after release, boats moved 1-km downstream and deployed paired, conical larval drift nets identical to those used in the shovelnose sturgeon recapture effort from four boats (8 nets total) at the bottom of the water column (Braaten et al. 2008). All drift nets were deployed for 5 min each. Collected pallid sturgeon larvae were preserved in a 10% formalin solution. Field counts were confirmed in the laboratory. After the "peak" had moved through the first transect, all four sampling boats moved 1.5 km downstream to join a fifth sampling boat already sampling in the headwaters (low-velocity area) of the reservoir. Like with the shovelnose sturgeon release, we standardized our active towing efforts to 5 min of sampling with each boat engine operating at low speeds (engine revolutions/minute = 1,000).

Results

Shovelnose sturgeon. – All larvae were caught in nets deployed just above the substrate, and we did not recapture any larvae in nets deployed in the middle of the water column. After sampling 5,148 m^3 of water over the course of 8.9 h, we recaptured 323 larval shovelnose sturgeon. This translated to a recapture rate of 0.14% and density of 0.063 larvae per m^3 of water sampled. River discharge on 1 July 2009 was 382 m^3/s, and we first recaptured larvae 27 min post-release. The peak (n = 17) of larval drift reached the first transect 49 min post-release (Figure 2.2). At the second transect, the peak (n = 72) arrived 2.8 h post-release (Figure 2.2). The peak (n=8) reached the third transect 4.7 h post-release, and arrived in the headwaters (n = 16) 7.9 h post-release (Figure 2.2). However, we continued to catch larval shovelnose sturgeon for an additional 0.8 h in the headwaters. One set of paired larval drift nets was deployed in the reservoir (< 1 km downstream from the headwaters) the following morning, but all larvae caught (n=3) were dead.

Distribution of released larval shovelnose sturgeon across the channel was uneven (Figure 2.3). Catches of larval sturgeon were highest in the thalweg (59%), similar to previous studies (Braaten et al. 2008). Drift nets positioned on the edge of the main channel near the flooded

vegetation collected larval shovelnose sturgeon (12%), but only at the first transect. Nine percent of the shovelnose sturgeon larvae were sampled from channel border and 19% were sample in the low velocity transition zone.

Pallid sturgeon. – We recaptured 397 larval pallid sturgeon in 6,787 m^3 of water sampled, a recapture rate of 0.29%. The density of recaptured larvae was 0.06 larvae per m^3 of water sampled. Missouri River discharge on 2 July 2009 was 371 m^3/s, and we began recapturing larvae 16 min post-release. The peak (n = 11) of drifting pallid sturgeon larvae reached the first transect 1.7 h post-release (Figure 2.4). Pallid sturgeon larvae began arriving in the headwaters area 3.21 h post-release, but the peak (n = 25) was not documented until 5.65 h post-release. We continued to recapture larval pallid sturgeon for 2 h after the peak arrived in the headwaters.

Similar to shovelnose sturgeon, larval pallid sturgeon were not evenly distributed across the channel by the time they reached the first transect (Figure 2.5). At the first transect, 98.7% of the larvae were sampled in the thalweg. Drift nets positioned on the edge of the main channel sampled 1.3% of the pallid sturgeon and no pallid sturgeon were sampled in the flooded vegetation.

Discussion

The 2009 drift study accomplished two goals: 1) it provided us with needed guidance for establishing habitat sampling design criteria and specific focus areas based on the drift and dispersal patterns of larvae as they entered the headwater habitat, and 2) it provided additional support that recruitment failure is likely a function of larvae drifting into reservoirs headwater habitat (given the that dead larvae were sampled, albeit a low sample size). The headwater environment, dependent on year, consists of a channel increasing in heterogeneity and complex hydraulics, a number of side channels, a large amount of flooded vegetation, and a variety of backwaters. In 2008, while performing the first effort to study and characterize the habitat larval sturgeon might encounter as they drift into the reservoir, it became evident that a better understanding of larval drift and dispersal would be needed to guide future habitat sampling design. Though the larval drift dynamics of shovelnose sturgeon and pallid sturgeon

had been studied in laboratory and field investigations, prior to our investigation there was no information as to exactly where larvae might drift and settle in the headwater environment.

The majority of the larvae we sampled remained in the thalweg and drifted into the headwater habitat. Specifically, larvae drifted mainly in the thalweg at the lowest 0.5 m (our information corroborates Braaten et al. 2008, 2012) and did not drift into flooded terrestrial vegetation in the floodplain. This was an important finding as we had initial concerns that the flooded, heavily-vegetated habitats (including side channels, backwaters, and pools) might be entraining larval sturgeon. The nets positioned along outside river bends against the vegetation line did not collect any larval pallid sturgeon or shovelnose sturgeon after the initial release site (release occurred at the surface and likely influenced the capture location at the first site). Larval pallid sturgeon and shovelnose sturgeon were found in disproportionately large numbers near the bottom in the thalweg, which corroborates Braaten et al. (2008, 2012). Finally, we recaptured three dead larval sturgeon in the transition zone the day after the release experiment. Although the sample size was low, it supports the findings in Chapter 1, Chapter 6, and Chapter 8 that anoxic conditions and sediment substrate are the mechanisms for recruitment failure.

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Figure 2.1. Sampling for shovelnose sturgeon larvae in the Missouri River 2 km upstream from Ft. Peck Reservoir.

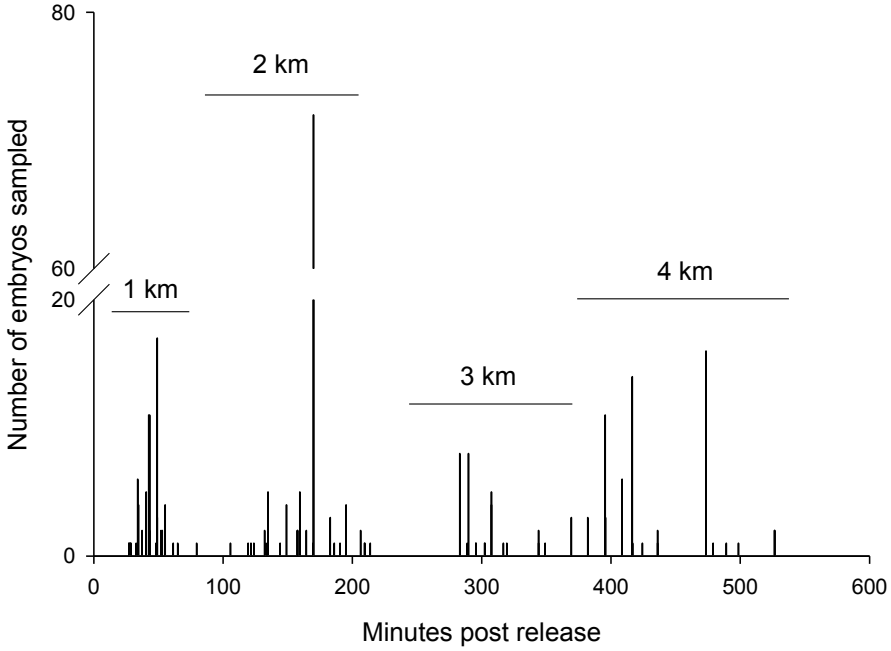

Figure 2.2. Number of larval shovelnose sturgeon recaptured by time after release. Horizontal lines represent time sampled at each transect. Value above a horizontal line represents the distance downstream from the release site.

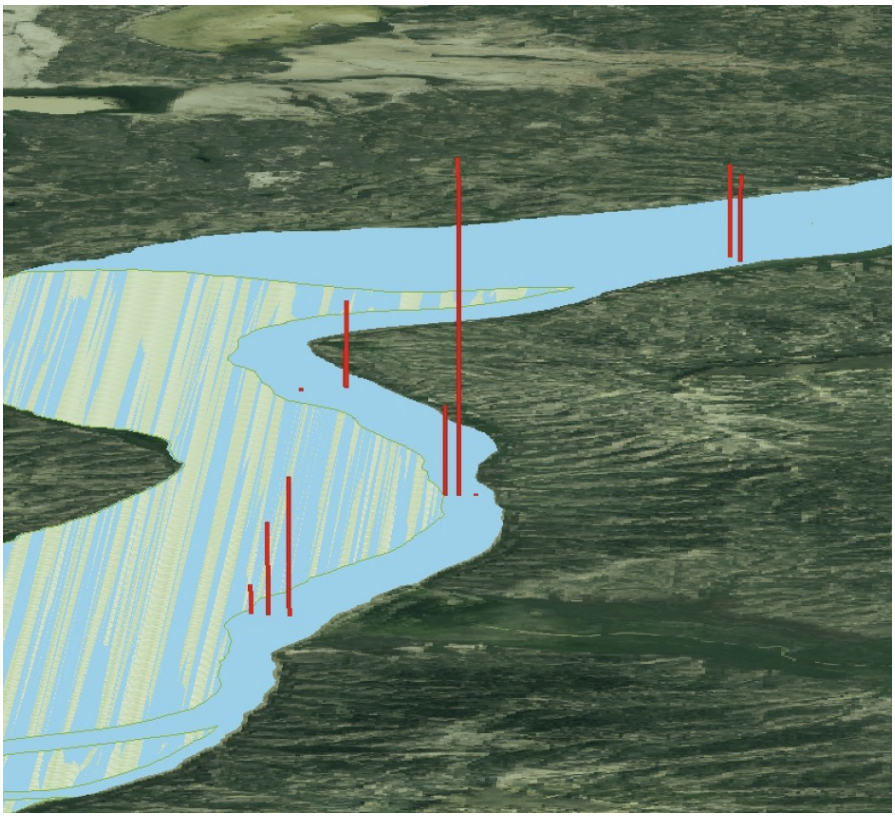

Figure 2.3. Relative distribution of shovelnose sturgeon larvae sampled during the drift experiment in the Missouri River and transition zone of Ft. Peck Reservoir in 2009. Light blue represents water and blue-yellow hashed area represents flood vegetation. Height of bar indicates relative abundance, higher the bar the more fish sampled.

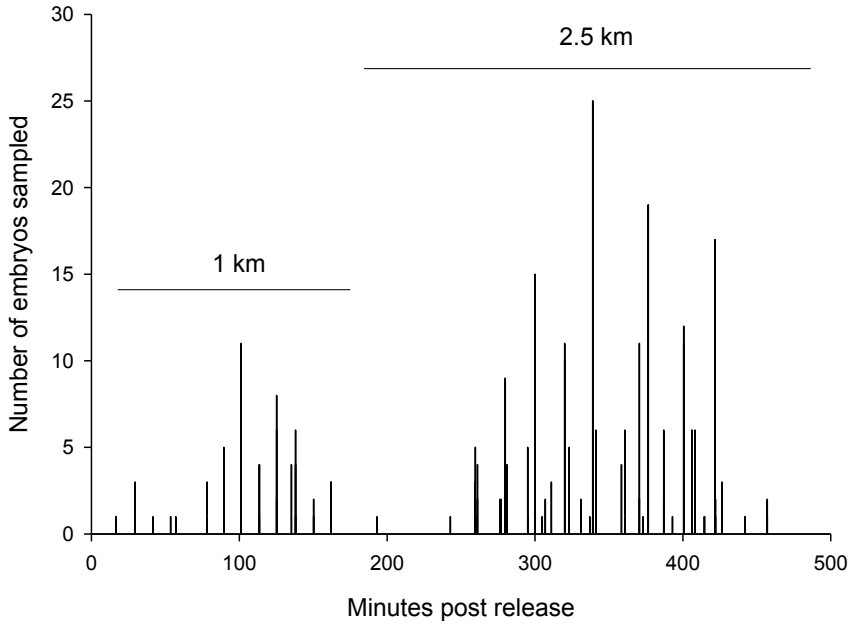

Figure 2.4. Number of larval pallid sturgeon recaptured by time after release. Horizontal lines represent time sampled at each transect. Value above a horizontal line represents the distance downstream from the release site.

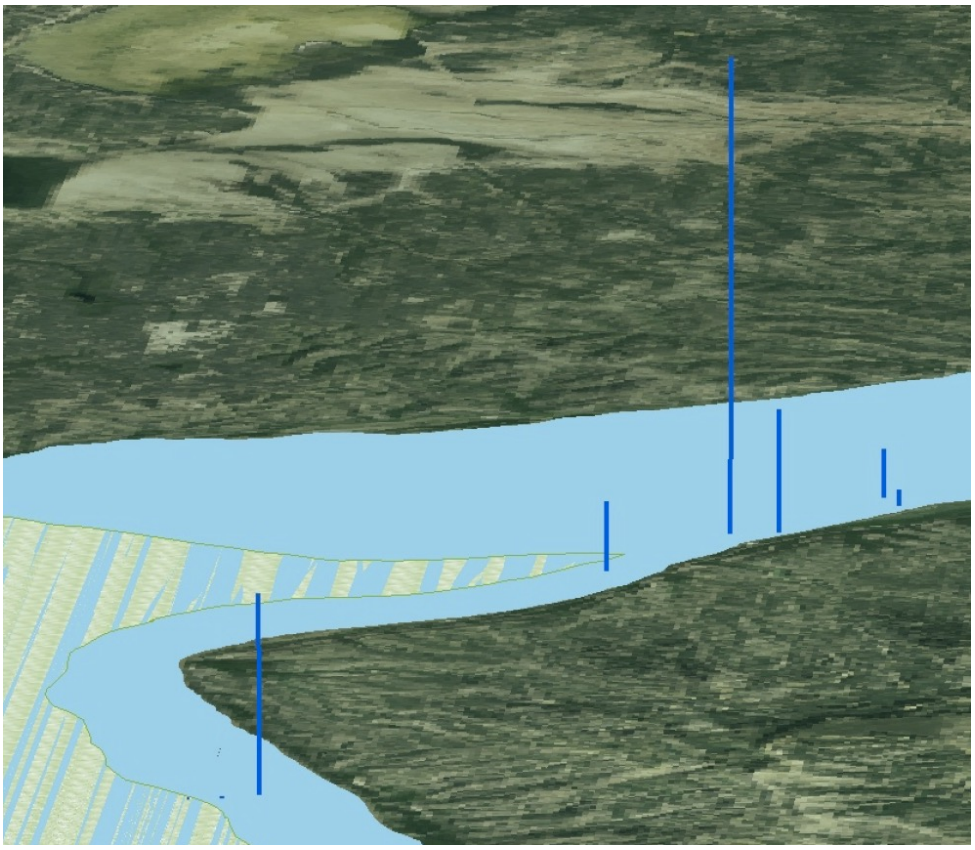

Figure 2.5. Relative distribution of pallid sturgeon larvae sampled during the drift experiment in the Missouri River and transition zone of Ft. Peck Reservoir in 2009. Light blue represents water and blue-yellow hashed area represents flood vegetation. Height of bar indicates relative abundance, higher the bar the more fish sampled.

Chapter 3
Effects of pH on Mortality

Preface

Empirical headwater pH levels varied from 5.5 to 8.6. High and low levels of pH have been shown to negatively influence fish species. Based on these data, we conducted laboratory investigations into the effects of varying pH levels on sturgeon larvae in 2010. We describe the investigations in this chapter.

Introduction

The effects of pH on mortality and growth have been studied in a number of fish species. For example, Wagner et al. (1997) documented greater mortality and increased stress response in rainbow trout *Oncorhynchus mykiss* in high pH (8.4-9.6) environments, with stress occurring faster in high water temperature (19-22 ^{0}C), high pH systems. Red drum *Sciaenops ocellatus* larvae (36 hours post hatch) demonstrated low survival (< 50%) at pH levels of 9.4; it is suggested in this same study that red drum mortality could be reduced in water with pH values less than 9.4 (Lyon and Fisher 1998). Several studies in Upper Klamath Lake, Oregon have addressed the effects of high pH on shortnose suckers *Chasmistes brevirostris* and Lost River suckers *Deltistes laxatus*. While Bellerud and Saiki (1995) found 96-hour LC_{50} values of 10.45 (larval Lost River suckers), 9.92 (juvenile Lost River suckers), 10.33 (larval shortnose suckers), and 9.85 (juvenile shortnose suckers), more recent studies demonstrated little to no effect of high pH on growth, survival, or respiratory ability (Lease 2003; Foott 2006).

Much of the literature with regard to effects of pH on sturgeon species suggests no significant individual responses within pH values varying from 6.5 to 7.5. Jian-Yi et al. (2006) found that Chinese sturgeon *Acipenser sinensis* embryos and larvae increased their rate of oxygen consumption as pH increased from 5.5 to 7.0, but decreased oxygen consumption as pH increased from 7.0-9.0; the optimal pH range was determined to be between 6.5 and 7.5. In a more recent study, Cope et al. (2011) determined that survival of shortnose sturgeon *Acipenser brevirostrum* was not affected by water conditions that included a pH range of 6.9-

7.3. However, there are no data on the effects of pH on larval pallid sturgeon or shovelnose sturgeon. Our objectives were to determine if the range of pH levels similar to what we observed in the headwater habitat (5.5-8.6) negatively influenced survival and growth of pallid sturgeon and shovelnose sturgeon. Additionally, we wanted to determine if fluctuations in pH level negatively influenced survival and growth.

Methods

Shovelnose Sturgeon Broodstock Collection, Spawning, and Incubation

In early May 2010, we collected ripe females and male shovelnose sturgeon from the Missouri River downstream of Coal Banks Recreation Area, MT (N 48.032004 W 110.235293) using 45.72-m long by 1.83-m deep drifting trammel nets. The outer mesh panel was 25.4 cm (bar measure) and the inner-mesh panel was 5.1 cm (bar measure). We drifted nets in 10-minute intervals over known spawning grounds to target spawning adults. We confirmed sexual maturity of collected shovelnose sturgeon by taking gonadal biopsies from each fish.

We transported fish in a 1,893 L oxygenated tank to the Bozeman Fish Technology Center (BFTC). Once there, fish were held at 14° C until we were able to sample oocytes from mature females and calculate individual oocyte polarization index (PI). Polarization index is used as an indicator of spawning readiness, and is defined as the ratio of the distance of the germinal vesicle from the animal pole to the oocyte diameter(Dettlaff et al. 1993). Females that are ready to spawn will have a PI ≤ 0.07 (Dettlaff et al. 1993). Based on individual PI, we assigned each female to a potential spawning date and manipulated water temperature to entrain spawning readiness.

To induce ovulation, each female was injected with 10% (priming dose) of a total dose of Leutinizing Hormone Releasing Hormone (LHRH) at a volume of 20 µg/kg body weight followed 12 hours later with the remaining 90% (resolving dose) of the dose. At the time females were administered their priming dose, males were injected with LHRH [20 µg/kg body weight]. We collected milt from males through catheterization 24 hours prior to expected female ovulation. When females began ovulating, we collected released oocytes via hand-

stripping, fertilized them with milt (5 ml of milt to 1000 ml of ambient-temperature hatchery water), and stirred the mixture for two min. We drained the milt-water mixture to prevent polyspermy and then added an aqueous solution of Fuller's Earth to de-adhese the embryos. De-adhesion prevents fertilized embryos from clumping together during the incubation process. After de-adhesion, the Fuller's Earth solution was replaced with BFTC water, and the embryos were transferred into McDonald hatching jars. Embryos were incubated at 18-20 °C for up to 6 days, and dead eggs were removed from the hatching jar. After final hatch was reached, larvae were collected with a 1-L beaker and randomly transferred into test tanks.

Pallid Sturgeon Embryo Shipments and Incubation

We received pallid sturgeon embryos from the Miles City State Fish Hatchery, Miles City, Montana and Gavins Point National Fish Hatchery, Yankton, South Dakota. Embryos were transported to the BFTC via FedEx in 18.9-L plastic bags, and water in the bags was tempered by floating each bag in a tank filled with BFTC water. After tempering, each group of pallid sturgeon embryos was immersed in an Ovadine-water solution and then transferred into a McDonald hatching jar for incubation. Incubation procedures continued identical to the shovelnose sturgeon incubation. After final hatch was reached, larvae were collected with 1-L beaker and randomly transferred into test tanks.

Dose Response Experiments

We transferred 140 individual immediate post-hatch pallid sturgeon and shovelnose sturgeon larvae into each of 30 different McDonald hatching jars (hereafter referred to as tanks), separated by species. For each species, tanks were randomly assigned to one of five pH treatment levels: 6.5, 7, 7.5, 7.8, and control (pH = 8). Exposures lasted for six days (day 0 – 6) and each treatment level was replicated three times. We controlled pH level in each tank by dripping a 1% stock solution of sulfuric acid (H_2SO_4) into five different 18.9 L head tanks. Each head tank was randomly assigned to one of the five treatment levels and filled each of its replicate tanks at a rate of 5 L/min. Water from the BFTC was directed into each head tank and the assigned pH level was achieved by controlling the flow of the 1% H_2SO_4 solution with

a 19.05 mm PVC ball valve over each head tank. Overflow water was routed into a drain and was not reused.

We collected data on mortality during each trial. Mortalities were counted and recorded. On day 6, the total number of individuals that remained alive were counted and recorded. Percent survival was calculated by dividing the total number alive at the end of the experiment by the total number of larvae initially put in each tank.

Diel Fluctuation Experiments

We transferred 50 individual IPH pallid sturgeon and shovelnose sturgeon larvae into each of 30 different tanks, separated by species. Tanks were randomly assigned to one of five treatment levels for both species, and each treatment level was replicated three times. Diel fluctuation treatments for the pH experiment were designed to mimic natural fluctuations between 0900 and 2100 hours. Treatment levels for pH were also controlled identically to the dose response experiments as described above, except we adjusted pH levels in each tank at 500, 700, 900, 1300, 1700, 2100, and 2300 hours. Target pH values at time (h) for each treatment level are shown in Table 3.1, with target fluctuations varying from 8.0 to 8.0 (control), 7.8 to 8.0, 7.5 to 8.0, 7.0 to 8.0, and 6.5 to 8.0. We collected data mortality for each species similar to static trials.

Statistical Analyses

All analyses were conducted using the statistical software R (Version 2.13.1). Data were tested to determine if the assumptions of normality were met, and, when data did not meet these assumptions, a transformation of the data was performed and analyses conducted on the transformed data. Tank effects were tested for in all analyses of laboratory data.

Average mortality (± 95% Confidence Interval) at each treatment level was determined for each species at each age group. Treatment, age, sampling day, and species/family effects were assessed with a factorial ANOVA, with the three afore-mentioned factors as the

categorical predictor variables and mortality as the continuous response. Tukey tests were used to determine differences between treatment levels for mortality.

Results

Dose Response Experiments

Mortality

There was no effect of pH level on larval mortality ($P > 0.05$; Figure 3.1). Shovelnose sturgeon larvae had significantly lower mortality among pH levels than pallid sturgeon larvae ($P < 0.00$).

Diel Fluctuation Experiments

Mortality

We were unable to conduct statistical analyses on the diel pH mortality data. A substantial number of larvae escaped from the experimental tanks, and, as a result, we were unable to account for their survival. The data that was successfully collected can be found in Table 3.2.

Discussion

The pH levels we used did not influence larval mortality. Recorded pH values in the headwaters of the Fort Peck reservoir in summer of 2008 varied from 7.21 – 8.64 (average pH of 8.30 +/- 0.23). The low end of this range, most likely caused by the emptying of a side pool into the main channel, is well within the range of pH values found not to negatively impact sturgeon (Jian-Yi 2006; Cope et al. 2011). The high end of this range is slightly greater than the high end of the range of pH values in the sturgeon studies discussed above. Based on the empirical data collected in the headwater habitat of Ft. Peck, pH levels likely do not negatively impact larval shovelnose sturgeon and pallid sturgeon.

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Table 3.1. Target pH levels in each treatment for diel-fluctuation trials.

	Time (h)												
Treatment Level	**2100**	**2300**	**100**	**300**	**500**	**700**	**900**	**1100**	**1300**	**1500**	**1700**	**1900**	**2100**
Control	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
7.8	8.0	7.97	7.94	7.91	7.88	7.85	7.82	7.85	7.88	7.91	7.94	7.97	8.0
7.5	8.0	7.92	7.83	7.75	7.67	7.59	7.50	7.59	7.67	7.75	7.83	7.92	8.0
7	8.0	7.84	7.68	7.52	7.36	7.20	7.04	7.20	7.36	7.52	7.68	7.84	8.0
6.5	8.0	7.75	7.50	7.25	7.0	6.75	6.50	6.75	7.0	7.25	7.50	7.75	8.0

Table 3.2. Mortality data collected during diel pH fluctuation trials in 2010. The "NA" denotes larvae for which we could not account on a given sampling day.

Tank Number	Treatment Level	Sampling Day	Number of Mortalities	Number of Survivors
1	7.5	2	3	NA
3	7.5	2	3	NA
5	7.5	2	0	NA
1	6.5	2	0	NA
3	6.5	2	0	NA
5	6.5	2	2	NA
5	7.85	2	5	NA
3	7	2	0	NA
5	7	2	0	NA
6	7	2	0	NA
2	8	2	0	NA
3	8	2	2	NA
4	8	2	4	NA
1	7.5	4	1	NA
3	7.5	4	5	NA
5	7.5	4	1	NA
1	6.5	4	2	NA
3	6.5	4	4	NA
5	6.5	4	0	NA
5	7.85	4	16	NA
3	7	4	0	NA
5	7	4	0	NA
6	7	4	NA	NA
2	8	4	0	NA
3	8	4	2	NA
4	8	4	9	NA
1	7.5	6	1	11
3	7.5	6	2	12
5	7.5	6	NA	23
1	6.5	6	NA	NA
3	6.5	6	11	5
5	6.5	6	3	13
5	7.85	6	4	1
3	7	6	2	15
5	7	6	NA	16
6	7	6	NA	NA
2	8	6	1	18
3	8	6	1	16
4	8	6	NA	4

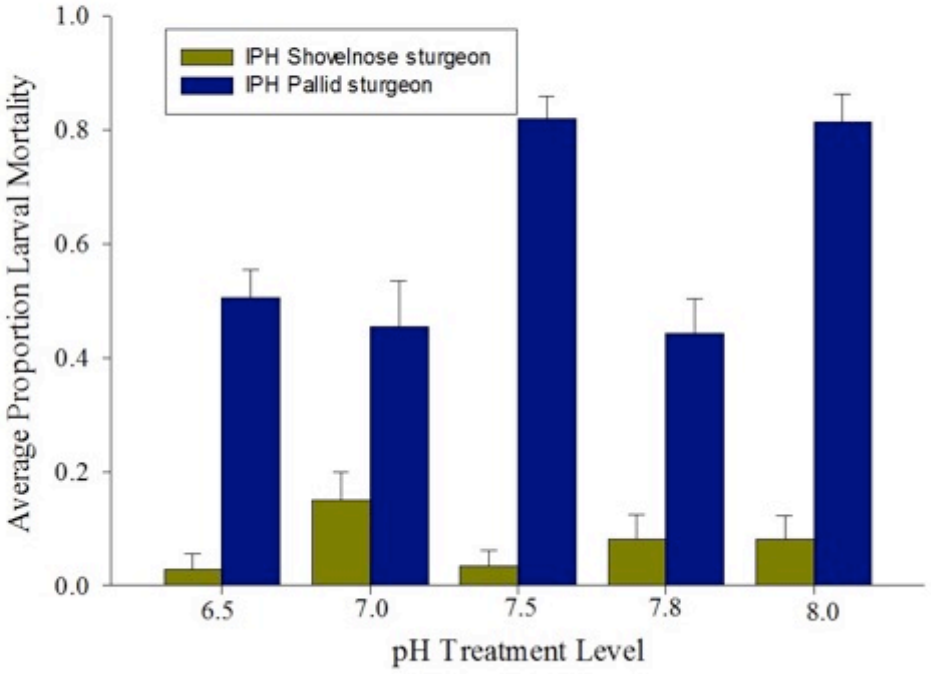

Figure 3.1. Average proportion mortality of immediate post-hatch shovelnose sturgeon and pallid sturgeon larvae in the static pH trials. Control treatment had a pH of 8.0.

Chapter 4
Effects of Dissolved Oxygen on Growth and Mortality

Preface

The water-quality examination of the headwater habitat in 2008 and 2009 revealed levels of oxygen in the reservoir varying from 1.5 mg/L to 8.6 mg/L. The low values observed in the headwater habitat represent hypoxic conditions that were significantly different enough from the river habitat as to warrant investigation. In 2011, we conducted laboratory investigations to determine the effect of varying dissolved oxygen levels (1.5 mg/L to 7.0 mg/L) on larval sturgeon. The results of the investigations are discussed in this chapter.

Introduction

Hypoxic conditions can have varying effects on fish. In juveniles and adults, the physiological response to hypoxia consists of increased ventilation and bradycardia, which serve to increase gas exchange and reduce damage to cardiac muscle (Taylor et al. 2010). The results of these changes can, in turn, negatively affect multiple processes. Under conditions of less than 2 mg/L O_2, juvenile yellow perch *Perca flavescens* in Lake Erie, Michigan, experienced diminished growth and decreased feeding rates (Roberts et al. 2011). Similarly, juvenile shortnose sucker *Chasmistes brevirostrum* and Lost River sucker *Deltistes luxatus* experienced limited growth under hypoxic conditions and high water temperatures (Terwilliger et al. 2003). Hypoxic conditions can also cause fish to be more susceptible to the negative effects of other abiotic factors. For example, the synergistic effects of high un-ionized ammonia levels and low dissolved oxygen concentration (1.65 mg/L) greatly increased mortality of juvenile dourado *Salminus brasiliensis* (Serafini et al. 2009).

The effects of hypoxia on sturgeon embryos have been well documented. Dissolved oxygen levels of 4-5 mg/L resulted in slowed development and an increase in developmental abnormalities in *Acipenser gueldenstaedtii* and *Acipenser persicus* embryos (Dettlaff et al. 1993). In this same study, 100% of embryos died under conditions of 3-4 mg/L dissolved oxygen (Dettlaff et al. 1993). Studies on the effects of hypoxia on several species of juvenile

sturgeon have demonstrated similar results (Jenkins et al. 1995; Cambell and Goodman 2004; Niklitschek and Secor 2009).

These studies provide important information on the effects of hypoxia on fishes at the juvenile and embryonic stages, but there is currently a knowledge gap on the dissolved oxygen tolerance of larval pallid sturgeon. Additionally, shovelnose sturgeon are a sympatric species that recruit in the upper Missouri River. It may be plausible that shovelnose sturgeon larvae have a different tolerance to dissolved oxygen than pallid sturgeon. The objective of this study was to address these information gaps by determining the effects of different dissolved-oxygen conditions on the growth and mortality of larval shovelnose sturgeon and pallid sturgeon under laboratory conditions. The knowledge gained from the experiments will provide insight into the effects of a reservoir headwater environment on larval pallid sturgeon recruitment failure, which is important to the overall conservation goal of the species.

Methods

Shovelnose Sturgeon Broodstock Collection, Spawning, and Incubation

In early May 2011, we collected ripe female and male shovelnose sturgeon from the Missouri River downstream of Coal Banks Recreation Area, Montana (N 48.032004 W 110.235293) using 45.72-m long by 1.83-m deep drifting trammel nets. The outer mesh panel was 25.4 cm (bar measure) and the inner-mesh panel was 5.1 cm (bar measure). We drifted nets in 10-minute intervals over known spawning grounds to target spawning adults. We confirmed sexual maturity of collected shovelnose sturgeon by taking gonadal biopsies from each fish.

We transported fish in a 1,893 L oxygenated tank to the BFTC. Once there, fish were held at 14° C until we were able to sample oocytes from mature females and calculate individual oocyte polarization index (PI). Polarization index is used as an indicator of spawning readiness, and is the ratio of the distance of the germinal vesicle from the animal pole to the oocyte diameter (Dettlaff et al. 1993). Females that are ready to spawn will have a PI ≤ 0.07 (Dettlaff et al. 1993). Based on individual PI, we assigned each female to a potential spawning date and manipulated water temperature to entrain spawning readiness.

To induce ovulation, each female was injected with 10% (priming dose) of a total dose of Leutinizing Hormone Releasing Hormone (LHRH) at a volume of 20 µg/kg body weight followed 12 hours later with the remaining 90% (resolving dose) of the dose. At the time females were administered their priming dose, males were injected with LHRH [20 µg/kg body weight]. We collected milt from males through catheterization 24 hour prior to expected female ovulation. When females began ovulating, we collected released oocytes via hand-stripping, fertilized them with milt (5 ml of milt to 1000 ml of ambient-temperature hatchery water), and stirred the mixture for two min. We drained the milt-water mixture to prevent polyspermy and then added an aqueous solution of Fuller's Earth to de-adhese the embryos. De-adhesion prevents fertilized embryos from clumping together during the incubation process. After de-adhesion, the Fuller's Earth solution was replaced with BFTC water and the embryos were transferred into McDonald hatching jars. Embryos were incubated at 18-20 °C for up to 6 days, and dead eggs were removed from the hatching jar. After final hatch was reached, larvae were collected with 1-L beaker and randomly transferred into test tanks.

Pallid Sturgeon Embryo Shipments and Incubation

We received pallid sturgeon embryos from the Miles City State Fish Hatchery, Miles City, Montana and Gavins Point National Fish Hatchery, Yankton, South Dakota. Embryos were transported to the BFTC via FedEx in 18.9 L plastic bags, and water in the bags was tempered by floating each bag in a tank filled with BFTC water. After tempering, each group of pallid sturgeon embryos was immersed in an Ovadine-water solution and then transferred into a McDonald hatching jar for incubation. Incubation procedures continued identical to the shovelnose sturgeon incubation. After final hatch was reached, larvae were collected with 1-L beaker and randomly transferred into test tanks.

Experimental Design

All trials were conducted at the Bozeman Fish Technology Center (BFTC), Bozeman, Montana. Desired dissolved oxygen (DO) concentration levels were achieved using a gas-stripping column design similar to Mount (1964) (Figure 4.1). Oxygen was removed from water

in the column with a JB Eliminator vacuum pump (Model Number DV-6E; JB Industries, Inc., Aurora, IL, USA) to achieve a minimum DO level of 1.5 mg/L. Deoxygenated water was then distributed to each of the plastic McDonald-type hatching jars (tanks) at a rate of 1 L/min with a J-Plus jet pump (Model Number J15; Goulds Pumps, ITT, Seneca Falls, NY, USA). This translates to a tank turnover of 3 min. Based on the assigned treatment level, the deoxygenated water was mixed with untreated (i.e., oxygen rich; 7 mg/L) water from the BFTC to maintain the needed DO level in each tank. We monitored DO levels each day of a trial with a YSI model 55/12 FT handheld DO meter (YSI, Inc., Yellow Springs, Ohio).

Exposure trials were designed as a 2 x 2 x 3 factorial experiment in which larvae (two species) at each of two ages – immediate post hatch (IPH) and 40-day post hatch (DPH) – were exposed to three treatments levels based on the 2008 and 2009 empirical data – 1.5 mg/L, 2.5 mg/L, and 7.0 mg/L (control) – for a period of 6 (IPH) or 5 (40-DPH) days. At the start of each trial, experimental tanks were randomly assigned to one of the three treatment levels, with each treatment level replicated three times, thus 18 tanks per trial (Figure 4.2). Larvae were collected in a 1-L glass beaker from the 1.83-m (diameter) round holding tanks and randomly assigned them to experimental tanks. One hundred IPH larvae and 20 individual 40-DPH larvae were distributed to each tank. In the case of the 40-DPH pallid sturgeon, there were 10 larvae per tank. Shovelnose sturgeon larvae came from two distinct genetic families, with each family replicated three times per treatment level. Pallid sturgeon larvae came from one family lot. Water temperature in the tanks varied between 18-20° C.

We measured the effects of our treatment levels on larval growth and mortality in the following ways. Larval growth for each species was assessed by comparing pre-trial larval lengths to post-trial lengths of surviving larvae from each replicate. Immediately prior to beginning a trial, we randomly selected larvae from their holding tanks, euthanized them with an overdose of tricaine methanesulfonate (MS-222), and preserved them in 10% phosphate-buffered formalin. At the end of a trial, selected larvae were similarly euthanized and preserved. Larval length was measured to the nearest 0.01 mm using a dissecting scope with image analysis capabilities. To prevent biasing variance in sample measurements, the same proportion of individuals was measured for IPH and 40-DPH trials. Larval mortality was determined by

calculating the difference between number of living larvae in each tank at the end of the trial and the initial number in each tank.

Statistical Analyses

Average length (± SE) by treatment level was calculated for each species and age group. Differences in length among treatment groups and species were determined for each age group using a factorial analysis of variance (ANOVA), with each treatment group and species as the categorical predictor variables and length as the continuous response. Larval behavior was assessed visually by graphing location across time for each species at each age group. Average mortality (± 95% confidence interval) at each treatment level was determined for each species at each age group. Treatment, age, and species effects were assessed with a factorial ANOVA, with the three afore-mentioned factors as the categorical predictor variables and mortality as the continuous response. The first analysis assessed the effects of species and treatment on mortality at the IPH group; the second assessed the effects of age, species, and treatment on mortality. Tukey's honest significance tests were used to determine differences between levels of significant predictor variables in both the length and mortality analyses. All analyses were conducted using the statistical software R (Version 2.13.1).

Results

Growth

Growth for IPH larvae was negatively affected by low dissolved oxygen concentration (Figure 4.3). Treatment and species each had significant effects on larval growth (treatment: $P < 0.01$; species: $P = 0.00$), and there was a significant interaction ($P < 0.01$). There was no difference in length between pre-treatment shovelnose sturgeon and pallid sturgeon ($P = 0.14$). Post-treatment IPH larvae of each species were longer than pre-treatment individuals in all treatment groups (all P-values < 0.05). Our results demonstrated significant differences between treatments in length of IPH larvae of both species (Figure 4.3). Immediate post-hatch shovelnose sturgeon larvae in the control treatment were longer than larvae exposed to either 1.5 mg/L or 2.5 mg/L (both P-values = 0.00), but there was no difference in length between larvae exposed to 1.5 mg/L and 2.5 mg/L ($P = 0.79$). Immediate post-hatch pallid sturgeon

larvae in the 2.5 mg/L treatment were significantly shorter than larvae in the control group (P = 0.00).

There was no effect of treatment on length between species in 40-DPH larvae (P = 0.96; Figure 4.4), but there was an effect of species on length (P = 0.00). Specifically, 40-DPH shovelnose sturgeon larvae were longer than pallid sturgeon larvae in the control group (P = 0.04).

Mortality

Larval survival was negatively influenced by low dissolved oxygen concentration (Figure 4.5). Specifically, there was a significant treatment effect at 1.5 mg/L on larval mortality between species and ages (P < 0.01). There was no effect at 2.5 mg/L compared to the control (7 mg/L). Treatment was a significant factor (P < 0.01). Additionally, we documented a significant interaction among treatment, species, and age (i.e., treatment x age x species) (P = 0.00). The interaction was a function of differences between species by age at 2.5 mg/L. For the 2.5 mg/L treatment, pallid sturgeon at 40-DPH had higher mortality than IPH (P = 0.00; Figure 4.5), whereas there was no difference between ages for shovelnose sturgeon. At the 40-DPH age group, there was a significant effect of species on mortality in fish exposed to the 2.5 mg/L treatment, with shovelnose sturgeon having lower mortality than pallid sturgeon (P = 0.00; Figure 4.5).

Discussion

Larval mortality at 1.5 mg/L for all species at each age was never lower than 83% for the 6-day period. By contrast, with the exception of 40-DPH pallid sturgeon, mortality of larvae between species and ages was never greater than 36% at the 2.5 mg/L treatment. This suggests that these species have a minimum oxygen threshold for survival near 2.5 mg/L for short-term survival. Interestingly, 40-DPH shovelnose sturgeon larvae experienced lower mortality than 40-DPH pallid sturgeon larvae at the 2.5 mg/L treatment.

Shovelnose sturgeon and pallid sturgeon larvae exhibited diminished growth under hypoxic conditions. We believe that the length data, in the context of the mortality results, are indicative of the trade-off organisms must make between allocating resources toward maintenance (i.e., survival) and growth (Roff 1992; Stearns 1992; Cech and Crocker 2002). In both species at the youngest age group, individuals exposed to 2.5 mg/L experienced mortality that was not statistically different than individuals in the control group, but growth in the 2.5 mg/L treatment group was significantly lower than the control. Additionally, surviving shovelnose sturgeon larvae exposed to 1.5 mg/L experienced similar growth as did individuals in the 2.5 mg/L group but had higher mortality. Larvae in the 2.5 mg/L and, to a lesser extent, 1.5 mg/L (shovelnose sturgeon only) treatments grew slower, potentially contributing to their survival. Similar results (i.e., suppression of growth at low DO) have been documented in Atlantic sturgeon (Crocker and Cech 2002; Niklitschek and Secor 2009). Despite the fact that IPH larvae appear to have the ability to survive hypoxic conditions, the resulting decrease in body size may compromise long-term survival by affecting immunological function, gape size, predator avoidance, and other metabolic, physiological, and behavioral processes (Norris and Evans 2000; Niklitschek and Secor 2009; Sparkman and Palacios 2009).

Overall, there did not appear to be species differences in tolerance to hypoxic conditions. Although our results demonstrated two instances of statistically relevant differences in length or mortality between species, it is unlikely that these differences are biologically significant. It is difficult to infer the reasons for these two cases, but further research could more conclusively determine whether this trend is biologically relevant or an artifact of the high variability associated with larval experiments.

The relevance of these data is most apparent in the context of the Ft. Peck Reservoir headwater environment. As the empirical data collected in the Ft. Peck Reservoir headwater in 2008 and 2009 showed that dissolved oxygen concentrations in headwater habitat can drop below the minimum threshold for survival of drifting larvae observed in these experiments. Furthermore, the observations in 2012 and 2013 showed anoxic conditions at the substrate-water interface (see Chapter 6), which indicate that DO is problematic for larval sturgeon in the transition zone.

We can make some inferences about larval ability to withstand or physically avoid hypoxic conditions. At approximately 18° C, immediate post-hatch *Acipenser* spp. larvae respire cutaneously and through a complex of blood vessels on the yolk sac (Dettlaff et al. 1993). The transition to gill respiration occurs at stage 41, though the timing of this is temperature dependent; at 18° C, this stage is reached 5 days after hatching (Dettlaff et al. 1993). Based on calculated drift duration for pallid sturgeon (11-15 DPH) and shovelnose sturgeon (4-6 DPH) (Braaten et al. 2008; 2012), larvae may drift into the Ft. Peck Reservoir headwater immediately before, during, or after the onset of gill respiration. As demonstrated by our mortality data, hypoxic conditions (i.e., < 2.5 mg/L) have the same effects on cutaneously respiring larvae as on gill-breathing larvae (i.e., 40-DPH age group), indicating that the degree of gill development does not affect the ability of larvae to withstand hypoxia. Structurally, initial components of fin ray supports develop within the first week (shovelnose sturgeon) to 10 d (pallid sturgeon) after hatching (at 18° C), but final development of fin rays is not completed until at most 35 (shovelnose sturgeon) to 93 days (pallid sturgeon) after hatching (Snyder 2002). Although juvenile and adult sturgeon are able to avoid hypoxic conditions (Niklitschek and Secor 2010), it is highly unlikely that IPH larvae drifting into the Ft. Peck Reservoir headwater would be capable of swimming out of hypoxic/anoxic areas in the headwaters.

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Figure 4.1. Gas stripping column similar to Mount (1964) used in dissolved oxygen trials.

Figure 4.2. Tank setup used in dissolved oxygen trials. A 2 x 2 x 3 factorial design was used to expose larvae (two species) to 1.5 mg/L, 2.5 mg/L, and 7.0 mg/L (control) – for a period of 6 (IPH) or 5 (40-DPH) days.

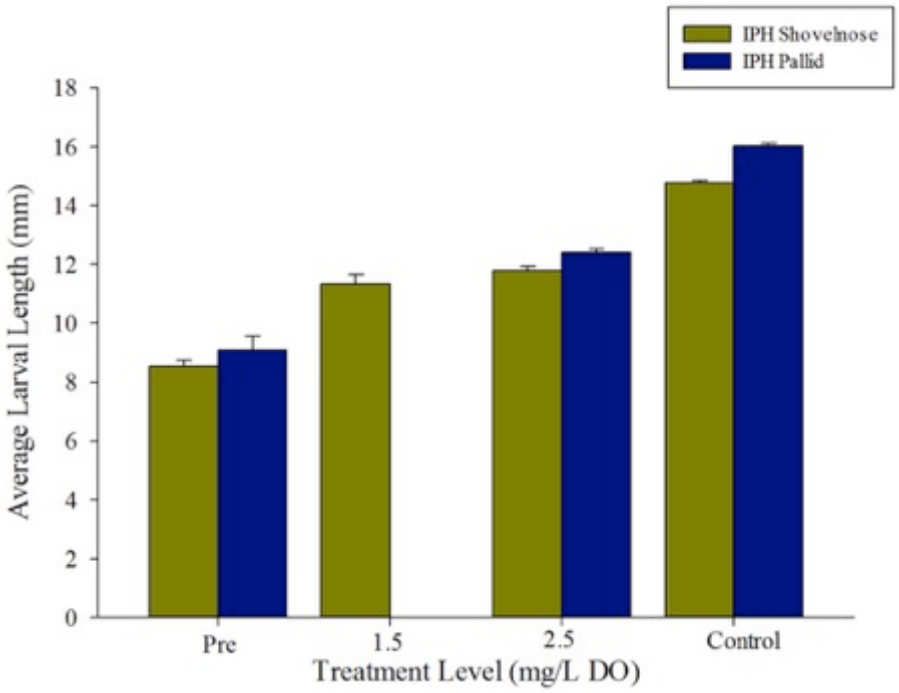


Figure 4.3. Average length (± SE) of Immediate Post-Hatch shovelnose sturgeon and pallid sturgeon in dissolved oxygen trials. The Control was 7.0 mg/L.

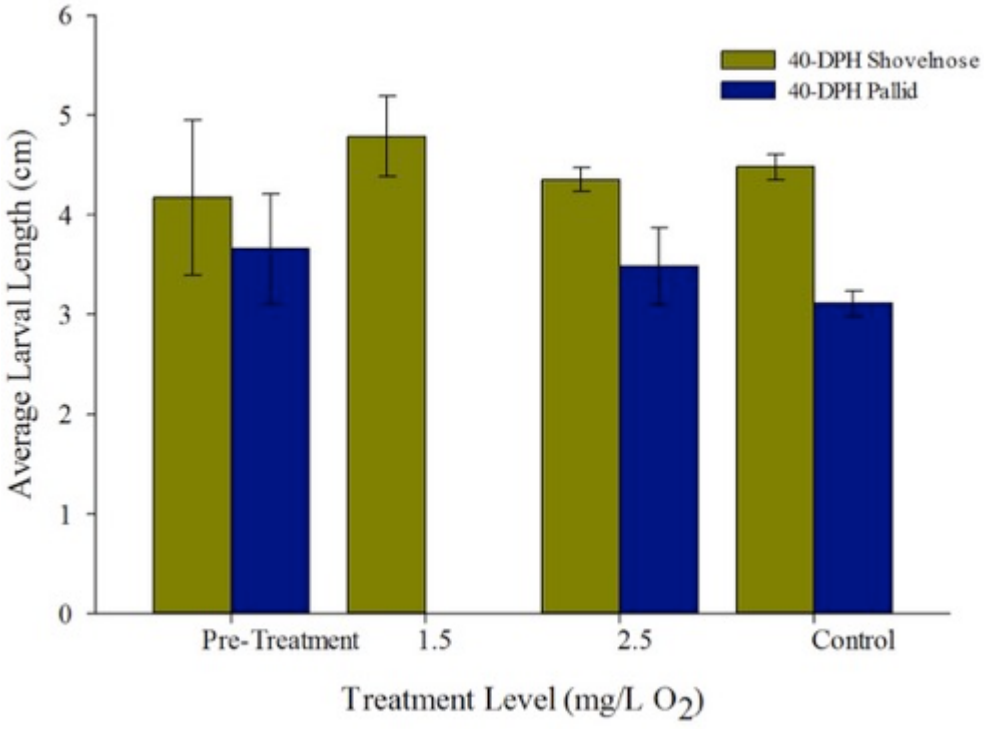
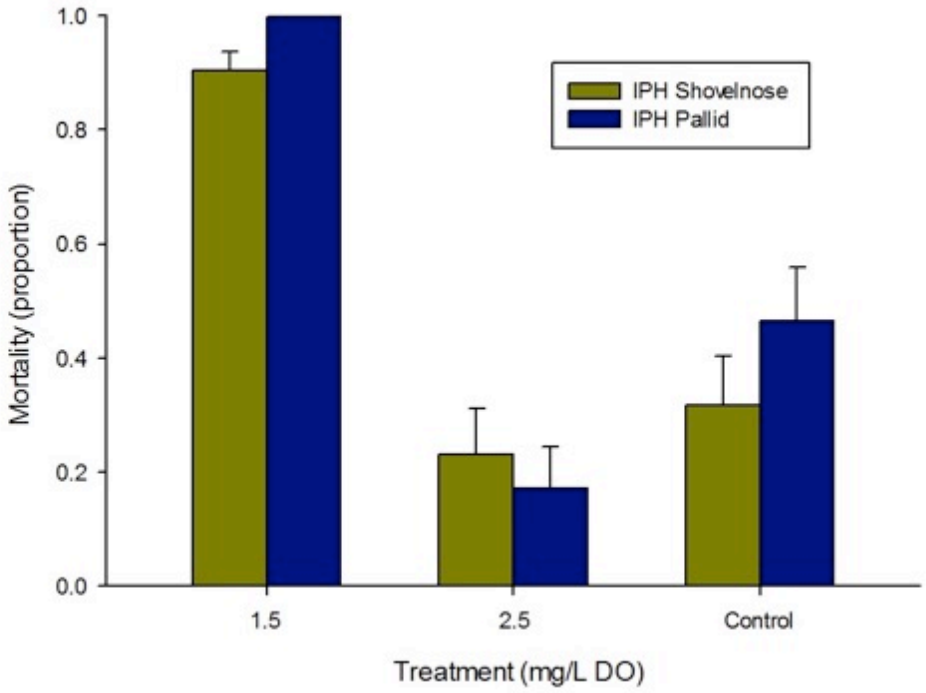


Figure 4.4. Average length (± SE) of 40-Days Post-Hatch shovelnose sturgeon and pallid sturgeon. The Control was 7.0 mg/L.

Immediate Post-Hatch



40-Days Post-Hatch

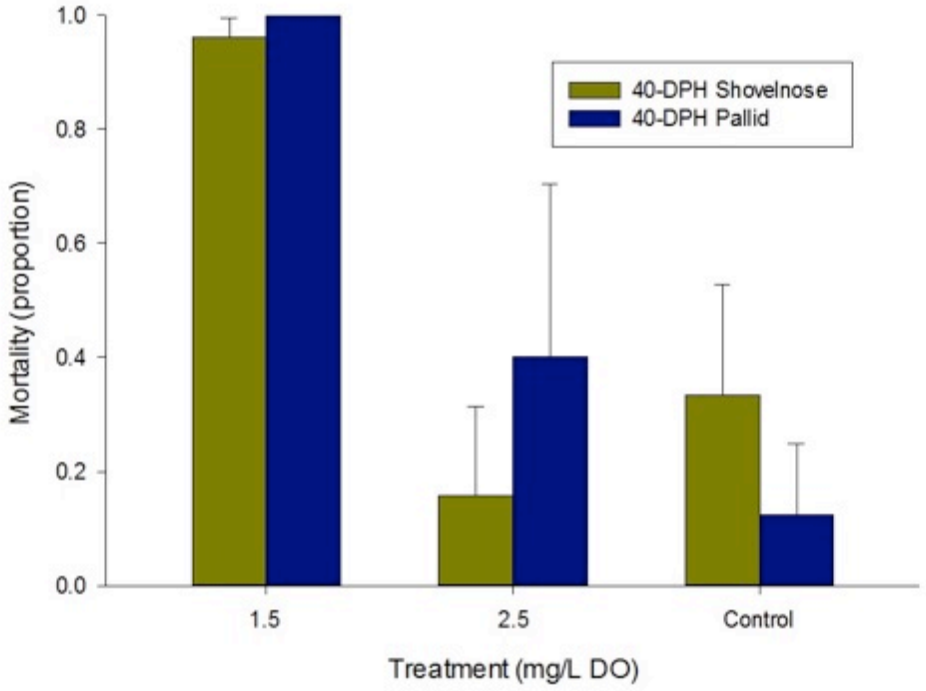


Figure 4.5. Average proportion mortality (± 95% Confidence Interval) of Immediate Post-Hatch (6-day experiment) and 40-Days Post Hatch (5-day experiment) shovelnose sturgeon and pallid sturgeon. Control treatment was 7.0 mg/L.

Chapter 5
Effects of Unionized Ammonia on Growth and Mortality

Preface

In 2011, we conducted laboratory experiments to examine the effects of substrate type on larval mortality. During these experiments, we observed high levels of unionized ammonia (UIA) in tanks containing fine particulate organic material (FPOM) from the Ft. Peck Reservoir headwater area. Ammonia is produced naturally through the decomposition of nitrogen-rich organic matter such as the substrate collected from the reservoir headwaters. We observed high mortality associated with the FPOM substrate, and hypothesized that UIA released from the FPOM might be causing the mortality. Because this substrate predominates in headwater habitat, we conducted an experiment to examine whether UIA might be the mechanism for larval pallid sturgeon mortality. We sampled for UIA in the Ft. Peck Reservoir headwater in the spring of 2011 and conducted dose response experiments described here in 2012.

Introduction

Ammonia (NH_3) is considered the most reactive form of nitrogen in lakes and streams, and is characterized by high toxicity and increased potential for retention in the environment. Ammonia is found mainly in the form of the dissociated ion NH_4^+ (ammonium). The positive charge associated with the ammonium ion is responsible for its high reactivity (in comparison to nitrate), and allows the ion to bond to clays and soil in stream substrates. Small quantities of NH_3 enter the aquatic environment because of animal excretions, and the amount of naturally occurring ammonia in a lake or stream at any given time is dependent on excretion rates (input), plant uptake (output), and bacterial oxidation (output). When dissolved in water, ammonia forms the toxic compound ammonium hydroxide (NH_4OH), which in turn dissociates to produce ammonium and hydroxyl ions (OH^-):

$$NH_3 + H_2O \leftrightarrow \mathbf{NH_4OH} \leftrightarrow NH_4^+ + OH^-.$$

The toxicity of ammonium hydroxide is affected by many environmental factors, such as pH, water temperature, DO, species, age, and time of day. For example, the amount of NH_4OH is low under acidic conditions, but increases in alkalinity push the reaction towards greater amounts of unionized ammonia, or ammonium hydroxide + NH_3 (dissolved)[1], and, therefore, higher toxicity.[2]

As mentioned previously, ammonia toxicity in fish is affected by a number of different environmental and physiological conditions. For every unit increase in pH and, to an extent, temperature, the percentage of unionized ammonia increases approximately by a magnitude of 10 (Burkhalter and Kaya 1977). At hypoxic conditions of ≤ 3.5 mg/L, susceptibility to ammonia toxicity increases, resulting in disruption of enzyme production (Kaizer et al. 2009), diminished growth, and physiological alteration (Magaud et al. 1997; Remen et al. 2008). Physiologically, fish are more susceptible to ammonia toxicity under conditions of stress (e.g., when they are not fed; Randall and Tsui 2002; Eddy 2005).

The lethal concentration of unionized ammonia in fish has been well-documented in controlled laboratory studies for a number of species and at varying life stages. For example, rainbow trout embryos and yolk-sac larvae exposed to unionized ammonia concentrations varying from 0.05 to 0.3 mg/L experienced slowed development (embryos), developmental abnormalities, and high mortality (Burkhalter and Kaya 1977). Laboratory trials conducted with adult Colorado pikeminnow *Ptychocheilus lucius*, razorback sucker *Xyrauchen texanus*, and fathead minnow *Pimephales promelas* resulted in mortality at concentrations of 0.14 to 0.31 mg/L unionized ammonia (Fairchild et al. 2002). A study on channel catfish *Ictalurus punctatus* found 96-h LC1 (lowest concentration for 1% of test population) values of 0.26 mg/L and 0.15 mg/L in 1 day post-hatch and 7 day post-hatch larvae, respectively; surviving individuals experienced lower growth rates as the concentration of unionized ammonia increased (Bader and Grizzle 1992). Finally, the lethal concentration of unionized ammonia in juvenile shortnose sturgeon *Acipenser brevirostrum* was determined to be 0.58 mg/L (Fontenot et al. 1998).

[1] Although unionized ammonia is comprised of NH_4OH and dissolved NH_3, the toxicity comes from the amount of NH_4OH; this is ultimately what is reported when discussing the percent of unionized ammonia in a sample at a given pH and temperature.
[2] Paragraph information from Horne and Goldman (1994).

Preliminary data from our 2011 substrate laboratory trials suggest that unionized ammonia levels of ≥ 0.05 mg/L may be connected to high mortality of immediate post-hatch pallid sturgeon and shovelnose sturgeon, thus this research was conducted to determine the effects of UIA on shovelnose sturgeon and pallid sturgeon (no results are presented for pallid sturgeon—poor hatching success resulted in too few pallid sturgeon to reliably conduct the experiments).

Methods

Shovelnose Sturgeon Broodstock Collection, Spawning, and Incubation

In early May of 2012, we collected ripe female and male shovelnose sturgeon from the Missouri River downstream of Coal Banks Recreation Area, MT (N 48.032004 W 110.235293) using 45.72-m long by 1.83-m deep drifting trammel nets. The outer mesh panel was 25.4 cm (bar measure) and the inner-mesh panel was 5.1 cm (bar measure). We drifted nets in 10-minute intervals over known spawning grounds to target spawning adults. We confirmed sexual maturity of collected shovelnose sturgeon by taking gonadal biopsies from each fish.

We transported fish in a 1,893 L oxygenated tank to the BFTC. Once there, fish were held at 14° C until we were able to sample oocytes from mature females and calculate individual polarization index (PI). Polarization index is used as an indicator of spawning readiness, and is the ratio of the distance of the germinal vesicle from the animal pole to the oocyte diameter (Dettlaff et al. 1993). Females that are ready to spawn will have a PI ≤ 0.07 (Dettlaff et al. 1993). Based on individual PI, we assigned each female to a potential spawning date and manipulated water temperature to entrain spawning readiness.

To induce ovulation, each female was injected with 10% (priming dose) of a total dose of Leutinizing Hormone Releasing Hormone (LHRH) at a volume of 20 µg/kg body weight followed 12 hours later with the remaining 90% (resolving dose) of the dose. At the time females were administered their priming dose, males were injected with LHRH (20 µg/kg body weight). We collected milt from males through catheterization 24 hours prior to expected female ovulation. When females began ovulating, we collected released oocytes via hand-

stripping, fertilized them with milt (5 ml of milt to 1000 ml of ambient-temperature hatchery water), and stirred the mixture for two min. We drained the milt-water mixture to prevent polyspermy and then added an aqueous solution of Fuller's Earth to de-adhese the embryos. De-adhesion prevents fertilized embryos from clumping together during the incubation process. After de-adhesion, the Fuller's Earth solution was replaced with BFTC water and the embryos were transferred into McDonald hatching jars. Embryos were incubated at 18-20 °C for up to 6 days, and dead eggs were removed from the hatching jar. After final hatch was reached, larvae were collected with a 1-L beaker and randomly transferred into test tanks.

Pallid Sturgeon Embryo Shipments and Incubation

We received pallid sturgeon embryos from the Miles City State Fish Hatchery, Miles City, Montana and the Gavins Point National Fish Hatchery, Yankton, South Dakota. Embryos were transported to the BFTC via FedEx in 18.9 L plastic bags, and water in the bags was tempered by floating each bag in a tank filled with BFTC water. After tempering, each group of pallid sturgeon embryos was immersed in an Ovadine-water solution and then transferred into a McDonald hatching jar for incubation. Incubation procedures continued identical to the shovelnose sturgeon incubation, but resulted in poor survival and too few pallid sturgeon to conduct experiements.

Exposure Trials

Exposure trials addressing the effects of unionized ammonia concentration were conducted as a 96-h static-renewal experiment in which 100 individual IPH shovelnose sturgeon larvae and 10 individual 40-DPH shovelnose sturgeon larvae were exposed in 1-L glass beakers to the following treatment levels: 0 mg/L (control), 0.01 mg/L, 0.05 mg/L, 0.10 mg/L, 0.12 mg/L, 0.14 mg/L, 0.16 mg/L, 0.18 mg/L, and 0.20 mg/L unionized ammonia (Figure 5.1). Desired unionized ammonia concentrations were achieved by titrating ammonium hydroxide into 1 L of deionized water, and each treatment level was replicated three times. We conducted an 80% daily water exchange to prevent water quality issues and minimize dissipation of ammonium hydroxide. Beakers were held in a water bath so that water temperature was maintained between 18-20° C. Larvae came from one distinct genetic family.

Growth for each species was assessed by comparing pre-trial larval lengths and weights to post-trial lengths and weights from each replicate. Immediately prior to beginning a trial, we randomly selected larvae from their holding tanks, euthanized them with an overdose of tricaine methanesulfonate (MS-222), and preserved them in 10% phosphate-buffered formalin. At the end of a trial, selected larvae were similarly euthanized and preserved. Larval length was measured to the nearest 0.01 mm using a dissecting scope with image analysis capabilities. Larval weight was measured to the nearest 0.01 g using a digital scale. To prevent biasing variance in sample measurements, the same proportion of individuals was measured for IPH and 40-DPH trials. Larval mortality was determined by recording the number of living larvae in each tank at the end of the trial and subtracting that from the initial population in each tank.

Statistical Analyses

All analyses were conducted using the statistical software R (Version 2.13.1). Data were tested to determine if the assumptions of normality were met, and, when data did not meet these assumptions, a transformation of the data was performed and analyses conducted on the transformed data. Tank effects were tested for in all analyses.

Average length and weight (± SE) at each treatment level were calculated for each species at each age group and treatment level. Differences in length and weight among treatment groups, sampling day, and species-family were determined using a factorial analysis of variance (ANOVA), with each treatment group, sampling day, and species/family as the categorical predictor variables and length as the continuous response. Average mortality (± 95% confidence interval) at each treatment level was determined for by age group. Treatment, age, sampling day, and species-family effects were assessed with a factorial ANOVA, with the three aforementioned factors as the categorical predictor variables and mortality as the continuous response. Tukey tests were used to determine differences among treatment levels for length, weight, and mortality.

Results

Growth

Pre-treatment shovelnose sturgeon larvae were significantly shorter and weighed less than post-treatment larvae (all *P*-values < 0.05), but there were no differences in larval length or weight among ammonia treatments (all *P*-values > 0.05). At the 40-DPH age group, there was no significant effect of treatment on larval length or weight (all *P*-values > 0.05).

Mortality

Treatment and age had significant effects on larval shovelnose sturgeon mortality (treatment: $P = 0.00$; age: $P < 0.00$), and there was a significant interaction between the two factors ($P < 0.00$; Figure 5.2). Immediate post-hatch larvae in the 0.12, 0.14, 0.16, 0.18, 0.20, and 0.22 mg/L treatment groups had greater mortality than 40-DPH larvae exposed to the same treatments (*P*-values < 0.00). At the IPH age group, larvae exposed to 0.01 mg/L unionized ammonia experienced lower mortality than larvae in the 0.14, 0.16, 0.18, 0.20, and 0.22 mg/L treatments (*P*-values < 0.05). Similarly, IPH larvae exposed to 0.05 mg/L experienced significantly lower mortality than larvae in the 0.12, 0.14, 0.16, 0.18, 0.20, and 0.22 mg/L treatments (*P*-values < 0.05).

Discussion

Immediate post-hatch shovelnose sturgeon experienced higher mortality over varying unionized ammonia concentrations than the 40-DPH group. This potentially indicates that a high ammonia event in a headwater environment could have a greater influence on survival at an earlier age. Mortality in the lowest treatment levels of 0.01 mg/L and 0.05 mg/L was significantly lower than mortality in treatment levels above 0.12 mg/L. This could demonstrate a level of tolerance for low levels of unionized ammonia. Interestingly, there were no significant effects of treatment level on larval growth. While reductions in growth have been associated with unionized ammonia exposure in other fish species (Bader and Grizzle 1992), it

could be the case that shovelnose sturgeon are not susceptible to the sub-lethal effects of unionized ammonia at the tested concentrations.

During empirical data collection in the headwater in 2012 (after this study), unionized ammonia levels collected in sediment and water were never measured above 0.02 mg/L. While it is likely that unionized ammonia levels never consistently reach concentrations demonstrated to be toxic in our experiments, pulses brought about by increases in organic debris during spring run-off could create conditions harmful to larval sturgeon. Additionally, changes in water quality over time could result in increased unionized ammonia levels in the headwater. Specifically, increases in water temperature or pH could result in the release of unionized ammonia bound in soil and sediment. These trials addressed unionized ammonia toxicity under normal water-quality conditions, but did not investigate the effects of unionized ammonia under extreme scenarios, such as hypoxia. Hypoxic conditions have been associated with higher susceptibility to the negative effects of unionized ammonia exposure in multiple studies of fish (Magaud et al. 1997; Remen et al. 2008; Kaizer et al. 2009; Serafini et al. 2009). Despite these findings we believe that hypoxic and anoxic conditions in the headwater environment at the mechanism for recruitment failure for pallid sturgeon in the upper Missouri River basin.

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Figure 5.1. Glass beakers used in the 2012 ammonia trials.

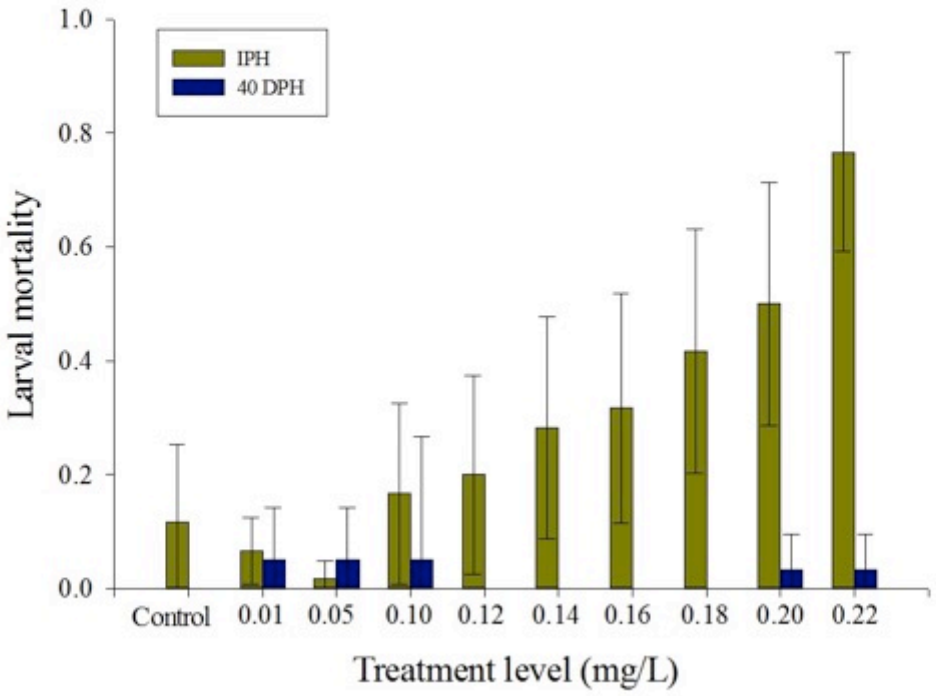

Figure 5.2. Average proportion mortality (± 95% confidence interval) of immediate post-hatch and 40-days post hatch shovelnose sturgeon larvae exposed to varying levels of unionized ammonia. Control treatment was 0.00 mg/L.

Chapter 6
Anoxic Conditions in the Transition Zone (Fisheries Article)

Preface

In 2011, we returned to the Ft. Peck headwater (also termed transition zone) to sample ammonia (see Chapter 5 for explanation). A van Dorn sampler was used to sample water near the water-substrate interface for ammonia. During the ammonia sampling, it was quickly discovered that the water at this location was anoxic. Thus, unlike the methods presented in Chapter 1, these samples were collected within 14 cm of the substrate (i.e., the water samples collected in 2008 and 2009 were approximately 500 cm above the substrate). Here we report the results of the 2012 and 2013 dissolved oxygen sampling and provide an explanation for pallid sturgeon recruitment failure. (Note: This chapter was submitted to *Fisheries* for publication and is similar to the final paper published in *Fisheries* 40:6-14).

Introduction

The proliferation of dams within the last century has been a response to human population growth in order to provide services such as flood control, hydropower, irrigation, water supply, navigation, and recreation. For example, dams account for 12-16% of the world's food production and generate 19% of the world's electricity—approximately a third of the countries in the world obtain 50% of their electricity from hydropower (WCD 2000). It is estimated that 60% of the world's rivers have been affected by dams and diversions (WCD 2000) with the contemporary number of reservoirs estimated at 16.7 million > 0.01 km^2 (2,249 km^3) and 2,094 > 10 km^2 (5,820 km^3) (Lehner et al. 2011). Dams influence more than 40% of the global discharge (Vörösmarty et al. 2003) and fragment the ecological integrity of river ecosystems (Nilsson et al. 2005). Given human population growth projections and global climate change, dam construction will continue, especially in developing countries because the most appropriate dam sites have already been exploited in developed countries (WCD 2000).

Undoubtedly, dams provide benefits to human development, but these benefits come at a cost to the natural environment.

Unfortunately, large-river fishes, such as species in the Order Acipenseriformes (Sturgeon and Paddlefish) live in regions of the world where rivers are highly regulated (Billard and Lecointre 2001; Nilsson et al. 2005; Lehner et al. 2011). Because of dams and overharvest (the primary mechanisms), 26 of the 27 species in the Order Acipenseriformes are listed as vulnerable, endangered, or critically endangered (Billard and Lecointre 2001; Lorke and Yew 2005) (also see International Union for Conservation of Nature (IUCN) Red List of Threatened Species at www.iucnredlist.org). Contemporary conservation of Acipenseriformes has focused on conservation propagation and altering dam discharge to simulate natural flow and temperature regimes (Billard and Lecointre 2001).

Elucidating the effects of dams on downriver habitat and aquatic biota has been an area of active science (e.g., Poff et al. 1997; Bunn and Arthington 2002; Poff et al. 2010) because it has been suggested that flow is the key variable that influences the patterns and processes observed in rivers (e.g., Power et al. 1995; Bunn and Arthington 2002). Furthermore, Poff et al. (2010) states “It is now widely accepted that a naturally variable regime of flow, rather than just a minimum low flow, is required to sustain freshwater ecosystems … and this understanding has contributed to the implementation of environmental flow management on thousands of river kilometres worldwide …” Given that much of the focus has been on downriver responses to flow management, less is understood regarding the upriver effects of dams on aquatic biota, especially on recruitment of large-river fishes such as the Pallid Sturgeon *Scaphirhynchus albus*.

The pallid sturgeon is endemic to the Missouri and Mississippi rivers and fossilized ancestors of the contemporary *Scaphirhynchus* spp. date 75-80 million years before the present (Grande and Hilton 2006; Grande and Hilton 2009; U.S. Fish and Wildlife Service 2014); however, within approximately two human generations the Pallid Sturgeon distribution and abundance has been reduced such that it was listed as endangered by the USA in 1990 (U.S. Fish and Wildlife Service 2014). It is estimated that less than 175 naturally produced adult Pallid

Sturgeon (i.e., heritage fish) live in the free flowing Missouri River above Lake Sakakawea (hereafter termed upper Missouri River which also includes the Missouri River above Fort Peck Reservoir) (U.S. Fish and Wildlife Service 2014). An important causal factor for the population reduction in the upper Missouri River is the lack of survival in naturally-produced pallid sturgeon (hereafter termed recruitment). After spawning, eggs hatch and the free embryo pallid sturgeon drift for long distances (approximately 200 to 500 km depending on water temperature and velocity), near the substrate, and in the river thalweg (Braaten et al. 2010, 2012). The transformation of the upper and middle Missouri River from a free-flowing river to one fragmented by six large mainstem dams is likely the cause for the lack or recruitment because there is not enough available drift distance for free embryos to mature and settle out of the ichythoplankton before entering reservoirs (Braaten et al. 2012). For example, the distance from the known spawning locations to the river-reservoir transition zone (hereafter-termed transition zone; an area where the lotic ecosystem transforms to a lentic ecosystem) of Lake Sakakawea at full pool was approximately 37 km (Braaten et al. 2012); similarly, the majority of adult pallid sturgeon telemetry locations were within 75 km of the transition zone above Fort Peck Reservoir (Richards 2011). Despite this knowledge, how the transition zone influences recruitment failure of Pallid Sturgeon was not understood.

Millions of US dollars are spent annually on research, conservation propagation, and habitat alterations (i.e., modification of discharge from the dams and creation of habitat) for recovery of pallid sturgeon; furthermore, it is estimated to cost approximately $239,000,000 to implement all recovery tasks (U.S. Fish and Wildlife Service 2014). The vast majority of funding is related to understanding the downriver effects of dams on pallid sturgeon; we argue that fisheries biologists, river managers, and policy makers must consider the upriver effects of dams to ensure successful recovery. We hypothesized that the upriver effects of dams (i.e., reservoirs and the associated transition zone) are as equally detrimental to the continued existence of many large-river species as the downriver effects. Thus, we focused our research efforts on the transition zone as an ecological sink for pallid sturgeon. Here we present the first evidence, via field measurements and laboratory experiments, that environmental conditions in the transition zone are the mechanism for the lack of pallid sturgeon recruitment, which underscores the importance of considering upriver effects on large-river fishes.

Methods

In 2012, a pilot study was initiated and water samples were collected at three depths (surface, 50% maximum depth, and 100% maximum depth) using a Van Dorn sampler in the river and transition zone above Fort Peck Reservoir, Montana, USA. Samples were collected on 19 and 20 June 2012 when water temperatures in the river were optimal for pallid sturgeon spawning and embryo survival (Kappenman et al. 2013). Samples were emptied into an 18.9-L plastic container and dissolved oxygen (DO), temperature, pH, and unionized ammonia were measured using an YSI Professional Plus meter. We were concerned about the dissolved oxygen measurements in 2012 because the meter would not stabilize for samples near the substrate (i.e., at low dissolved oxygen). Thus, in 2013, we used the YSI ProODO, which uses an optical sensor to measure dissolved oxygen and reduced uncertainty in our measurements.

In 2013, dissolved oxygen, water temperature, and velocity were systematically measured along transects in the river and transition zone above Fort Peck Reservoir, Montana, USA (Figure 1). Water velocity, substrate, and channel characteristics were used to delineate the river and transition zone. River was defined as having surface water velocity ≥ 0.5 m s^{-1}, sand substrate, and a river channel within the riverbanks. The transition zone was defined as having a surface water velocity ≥ 0.1 m s^{-1} and < 0.5 m s^{-1}, silt substrate, river channel not well confined, and the reach resembled a lentic environment. The transition zone habitat has been previously described by Thorton (1990). As in 2012, measurements were collected on 18 June when water temperatures in the river were optimal for pallid sturgeon spawning (Kappenman et al. 2013). Transects within each habitat type were spaced approximately 1 kilometer apart. Measurements at 50, 75, and 100% of the maximum depth were collected in the thalweg and immediately outside the thalweg on river left and right along each transect. In addition, vertical profile measurements were collected in the thalweg, and dissolved oxygen, water temperature, and velocity were measured at 0.25 m increments. All dissolved oxygen and water temperature measurements were measured using an YSI (Yellow Springs Instrument, Inc.) ProODO meter, and velocity was measured using a Marsh McBirney Flo-Mate 2000. Unlike 2012, all measurements in 2013 we collected in situ because meter sensors were attached to a sounding weight attached to the boat via cable. Measurements at the maximum depth were

14 cm above the substrate because meter sensors were attached to the hanger bar for the sounding weight. Kruskal-Wallis test was used to compare dissolved oxygen concentration between habitat types and among depths because these data were not normally distributed.

Sediment samples were collected from the surface of the benthos in the thalweg on 18 June 2013 by scraping the benthos using a 250-mL plastic bottle attached to a metal rod. Sediment was collected in the river (i.e., sand; N=5) and transition zone (i.e., silt; N=8) at the same locations as the dissolved oxygen, water temperature, and velocity measurements. Approximately 10 mL of sediment (only fine-grained) were placed in separate 50-mL screw-top centrifuge tubes and stored on ice in the dark until samples were analyzed in the lab for sediment respiration rates. Additionally, river water was collected in the same area and stored on ice in dark conditions until lab analysis. Laboratory analysis followed methods outlined by Hill et al. (2000). Sediment samples were transferred from ice to ambient conditions, set upright for the sediment to settle, and the water was decanted. Each tube was filled to the top (being careful to leave room for air bubbles) with filtered (0.7 µm pore size) river water, sealed, and incubated for at least 2 hours in ambient temperatures (mean 23.8°C) in the dark. The dissolved oxygen concentration and temperature of the filtered water were recorded before filling the samples to get an estimate of beginning water properties. Dissolved oxygen concentrations were measured using an YSI ProODO after at least 2 hours of incubation to estimate the changes of oxygen concentrations attributed to sediment respiration. Additionally, eight blanks consisting only of filtered river water with known dissolved oxygen and temperature were incubated and analyzed to account for changes in dissolved oxygen concentrations not associated with the sediment (i.e., those changes due to respiration by organisms in the filtered water). Following incubation, samples were corrected for changes unattributed to sediment respiration (by using the dissolved oxygen measurements in the blank samples), divided by sample volume, and divided by total time incubated to obtain estimates in oxygen consumption per hour (mg O_2 $hour^{-1}$).

Following respiration measurements, sediment was saved for analysis of ash free dry mass (AFDM). Sediments were transferred to aluminum drying pans and oven dried (60° C, 5 days), weighed, and combusted (600° C, 4 hours) in a muffle furnace. Following combustion,

samples were re-wetted with distilled water to rehydrate clays, dried (60° C, 5 days), and re-weighed to estimate AFDM. The percentage of organic matter in the samples was estimated by dividing AFDM by dry mass. Ash free dry mass and dry mass estimates were then used to estimate sediment respiration rates (mg O_2 g^{-1} AFDM hr^{-1}), allowing us to correct for differences in the volume of sediment among samples. Kruskal-Wallis test was used to compare sediment respiration between substrate types because these data were not normally distributed. Correlation analysis was used to evaluate the relationship between percent organic matter and oxygen consumption.

All laboratory experiments were conducted at the U.S. Fish and Wildlife Service, Bozeman Fish Technology Center (BFTC), Bozeman, Montana, USA. Desired dissolved oxygen concentrations were achieved using a gas-stripping column (Mount 1964). Oxygen was removed from water in the column with a vacuum pump to achieve a minimum dissolved oxygen level of 1.5 mg L^{-1}. The deoxygenated water was then distributed to McDonald-type hatching jars (tanks) at a rate of 1 L min^{-1}, and complete tank turnover occurred in three minutes. Based on the assigned treatment level, the deoxygenated water was mixed with untreated (i.e., oxygen rich; 7 mg L^{-1}) water from the BFTC to maintain the needed dissolved oxygen level in each tank. We monitored dissolved oxygen levels each day of an experiment with an YSI model 55/12 FT handheld dissolved oxygen meter.

Exposure experiments were designed as a 2 x 2 x 3 factorial (see Chapter 4 for shovelnose sturgeon data) in which larvae at each of two ages, free embryo and 40-day post hatch (40-DPH), were exposed to three treatments. Treatments were 1.5 mg L^{-1}, 2.5 mg L^{-1}, and 7.0 mg L^{-1} (control) and fish were exposed for a period of 6 (free embryo) or 5 (40-DPH) days. At the start of each experiment, experimental tanks were randomly assigned to one of the three treatments, with each treatment replicated three times. Larvae were collected in a 1 L glass beaker from 1.83 m (diameter) round holding tanks and randomly assigned to experimental tanks. One hundred free embryos and 20 40-DPH were distributed to each tank. In the case of the 40-DPH pallid sturgeon, there were 10 per tank. Water temperature in the tanks varied between 18-20° C. Mortality was determined by recording the number of living fish in each tank at the end of the experiment and calculating the difference from the initial number

introduced into the tank. A two-way ANOVA was used to evaluate the influence of age and dissolved oxygen treatment on mortality. All analyses were evaluated for normality and homogeneity of variances. All statistical analyses were performed using R (R Development Core Team 2012) and $\alpha = 0.05$.

Results

The transition zone was hypoxic or anoxic near the substrate in and outside the thalweg in the transition zone (Table 1). Conversely, in the river (i.e., representing natural conditions) dissolved oxygen concentrations were > 7 mg L^{-1} at all depths and lateral locations (Table 1). Dissolved oxygen differed significantly near the substrate (i.e., 100% of maximum depth) between the river and transition zone (2013; Kruskal-Wallis $\chi^2 = 24.9$, $P < 0.0001$, df = 1), but dissolved oxygen did not differ between the river and transition zone at shallower depths (2013; Kruskal-Wallis $\chi^2 = 0.33$, $P = 0.56$, df = 1 for 75% of maximum depth; Kruskal-Wallis $\chi^2 = 1.93$, $P = 0.16$, df = 1 at 50% of maximum depth). A clinograde dissolved-oxygen distribution occurred in the transition zone, whereas dissolved oxygen was homogenous among depth in the river (Figure 2). Similar to the transect data, dissolved-oxygen profiles only differed between the transition zone and river near the substrate (Figure 2). As expected, velocity was lower in the transition zone as compared to the river (Table 1 and Figure 2).

Mass-normalized microbial respiration rates were approximately four times higher in the transition zone (i.e., silt substrate) than the river (i.e., sand substrate; Figure 3a), and respiration rates differed significantly between substrate type (Kruskal-Wallis $\chi^2 = 7.7$, $P = 0.005$, df = 1). The transition zone substrate had a higher proportion of organic matter than the river substrate (Figure 3b). Furthermore, percent organic matter was significantly correlated with oxygen consumption ($P = 0.0005$, $r = 0.82$, df = 12).

In our laboratory experiments, pallid sturgeon experience 100% mortality at dissolved oxygen concentrations of 1.5 mg L^{-1} at the free embryo and 40-day post hatch life stages (Figure 4). Mortality differed significantly among dissolved oxygen treatments ($P = 0.000$, $F = 110.2$, df = 2) and there was no influence of age ($P = 0.23$, $F = 1.52$, df = 1), but there was a significant

treatment by age interaction (P = 0.000, F = 16.9, df = 2). The interaction was a function of higher mortality in the control than the 2.5 mg L^{-1} dissolved oxygen treatment for the free embryo pallid sturgeon. Nevertheless, mean mortality at the 2.5 mg L^{-1} treatment and control were half the mortality at the 1.5 mg L^{-1} treatment. Pallid sturgeon typically died within 1 hour after exposure to the 1.5 mg L^{-1} dissolved oxygen treatment, which can be considered a minimum duration for dissolved oxygen levels below 1.5 mg L^{-1}.

Discussion

Given that free embryo pallid sturgeon drift into reservoirs (see Braaten et al. 2012); we have provided the data necessary to explain the mechanism for pallid sturgeon recruitment failure in the upper Missouri River. Prior to the fragmentation of the Missouri River by dams, pallid sturgeon free embryos would drift for hundreds of kilometers near the thalweg and settle out of the drift as they aged and could negotiate the flow (Figure 5a). Patches of suitable habitat (low velocity with high dissolved oxygen) existed within the thalweg, most likely behind velocity breaks such as woody debris or underwater sand dunes (Figure 5a). Under natural conditions, it is believed that drifting near the thalweg substrate was a mechanism to avoid predation, which evolved over millions of years (Braaten et al. 2012). In the current human-altered ecosystem, the river enters the transition zone, velocity slows, and fine particulate organic matter (FPOM) settles to form a flocculent that is anoxic (i.e., dead zone) because of high microbial respiration (Figure 5b). This is also the area where free embryo pallid sturgeon prematurely settle (Figure 5b) because the needed drift distance is hindered by river fragmentation from dams (Braaten et al. 2012).

For the upper Missouri River, the FPOM that concentrates in the transition zone is naturally occurring and likely lower than historical conditions given the dominate land use and occurrence of reservoirs on several tributaries in the upper Missouri River basin. The size of the transition zone is currently unknown because we did not measure the most downriver extent because of monetary and logistic constraints. Is it possible for free embryos to drift through the transition zone? We argue that this is highly unlikely. For example, our study reach was approximately 3-km long and it would take approximately 2.5 hours for a pallid

sturgeon free embryo to drift between the first and last transects—using the mean drift velocity for pallid sturgeon free embryos calculated as 95% of mean column velocity in the thalweg from our study (see Braaten et al. (2012) for using values slightly less than full velocity). This estimate is the best-case scenario because if the free embryos were drifting near the bottom it would take approximately 10 hours using the 95% mean bottom velocity. Both drift velocity estimates would be lethal for pallid sturgeon free embryos given that we discovered 100% mortality within 1 hour of being exposed to dissolved oxygen concentrations at 1.5 mg L^{-1}.

Others have suggested that reservoirs can influence fish assemblages (e.g., Martinez et al. 1994; Matthews et al. 1994; Falke and Gido 2006), Winston et al. (1991) hypothesized that recruitment failure in Speckled Chub *Macrhybopsis aestivalis* and Plains Minnow *Hybognathus placitus* may be a function of free embryos drifting into reservoirs, U.S. Army Corps of Engineers generally described the transition zone in the Missouri River mainstem water-quality report but provided no empirical measurements (U.S. Army Corps of Engineers 2006a), and Cole and Hannan (1990) describe the likelihood of low dissolved oxygen in the transition zones—hence the "forgotten dead zone" in our title. We believe our study is unique from those listed above because we make direct links between human-induced changes in sediment transport and the subsequent effects on dissolved oxygen and the survival of an endangered species. This underscores the need for a better understanding of upriver effects of dams on aquatic biota.

The upriver effects of dams likely influence other large-river species; for example, other species in the Order Acipenseriformes exhibit high mortality when exposed to hypoxic conditions (Detlaff et al. 1993; Cambell and Goodman 2004; Niklitschek and Secor 2009). Historically, most of the species in the Order Acipenseriformes migrated long distances in rivers to complete their life-history requirements (Bemis and Kynard 1997). However, given the globalization of dam construction many species are isolated from historical spawning areas or occur in fragmented river ecosystems (Billard and Lecointre 2001). Furthermore, dams have reduced the global flux of sediment reaching the oceans by over 100 billon metric tons (Syvitski et al. 2005). We contend that sediment and anoxic conditions in transition zones are global threats to many species that evolved in large, turbid free-flowing rivers. Ecologists,

engineers, and policy makers need to broaden the regulated-river paradigm to consider upriver and downriver effects of dams equally to comprehensively mitigate altered ecosystems for the benefit of large-river fishes.

Specifically for the pallid sturgeon, it is unlikely that it can be recovered in all management units as outlined in the *Recovery Plan for the Pallid Sturgeon* (U.S. Fish and Wildlife Service 2014) without sizable modifications to how mainstem reservoir water levels are managed by the U.S. Army Corps of Engineers. Is managing reservoir water levels to increase the drift distance available for pallid sturgeon free embryos a viable management action? To achieve the needed drift distance for pallid sturgeon free embryos, reservoirs would need to be operated at a much-reduced capacity and this would likely influence the current benefits to society that dams provide as outlined in the Missouri River Mainstem Reservoir System Master Control Manual (U.S. Army Corps of Engineers 2006b). If natural resource agencies are serious about recovering the pallid sturgeon as outline in the pallid sturgeon recovery plan, then all stakeholders need to begin thoughtful discussions and take creative action regarding innovate approaches to managing Missouri River reservoirs. We argue that creative approaches are needed to conserve pallid sturgeon in the upper Missouri River and could be used as a model to benefit other large-river fishes worldwide. Change is required given our results, because simply modifying discharge from dams to reflect a more natural hydrograph is presently shortsighted in terms of large-river fish conservation.

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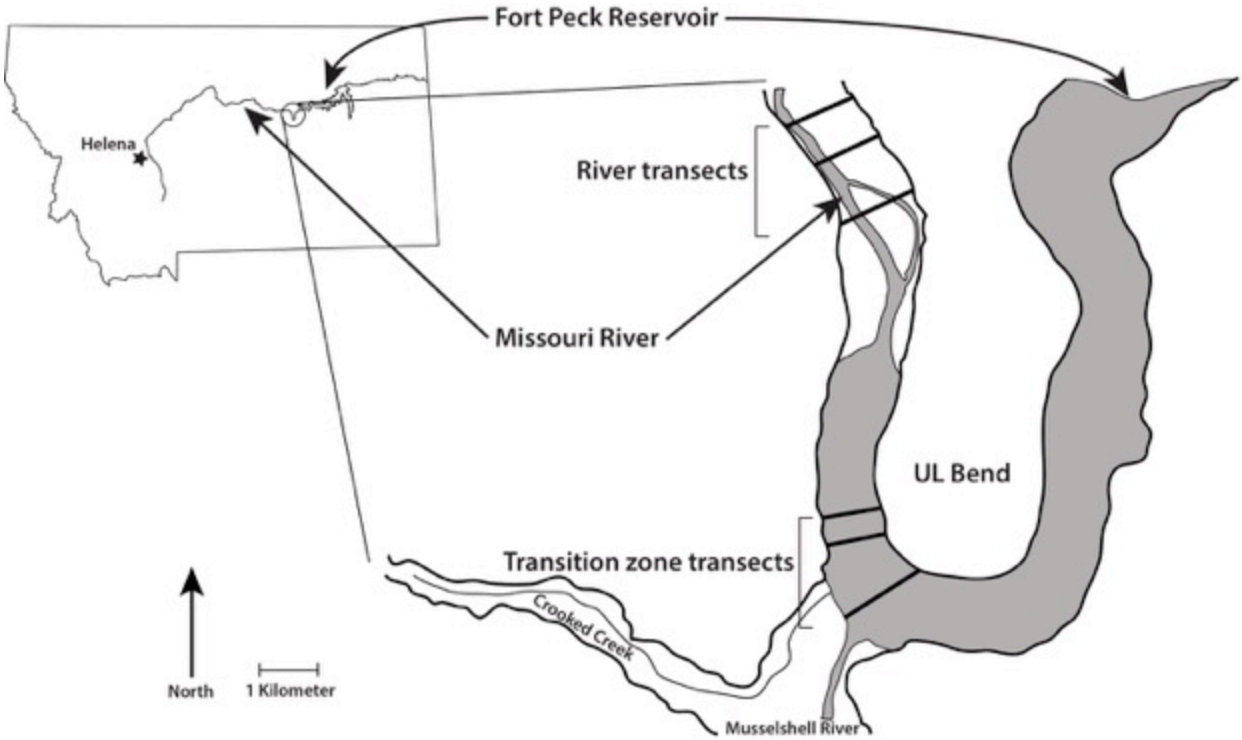
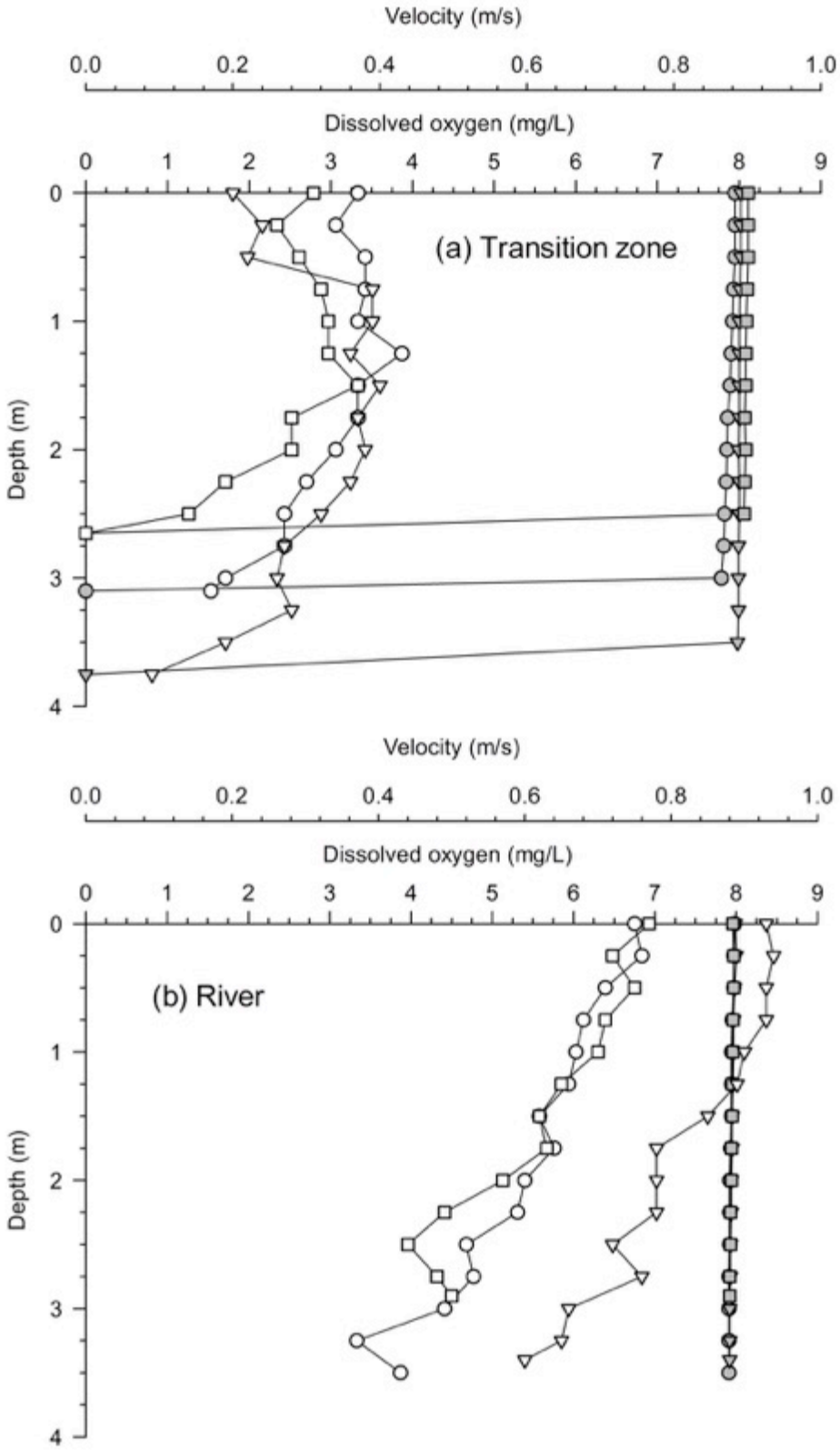
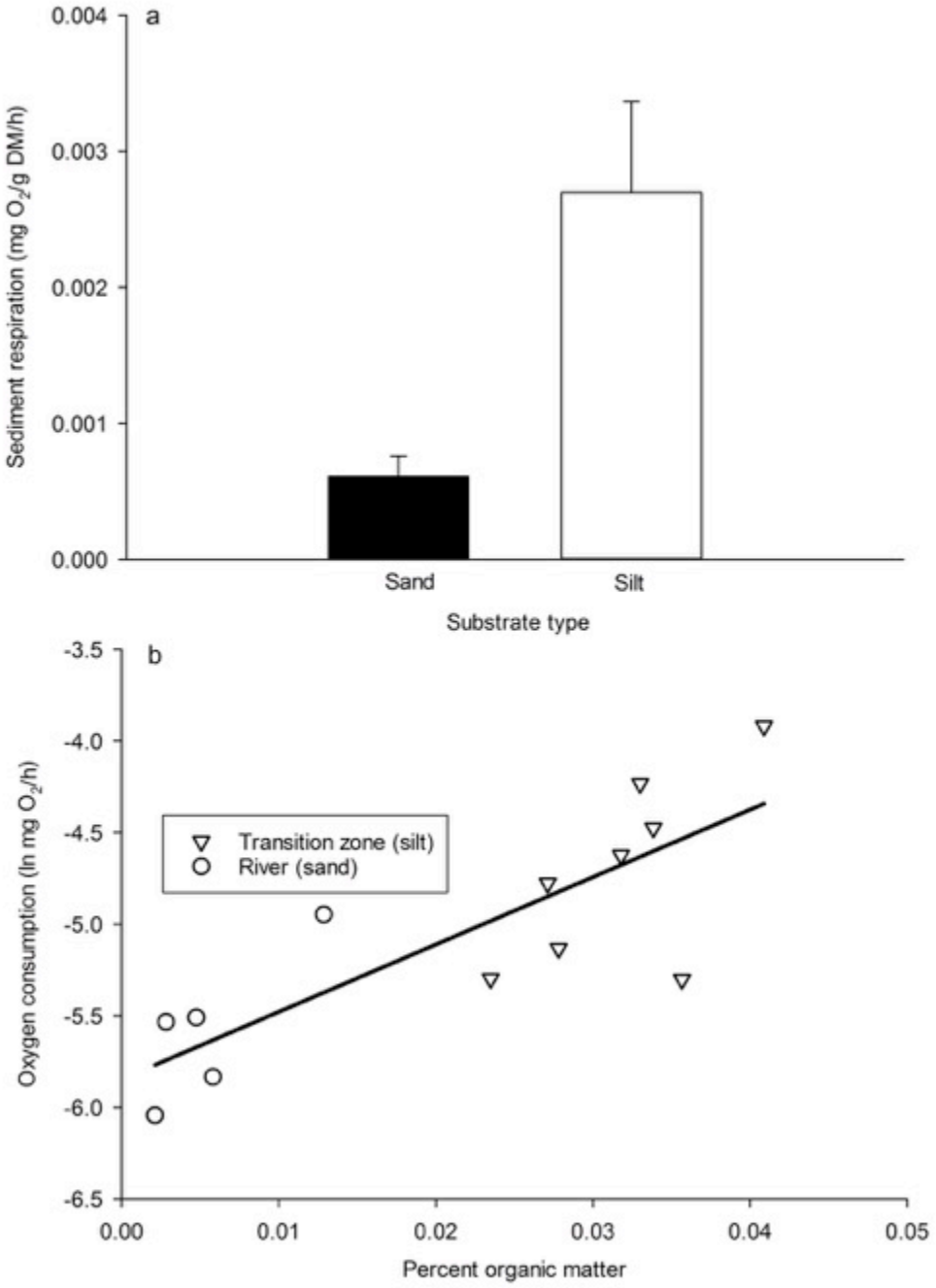
Table 1. Results of dissolved oxygen (mg L^{-1}), temperature (°C), and velocity (m s^{-1}) field measurements by depth (values in parentheses are 95% confidence intervals).

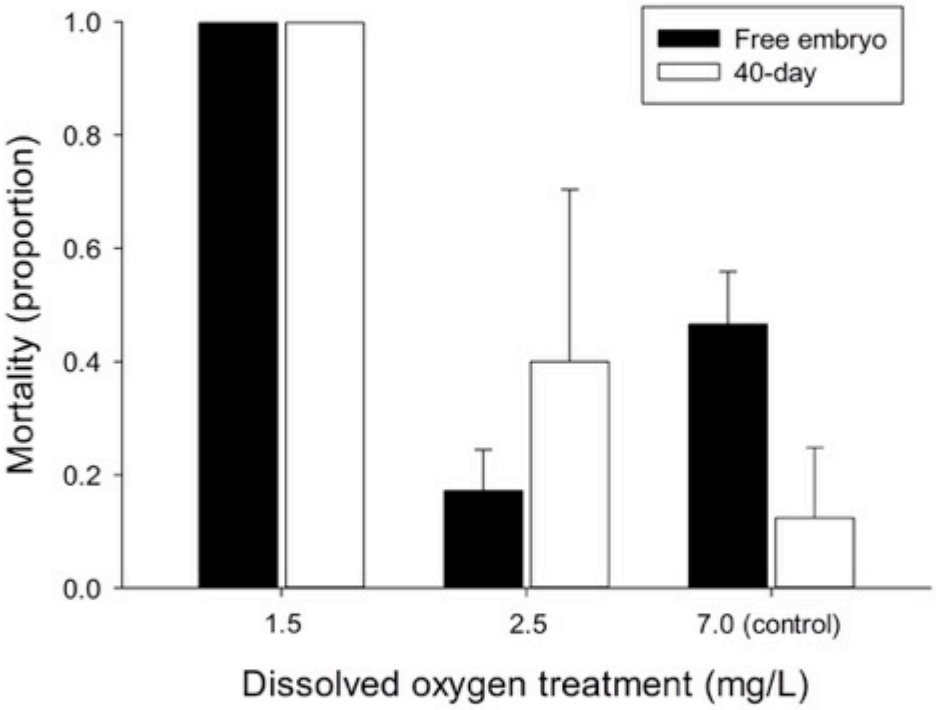
			Percent of maximum depth		
Year	Location[a]	Variable	50	75[a]	100[b]
2012	Transition zone				
	Thalweg				
		Dissolved oxygen	8.55 (0.17)		1.32 (1.49)
		Temperature	18.30 (0.00)		17.83 (0.32)
	River				
	Thalweg	Dissolved oxygen	8.25 (0.37)		7.61 (1.02)
		Temperature	18.15 (0.08)		18.03 (0.11)
2013	Transition zone				
	Thalweg				
		Dissolved oxygen	7.95 (0.07)	7.93 (0.07)	0.00 (0.00)
		Temperature	20.6 (0.3)	20.6 (0.2)	19.9 (0.2)
		Velocity	0.34 (0.04)	0.30 (0.05)	0.08 (0.06)
	Outside thalweg				
		Dissolved oxygen	8.02 (0.06)	7.94 (0.12)	0.00 (0.00)
		Temperature	20.9 (0.6)	20.6 (0.7)	20.5 (0.7)
		Velocity	0.25 (0.12)	0.19 (0.08)	0.04 (0.07)
	River				
	Thalweg				
		Dissolved oxygen	7.94 (0.01)	7.91 (0.05)	7.92 (0.01)
		Temperature	21.9 (0.3)	21.9 (0.3)	21.9 (0.3)
		Velocity	0.68 (0.09)	0.59 (0.07)	0.38 (0.14)
	Outside thalweg				
		Dissolved oxygen	7.96 (0.02)	7.96 (0.02)	7.96 (0.02)
		Temperature	22.0 (0.2)	22.0 (0.2)	22.0 (0.2)
		Velocity	0.57 (0.10)	0.52 (0.10)	0.27 (0.15)

[a] No measurements were made outside the thalweg and at 75% of maximum depth in 2012.
[b] In 2013, measurements were 14 cm above the substrate given where the meter sensors were attached to the sounding weight.


Fort Peck Reservoir
Helena
River transects
Missouri River
UL Bend
Transition zone transects
Crooked Creek
North
1 Kilometer
Musselshell River


Velocity (m/s)
0.0
0.2
0.4
0.6
0.8
1.0
Dissolved oxygen (mg/L)
0
1
2
3
4
5
6
7
8
9
Depth (m)
(a) Transition zone
Velocity (m/s)
Dissolved oxygen (mg/L)
(b) River
Depth (m)


a
0.004
0.003
0.002
0.001
0.000
Sediment respiration (mg O_2/g DM/h)
Sand
Silt
Substrate type
b
-3.5
-4.0
-4.5
-5.0
-5.5
-6.0
-6.5
Oxygen consumption (ln mg O_2/h)
Transition zone (silt)
River (sand)
0.00
0.01
0.02
0.03
0.04
0.05
Percent organic matter


Free embryo
40-day
Mortality (proportion)
1.0
0.8
0.6
0.4
0.2
0.0
1.5
2.5
7.0 (control)
Dissolved oxygen treatment (mg/L)

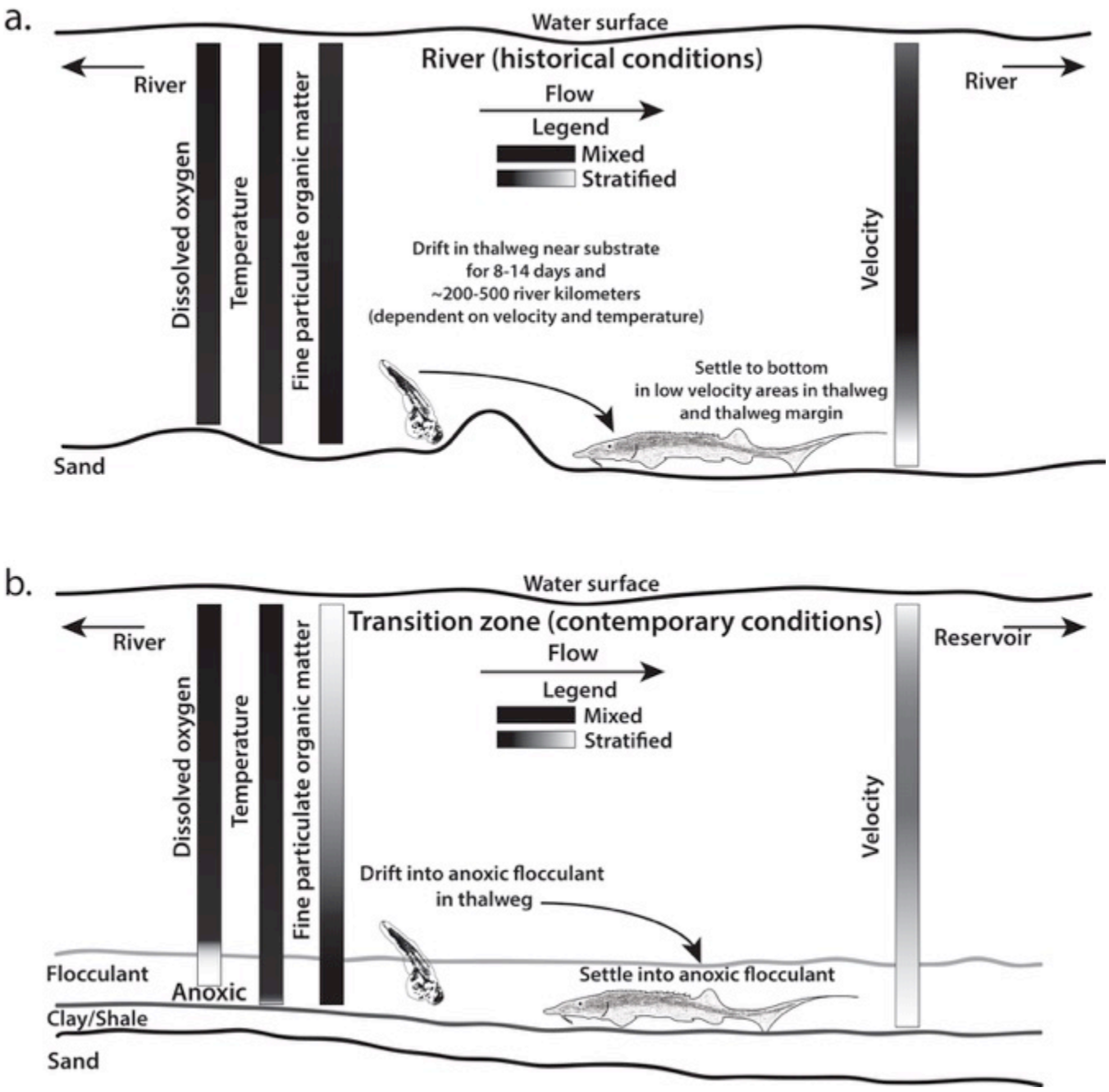

a.
Water surface
River (historical conditions)
River
River
Flow
Legend
Mixed
Stratified
Dissolved oxygen
Temperature
Fine particulate organic matter
Velocity
Drift in thalweg near substrate
for 8-14 days and
~200-500 river kilometers
(dependent on velocity and temperature)
Settle to bottom
in low velocity areas in thalweg
and thalweg margin
Sand
b.
Water surface
Transition zone (contemporary conditions)
River
Reservoir
Flow
Legend
Mixed
Stratified
Dissolved oxygen
Temperature
Fine particulate organic matter
Velocity
Drift into anoxic flocculant
in thalweg
Settle into anoxic flocculant
Flocculant
Anoxic
Clay/Shale
Sand

Figure captions (captions too large to have below figures)

Figure 1. Sampling locations in the Missouri River and river-reservoir transition zone in Fort Peck Reservoir, Montana.

Figure 2. Dissolved oxygen and velocity profiles for the transition zone (a) and river (b). Shaded symbols delineate dissolved oxygen measurements and open symbols delineate velocity measurements. Symbol shapes correspond to dissolved oxygen and velocity measurements collected in the same profile. The variation in where conditions become anoxic in the transition zone is a function of varying maximum depth (see main text). Symbols for dissolved oxygen are overlaid in the river because of similarities in the measurements.

Figure 3. Mean (standard error) sediment microbial respiration estimates per dry mass (DM) (mg O_2 g^{-1} DM hr^{-1}) for sand and silt (a). Relationship between natural log of oxygen consumption and percentage of organic matter for all empirical samples (b).

Figure 4. Mortality of free embryo and 40-day old pallid sturgeon by dissolved oxygen treatment.

Figure 5. Schematic of historical river conditions and the contemporary ecological sink as a function of reservoirs. Historically, river habitat was dynamic with dissolved oxygen, temperature, and fine particulate organic matter mixed throughout the water column as a function of complex velocity currents. Velocity is lowest near the substrate as a function of sheer stress and drag forces. Free embryos drift along the thalweg for hundreds of kilometers prior to settling at the substrate. Pockets of low-velocity microhabitat with high dissolved oxygen exist in the river thalweg, and these are likely the locations that pallid sturgeon select once they reach an age and size where they can negotiate flow (a). In the current conditions, the transition zone habitat is a human-made habitat that differs from the river by having stratified dissolved oxygen concentrations and fine particulate organic matter. Free embryo pallid sturgeon drift into these habitats because drift distance is limited and are involuntarily exposed to habitat that is anoxic (b).

Chapter 7
Effects of Sedimentation Rate on Growth and Mortality

Preface

Habitat sampling performed in 2008 and 2009 lead us to hypothesize that altered sediment load and sedimentation rate, associated with pallid sturgeon drift dynamics and larval behavior when entering the reservoir headwater habitat, might negatively affect pallid sturgeon larval survival. We performed a series of experiments in 2010, 2011, and 2012 to examine the effects of suspended sediment and sedimentation rate on sturgeon growth and survival. In this chapter we describe the results of those laboratory trials.

Introduction

Pallid sturgeon evolved in a free flowing river with high turbidity, seasonal flooding, and a dynamic river channel consisting of sandbars, islands, and shifting banks that developed and disappeared from deposition and erosion. Dams on the Missouri River, such as Fort Peck Dam, have interrupted natural river geomorphology. Above Gavins Point Dam (the most downstream dam on the Missouri River), much of the sediment that was once transported to the Mississippi River is now deposited in reservoir headwaters (also defined as transition zones, see Chapter 6). It has been estimated that dams have reduced the global flux of sediment reaching the oceans by over 100 billon metric tons (Syvitski et al. 2005).

Drifting pallid sturgeon larvae are governed by much of the same fluvial transport mechanisms that carry sediments. Given the altered hydraulic scenario, sediment and pallid sturgeon larvae are unnaturally deposited into reservoirs that present a much different environment than the upstream river channel (see Chapter 6). In the reservoir headwaters, river channel functionality ceases and sediment deposition occurs at a rapid rate, thus the bottom substrate consists of fine sediment, and water-quality parameters undergo subsequent change. We surmise that drifting pallid sturgeon larvae are incapable of escaping this unnatural environment. The developing larvae have many physiological characteristics common to an

unhatched embryo. That is, the larvae are dependent on a yolk sac for food, respire through the body surface, and are not neutrally buoyant.

Changes in suspended sediments and sedimentation rate of aquatic systems can have significant effects on the survival, growth, and behavior of resident fish species at varying life stages. Reviews of the biological effects of suspended and bedded sediment (SABS) have stated the major effects of SABS on fishes include: 1) direct physiological effects of suspended sediment, such as suffocation and abrasion, (2) effects due to decreases in water clarity and light penetration, and 3) effects due to sediment deposition such as increased embeddedness, burial of eggs and larvae, and provision of a growth surface for bacteria and fungi, and 4) absorption and adsorption of chemicals (Appleby and Scarratt 1989; Berry et al. 2003). Current evidence suggests that sedimentation can negatively influence sturgeon and other fish species at the embryo and juvenile life stages. For example, white sturgeon *Acipenser transmontanus* embryos had reduced survival (0-5%) in sediment of both 5 and 20 mm, while embryos under control conditions had 80% survival; additionally, there was a negative relationship between white sturgeon embryo survival and duration of sedimentation (Kock et al. 2006). In the same study, sedimentation was also demonstrated to delay time to hatch and decrease larval length. In similar research, high sediment loads were demonstrated to decrease salmonid embryo survival within redds (Heywood and Walling 2007) and significantly alter the emergence pattern of rainbow trout fry from redds (Fudge et al. 2008). High sedimentation rates negatively influence fish at the juvenile stage, for example Garakouei et al. (2009) determined the LC50 for suspended sediment in two species of juvenile sturgeon (average length of 70-100 mm). Persian sturgeon *A. persicus* juveniles experienced 24 and 96 hour LC50 values of 60,802 mg/L and 15,367 mg/L, respectively, while starry sturgeon *A. stellatus* juveniles experienced 24 and 96 hour LC50 values of 46,294 mg/L and 8,539 mg/L, respectively (Garakouei et al. 2009). These values are relatively high in relation to suspended solid levels found in turbid North American rivers, and give some indication of the general tolerance of juvenile sturgeon to high levels of suspended sediments. Sedimentation has also had measurable effects on fish stress response and growth. Sutherland (2003) found a negative relationship between growth and increasing sedimentation and a positive relationship between

cortisol levels and increasing sedimentation in juvenile whitetail shiner *Cyprinella galactura*, indicating that higher sediment loads inhibited growth and increased stress.

While these studies provide valuable information on the effects of sedimentation on embryos and juveniles, research on effects to sturgeon larvae is still needed. Pallid sturgeon evolved in a highly turbid river system, and the presence of high sediment loads itself likely does not negatively alter their survival. However, the current alteration of fluvial transport in the Missouri River has led to an unnatural process in which sediment – and larvae – are no longer transported (see Chapter 6). As such, sturgeon larvae are deposited with sediment into the low velocity reservoir environment, making it especially important to understand potential effects in order to guide conservation and management efforts. We designed a laboratory study based on empirical values from the upper Missouri River to examine the immediate effects of suspended sediment and sedimentation rate on survival of sturgeon larvae.

Methods

Shovelnose Sturgeon Broodstock Collection, Spawning, and Incubation

In early May of 2012, we collected ripe female and male shovelnose sturgeon from the Missouri River downstream of Coal Banks Recreation Area, MT (N 48.032004 W 110.235293) using 45.72-m long by 1.83-m deep drifting trammel nets. The outer mesh panel was 25.4 cm (bar measure) and the inner-mesh panel was 5.1 cm (bar measure). We drifted nets in 10-minute intervals over known spawning grounds to target spawning adults. We confirmed sexual maturity of collected shovelnose sturgeon by taking gonadal biopsies from each fish.

We transported fish in a 1,893 L oxygenated tank to the BFTC. Once there, fish were held at 14°C until we were able to sample oocytes from mature females and calculate individual polarization index (PI). Polarization index is used as an indicator of spawning readiness, and is the ratio of the distance of the germinal vesicle from the animal pole to the oocyte diameter (Dettlaff et al. 1993). Females that are ready to spawn will have a PI ≤ 0.07 (Dettlaff et al. 1993). Based on individual PI, we assigned each female to a potential spawning date and manipulated water temperature to entrain spawning readiness.

To induce ovulation, each female was injected with 10% (priming dose) of a total dose of Leutinizing Hormone Releasing Hormone (LHRH) at a volume of 20 µg/kg body weight followed 12 hours later with the remaining 90% (resolving dose) of the dose. At the time females were administered their priming dose, males were injected with LHRH [20 µg/kg body weight]. We collected milt from males through catheterization 24 hours prior to expected female ovulation. When females began ovulating, we collected released oocytes via hand-stripping, fertilized them with milt (5 ml of milt to 1000 ml of ambient-temperature hatchery water), and stirred the mixture for two min. We drained the milt-water mixture to prevent polyspermy and then added an aqueous solution of Fuller's Earth to de-adhese the embryos. De-adhesion prevents fertilized embryos from clumping together during the incubation process. After de-adhesion, the Fuller's Earth solution was replaced with BFTC water and the embryos were transferred into McDonald hatching jars. Embryos were incubated at 18-20 °C for up to 6 days, and dead eggs were removed from the hatching jar. After final hatch was reached, larvae were collected with a 1 L beaker and randomly transferred into test tanks.

Pallid Sturgeon Embryo Shipments and Incubation

We received pallid sturgeon embryos from the Miles City State Fish Hatchery, Miles City, Montana and the Gavins Point National Fish Hatchery, Yankton, South Dakota. Embryos were transported to the BFTC via FedEx in 18.9 L plastic bags, and water in the bags was tempered by floating each bag in a tank filled with BFTC water. After tempering, each group of pallid sturgeon embryos was immersed in an Ovadine-water solution and then transferred into a McDonald hatching jar for incubation. Incubation procedures continued identical to the shovelnose sturgeon incubation. After final hatch was reached, larvae were collected with 1 L beaker and randomly transferred into test tanks.

Exposure Trials

Sediment deposition occurs when the forces responsible for sediment transportation are no longer sufficient to overcome the forces of particle weight and friction. We conducted pilot studies in each of 2010 and 2011 to develop a laboratory system that would emulate the deposition effect observed in the reservoir headwater environment. Experimental tanks were

constructed using 7.62-cm by 1.5-m long clear PVC tubes to simulate cross sections of the Ft. Peck Reservoir headwaters (Figure 7.1). Treatments were controlled by manipulating the sediment-laden water ball valve and the freshwater ball valve, and each tank received 2000 ml of water per minute. The suspended sediment of the headtank was kept constant by adding 10.8 Kg of Fuller's Earth twice daily for the duration of each trial. Sedimentation rate treatment levels were 0.00 g/cm^2 (control), 0.39 g/cm^2 (medium), and 0.77 g/cm^2 (high), maintaining suspended sediment at 0.00 mg/L (control), 482.75 mg/L (medium), and 984.6 mg/L (high), respectively. The medium sedimentation rate approximated sediment depostion rates observed in the Ft. Peck Reservoir headwater in 2008 and 2009, while the high sedimentation rate was based on 30 years of USGS data collected near Landusky, Montana. None of the trails in 2010 or 2011 produced reliable data, which was likely a function of tank design.

In 2012, trials addressing the effects of sedimentation rate were conducted using 9.5 L plastic zebrafish tanks (Figure 7.2). Treatments were controlled by manipulating the sediment-laden water ball valve and the freshwater ball valve, and each tank received 2 L of water per minute. The suspended sediment of the headtank was kept constant by adding 10.8 kg of Fuller's Earth twice daily for the duration of each trial. Sedimentation rate treatment levels were 0.00 g/cm^2 (control), 0.39 g/cm^2 (medium), and 0.77 g/cm^2 (high), maintaining suspended sediment at 0.00 mg/L (control), 482.75 mg/L (medium), and 984.6 mg/L (high), respectively. Each treatment level was replicated three times. We transferred 100 individual IPH larvae and 20 individual 40-DPH larvae of each species into each tank, as described above. Pallid sturgeon larvae came from two distinct genetic families, with each family replicated three times per treatment level. There was only one family of shovelnose sturgeon larvae. Water quality parameters – DO (mg/L), pH, temperature (C), and unionized ammonia (mg/L) – were monitored daily to ensure that observed mortality was attributed to the treatment levels.

We measured the effects of our treatment levels on larval growth and mortality in the following ways. Larval growth for each species was assessed by comparing pre-trial larval lengths and weights to post-trial lengths and weights of surviving larvae from each replicate. Immediately prior to beginning a trial, we randomly selected larvae from their holding tanks, euthanized them with an overdose of tricaine methanesulfonate (MS-222), and preserved them in 10%

phosphate-buffered formalin. At the end of a trial, selected larvae were similarly euthanized and preserved. Larval length was measured to the nearest 0.01 mm using a dissecting scope with image analysis capabilities. Larval weight was measured to the nearest 0.01 g using a digital scale. To prevent biasing variance in sample measurements, the same proportion of individuals was measured for IPH and 40-DPH trials. Larval mortality was determined by recording the number of living larvae in each tank at the end of the trial and subtracting that from the initial population in each tank.

Statistical Analyses

All analyses were conducted using the statistical software R (Version 2.13.1). Data were tested to determine if the assumptions of normality were met, and, when data did not meet these assumptions, a transformation of the data was performed and analyses conducted on the transformed data. Tank effects were tested for in all analyses of laboratory data.

Average length and weight (± SE) at each treatment level were calculated for each species at each age group and treatment level. Differences in length and weight among treatment groups, sampling day, and species-family were determined using a factorial analysis of variance (ANOVA), with each treatment group, sampling day, and species-family as the categorical predictor variables and length as the continuous response. Average mortality (± 95% confidence interval) at each treatment level was determined for each species at each age group. Treatment, age, sampling day, and species-family effects were assessed with a factorial ANOVA, with the three aforementioned factors as the categorical predictor variables and mortality as the continuous response. Tukey tests were used to determine differences between levels for length, weight, and mortality.

Results

Water quality parameters were similar among treatment levels and trials, and values remained within normal levels for sturgeon.

Growth

Pre-treatment larvae were shorter than post-treatment larvae (all *P*-values = 0.00), but there were no significant differences in larval length among sedimentation rate treatments (all *P*-values > 0.05; Figure 7.3). Pre-treatment larvae were similar in length between species (*P*-values > 0.05). Post-treatment shovelnose sturgeon larvae were longer than pallid sturgeon larvae from either family (*P*-values = 0.00). Additionally, post-treatment larvae from pallid sturgeon family #2 were significantly longer than those from pallid sturgeon family #1 (*P* = 0.01).

For 40-DPH, pre-treatment larvae were shorter than larvae in the control and high treatment groups (*P*-values < 0.05), but not the medium treatment group (*P* > 0.05). Shovelnose sturgeon larvae were longer than pallid sturgeon larvae from either family (*P*-values = 0.00), except in the medium and high sedimentation rate treatments where shovelnose sturgeon larvae were similar in length to larvae from pallid sturgeon family #1 (*P* > 0.05) and shorter than larvae from pallid sturgeon family #2 (*P* = 0.00) (Figure 7.4). Additionally, post-treatment larvae from pallid sturgeon family #2 were significantly longer than those from pallid sturgeon family #1 (*P* = 0.01).

For both age groups, weight did not differ among sedimentation treatments for shovelnose sturgeon or pallid sturgeon. For the IPH age group, shovelnose sturgeon larvae weighed significantly more than larvae from pallid sturgeon family #1 (*P* < 0.00; Figure 7.5). For the 40-DPH age group, shovelnose sturgeon larvae were heavier than larvae from pallid sturgeon family #1 (*P* < 0.00), but weighed less than larvae from pallid sturgeon family #2 (*P* < 0.05; Figure 7.6). Additionally, larvae from pallid sturgeon family #1 weighed less than those from family #2 (*P* < 0.05).

Mortality

There was no effect of sedimentation rate (i.e., no difference between treatments and control) on larval mortality (Figure 7.7). However, there were individually significant effects of larval family (*P* = 0.00) and age (*P* < 0.00) on mortality, and there was a significant interaction (*P* <

0.00). There was no effect of age on shovelnose sturgeon larval mortality (P = 0.99), but individuals from both pallid sturgeon families experienced higher average mortality at the IPH age group than at the 40-DPH age group (P-values = 0.00). At the IPH age group, shovelnose sturgeon larvae experienced lower mortality than larvae from either pallid sturgeon family (P-values = 0.00), but there was no difference in larval mortality between pallid sturgeon families (P = 0.99). At the 40-DPH age group, there were no differences in larval mortality between pallid sturgeon families (P = 0.76) and between shovelnose sturgeon larvae and pallid sturgeon larvae from either family (P-values > 0.05).

Discussion

The effects of fish species exposure to elevated suspended sediments are somewhat varied and are dependent on factors such as suspended sediment level, sedimentation rate, duration, life stage, and species, but immediate mortality is in general caused by oxygen starvation and/or damage to respiratory tissue (Appleby and Scarratt 1989). The suspended sediment load, sedimentation rate, and duration period used in this study did not influence larval mortality in either age group in both species. The IPH larvae, because they were able to regularly swim off of the bottom substrate or remain in the water column by utilizing tank flow patterns and burst swimming, were not covered and buried in the sediment regardless of sedimentation rate used in the trials. This is in contrast to pre-hatch sturgeon embryos (unable to swim) that were buried in sediment (Koch et al. 2006). Koch et al. (2006) suggested that the mortality of pre-hatched white sturgeon embryos resulted from restricted rates of water renewal around the embryos and a reduction in ability to exchange respiratory gases (oxygen and carbon dioxide). The IPH larvae in the trials appeared to have maintained respiratory exchange rates at rates similar to the control treatment and were not subjected to high mortality due to increased suspended sediment load. The 40-DPH sturgeon also avoided being buried in sediment and while we had hypothesized that suspended sediment load may have a negative effect on gill function and respiration ability there were no differences among levels. Overall, pallid sturgeon and shovelnose sturgeon evolved in and inhabit a highly turbid, large river environment in which the sediment is in suspension; thus, they may have some resistance to high suspended sediment levels.

At both ages and families, larvae grew over the course of each trial and sedimentation rate did not influence larval length or weight. Similarities in length between species and families immediately after hatching make sense given general constraints on embryo size, but similar to the sub-lethal effects of hypoxic conditions, the size differences between shovelnose sturgeon larvae and pallid sturgeon larvae and between larvae of different families are important to consider in the context of the Missouri River environment. It is possible that being small in a big river environment can affect survival by making an individual more susceptible to disease and less competitive.

Our investigations focused on the immediate observable effects of increased sediment load on sturgeon larval survival and growth. Further investigation into longer term interactions of increased sediment load and sedimentation effects on larval sturgeon are warranted. Increases in suspended sediment and sedimentation have been linked to decreased quality and quantity of sturgeon habitat because of changes to depth, bottom topography, current velocity pattern, turbidity, dissolved oxygen, substrate type, and benthic community composition (Hatin et al. 2007).

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Figure 7.1. Tank design for 2010 and 2011 sedimentation rate trials.

Figure 7.2. Tank design for 2012 sedimentation rate trials.

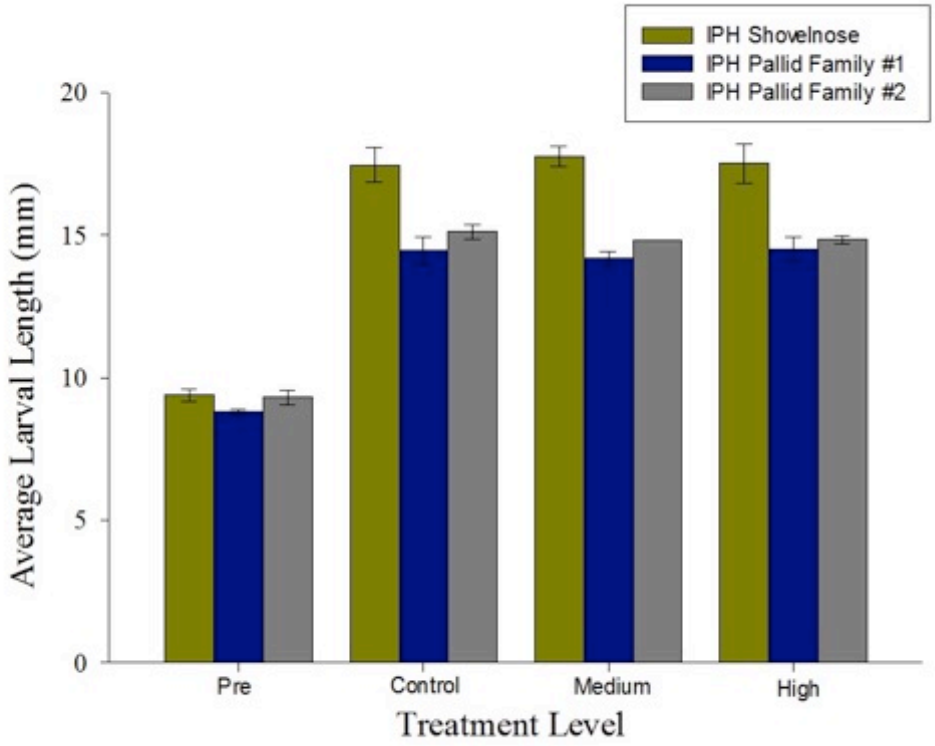

Figure 7.3. Average larval length of immediate post-hatch shovelnose sturgeon, pallid sturgeon family #1, and pallid sturgeon family #2 larvae exposed to different sedimentation rate treatments. Sedimentation rate treatment levels were 0.00 g/cm^2 (control), 0.39 g/cm^2 (medium), and 0.77 g/cm^2 (high), maintaining suspended sediment at 0.00 mg/L (control), 482.75 mg/L (medium), and 984.6 mg/L (high), respectively.

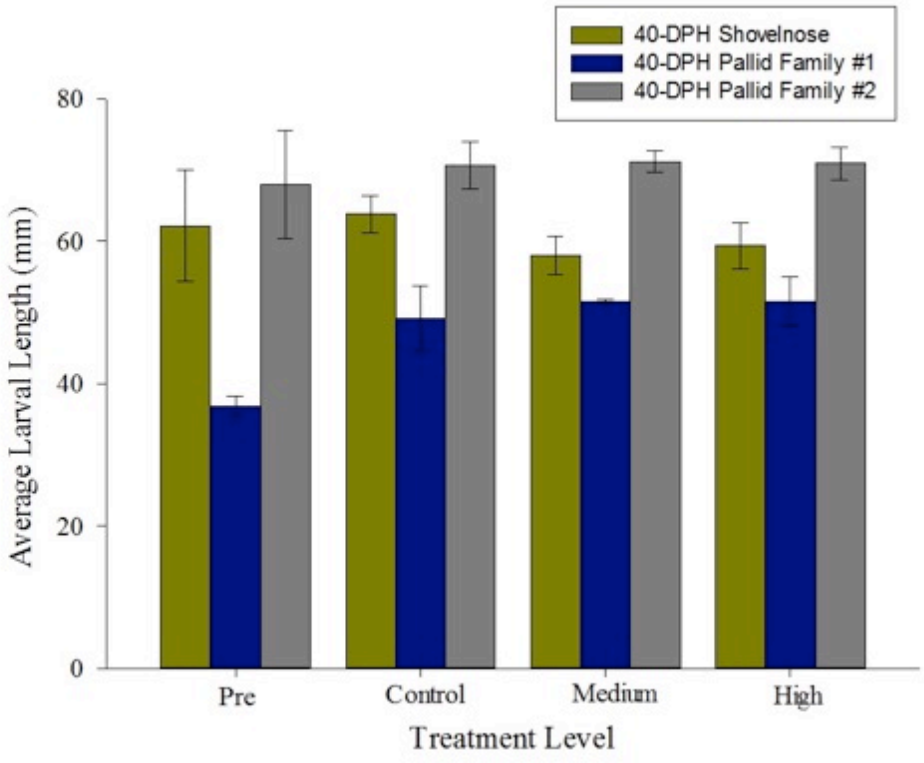

Figure 7.4. Average larval length of 40-days post-hatch shovelnose sturgeon, pallid sturgeon family #1, and pallid sturgeon family #2 larvae exposed to different sedimentation rate treatments. Sedimentation rate treatment levels were 0.00 g/cm^2 (control), 0.39 g/cm^2 (medium), and 0.77 g/cm^2 (high), maintaining suspended sediment at 0.00 mg/L (control), 482.75 mg/L (medium), and 984.6 mg/L (high), respectively.

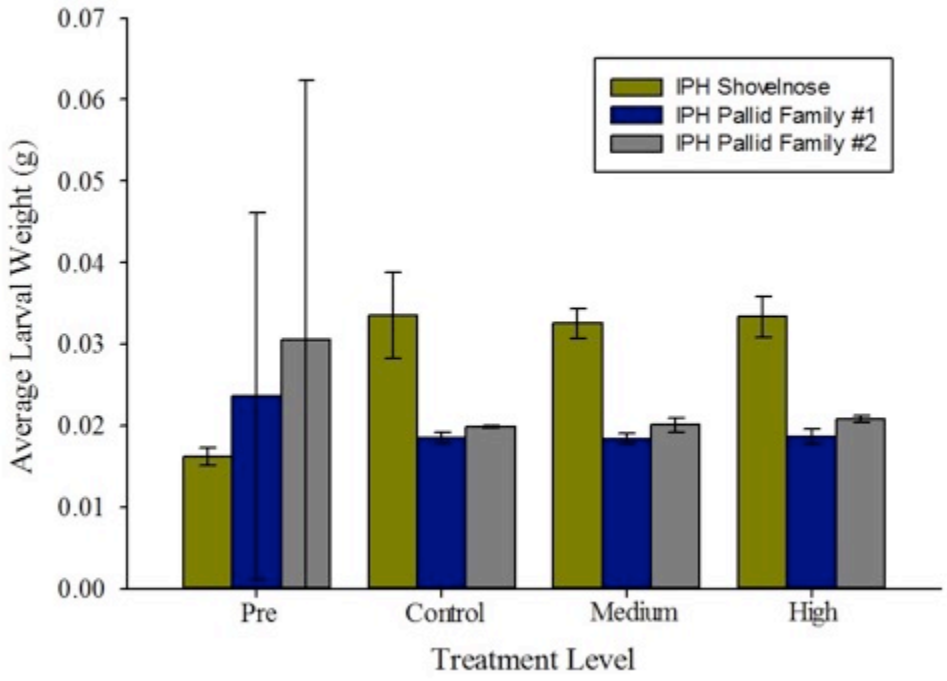

Figure 7.5. Average larval weight of immediate post-hatch shovelnose sturgeon, pallid sturgeon family #1, and pallid sturgeon family #2 larvae exposed to different sedimentation rate treatments. Sedimentation rate treatment levels were 0.00 g/cm^2 (control), 0.39 g/cm^2 (medium), and 0.77 g/cm^2 (high), maintaining suspended sediment at 0.00 mg/L (control), 482.75 mg/L (medium), and 984.6 mg/L (high), respectively.

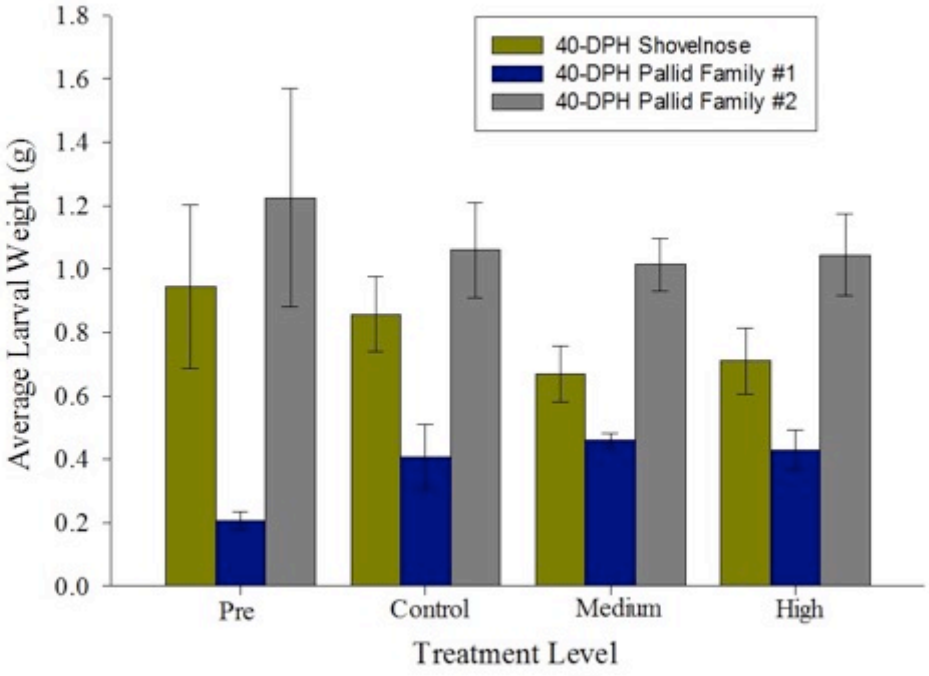

Figure 7.6. Average larval weight of 40-days post-hatch shovelnose sturgeon, pallid sturgeon family #1, and pallid sturgeon family #2 larvae exposed to different sedimentation rate treatments. Sedimentation rate treatment levels were 0.00 g/cm^2 (control), 0.39 g/cm^2 (medium), and 0.77 g/cm^2 (high), maintaining suspended sediment at 0.00 mg/L (control), 482.75 mg/L (medium), and 984.6 mg/L (high), respectively.

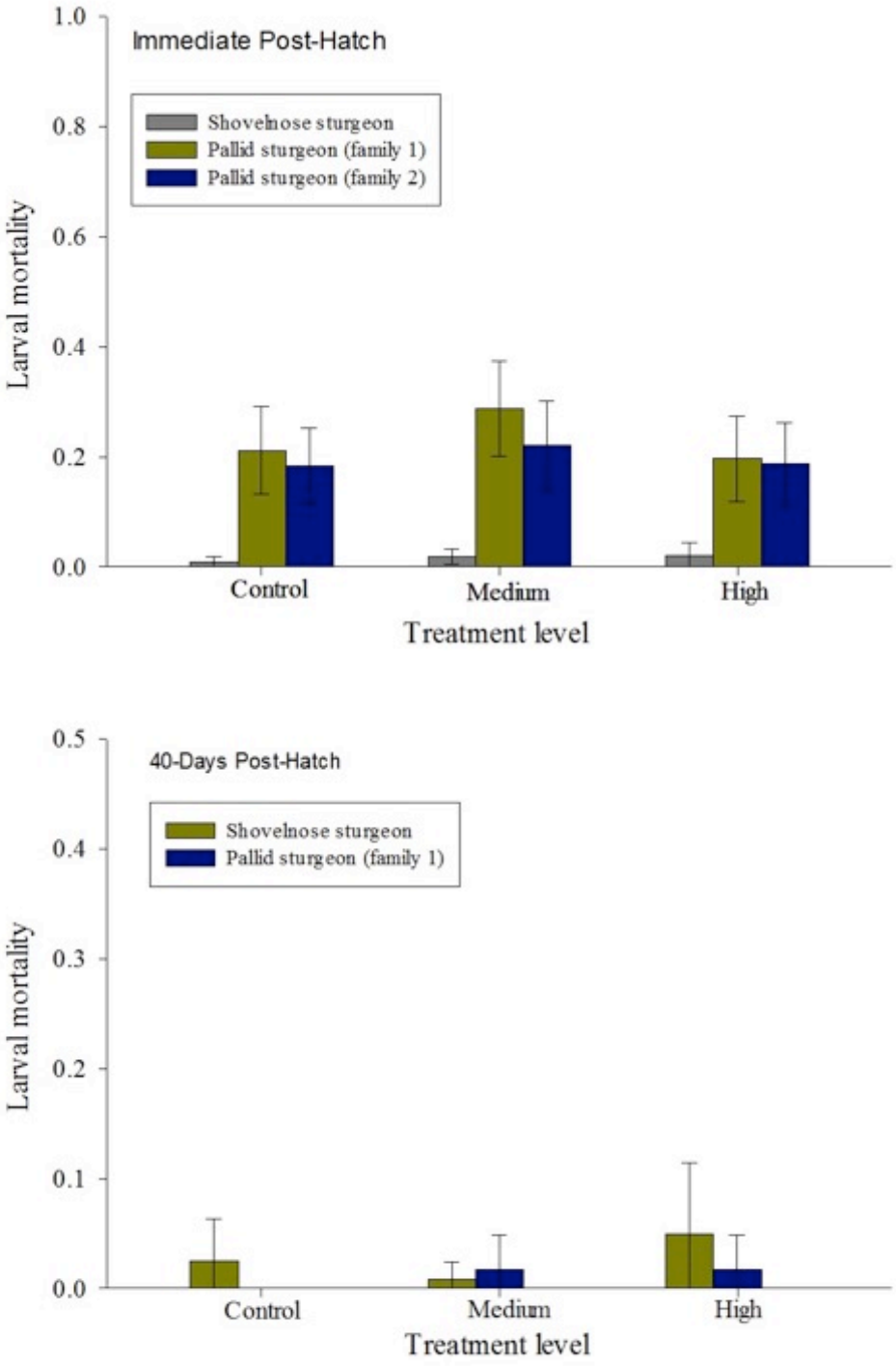

Figure 7.7. Average proportion mortality (± 95% Confidence Interval) of Immediate Post-Hatch and 40-Days Post Hatch shovelnose sturgeon and pallid sturgeon. Sedimentation rate treatment levels were 0.00 g/cm^2 (control), 0.39 g/cm^2 (medium), and 0.77 g/cm^2 (high), maintaining suspended sediment at 0.00 mg/L (control), 482.75 mg/L (medium), and 984.6 mg/L (high), respectively.

Chapter 8
Effects of Substrate on Growth and Mortality

Preface

Field observations of reservoir habitat revealed that the substrate present in the reservoir habitat is made up of fine particulate matter. The pilot studies we conducted in 2010 and 2011 to emulate reservoir conditions and examine the effect of sediment rate on larval survival and growth were difficult because of experimental-design challenges. The difficult design challenges led us to construct a simpler sedimentation rate design (see Chapter 7) and a static system (this Chapter). The simpler static design (i.e., representing lentic habitat) will provide information on the effect of sediment (substrate) without the complications of mimicking sediment deposition rate and flow. In 2011 and 2012, we performed laboratory studies to examine the effect of substrate type on mortality and growth.

Introduction

Several studies have addressed juvenile and adult pallid sturgeon and shovelnose sturgeon habitat use in the upper Missouri River (e.g., Bramblett and White 2001; Gerrity et al. 2008). Conversely, there is little information on substrate use by larval pallid sturgeon and shovelnose sturgeon. To date, much of the research on pallid sturgeon and shovelnose sturgeon larvae has focused on drift dynamics (Kynard et al 2002, 2007; Bratten et al. 2008, 2012). To our knowledge no study has described how larval sturgeon, once they become benthically oriented or exit the fluvial drift, interact with the substrate. Thus, the effects of altered substrate, resulting from reservoirs, on larval survival, growth, and behavior are unknown.

Substrate (e.g., sediment particle size) has an effect on larval fish survival, growth, and behavior and influences water-quality parameters that affect a larval fishes environment (Berry et al. 2003). Substrate particle size determines interstitial space availability and can influence health and resistance to parasitism and disease, physiological processes (such as reparation, growth), and behavior (ability to see prey). In studies of larval white sturgeon, substrate type

(e.g., sand, cobble, gravel) influenced the proportion of larvae that drifted at different ages post hatch, suggesting larval sturgeon have the ability to select for substrate, and indicating that substrate influences behavior (McAdam 2011).

Substrates affect water quality by releasing substances into the water, absorbing substances from the water, or reacting chemically with substances from other sources. Framework sediment that remain bedded, such as gravel, have less potential negative impact on water quality parameters than fine sediment deposits and suspended load sediments. The headwaters reservoir substrate is made up of fine deposits and because of sediment deposition rate and chemical interactions, reservoir substrate has different properties than what larval sturgeon encounter in the river. The altered substrate that exists in the reservoir headwater environment might function as a physical or chemical stressor to larval sturgeon (see Chapter 6).

There is a knowledge gap on the influence of substrate on pallid sturgeon and shovelnose sturgeon larvae in the months immediately following initiation of the benthic life stage and a gap in the knowledge of the effects of substrate on drifting pallid sturgeon and shovelnose sturgeon larvae that are deposited into the reservoir headwater environment when they involuntarily exit the fluvial drift. The objectives of this study were to examine varying substrate treatments on growth and mortality in pallid sturgeon and shovelnose sturgeon larvae. Specifically, we were interested in how fine sediment (the reservoir sediment) might affect survival and physiological condition (determined by assessing growth).

Methods

Shovelnose Sturgeon Broodstock Collection, Spawning, and Incubation

We collected ripe female and male shovelnose sturgeon from the Missouri River downstream of Coal Banks Recreation Area, MT (N 48.032004 W 110.235293) using 45.72-m long by 1.83-m deep drifting trammel nets. The outer mesh panel was 25.4 cm (bar measure) and the inner-mesh panel was 5.1 cm (bar measure). We drifted nets in 10-minute intervals over

known spawning grounds to target spawning adults. We confirmed sexual maturity of collected shovelnose sturgeon by taking gonadal biopsies from each fish.

We transported fish in a 1,893 L oxygenated tank to the BFTC. Once there, fish were held at 14° C until we were able to sample oocytes from mature females and calculate individual polarization index (PI). Polarization index is used as an indicator of spawning readiness, and is the ratio of the distance of the germinal vesicle from the animal pole to the oocyte diameter (Dettlaff et al. 1993). Females that are ready to spawn will have a PI ≤ 0.07 (Dettlaff et al. 1993). Based on individual PI, we assigned each female to a potential spawning date and manipulated water temperature to entrain spawning readiness.

To induce ovulation, each female was injected with 10% (priming dose) of a total dose of Leutinizing Hormone Releasing Hormone (LHRH) at a volume of 20 µg/kg body weight followed 12 hours later with the remaining 90% (resolving dose) of the dose. At the time females were administered their priming dose, males were injected with LHRH [20 µg/kg body weight]. We collected milt from males through catheterization 24 hours prior to expected female ovulation. When females began ovulating, we collected released oocytes via hand-stripping, fertilized them with milt (5 ml of milt to 1,000 ml of ambient-temperature hatchery water), and stirred the mixture for two min. We drained the milt-water mixture to prevent polyspermy and then added an aqueous solution of Fuller's Earth to de-adhese the embryos. De-adhesion prevents fertilized embryos from clumping together during the incubation process. After de-adhesion, the Fuller's Earth solution was replaced with BFTC water and the embryos were transferred into McDonald hatching jars. Embryos were incubated at 18-20 °C for up to 6 days, and dead eggs were removed from the hatching jar. After final hatch was reached, larvae were collected with a 1 L beaker and randomly transferred into test tanks.

Pallid Sturgeon Embryo Shipments and Incubation

We received pallid sturgeon embryos from the Miles City State Fish Hatchery, Miles City, Montana and the Gavins Point National Fish Hatchery, Yankton, South Dakota. Embryos were transported to the BFTC via FedEx in 18.9 L plastic bags, and water in the bags was tempered by floating each bag in a tank filled with BFTC water. After tempering, each group of pallid

sturgeon embryos was immersed in an Ovadine-water solution and then transferred into a McDonald hatching jar for incubation. Incubation procedures continued identical to the shovelnose sturgeon incubation. After final hatch was reached, larvae were collected with 1 L beaker and randomly transferred into test tanks.

Exposure Trials

The experiments were designed as a static exposure in which each 9.5 L plastic tank was a closed system that contained a specific amount of substrate and water (Figure 8.1). The ratios of substrate to water in each tank were kept as similar as possible for even treatment application. Each tank was aerated with a glass bead air diffuser at a level that provided sufficient oxygenation, but did not disturb the substrate. In 2011, treatment levels were no substrate (control), 3.78 metric cups sand, gravel (3.78 metric cups coarse gravel + 0.24 metric cup pea gravel), 1.89 metric cups Fuller's Earth, and 1.89 metric cups Missouri River "FPOM" collected from the headwaters of the Ft. Peck Reservoir (Figure 8.2). In 2012, treatment levels were no substrate (control), 3.78 metric cups sand, gravel (3.78 metric cups coarse gravel + 0.24 metric cup pea gravel), cobble, 1.89 metric cups Fuller's Earth, and 1.89 metric cups Missouri River "FPOM" collected from the headwaters of the Ft. Peck Reservoir. Additionally, to maintain non-toxic levels of ammonia, 30 g of Proline Zeolite Ammonia Removing crystals (Aquatic Eco-Systems, Inc., Apopka, Florida, USA) were packed into porous, nylon pouches and placed in each tank; zeolite packs were replaced every day of each trial. Each substrate was replicated three times. We transferred 100 individual IPH larvae and 20 individual 40-DPH larvae of each species into each tank, as described above. Shovelnose sturgeon and pallid sturgeon larvae were represented by one distinct genetic family. Water-quality parameters –DO (mg/L), pH, temperature (C), and unionized ammonia (mg/L)– were monitored daily to ensure that observed mortality was attributed to the treatment levels. All experiments were conducted during a six-day period.

We measured the treatment effects on larval growth and mortality in the following ways. Larval growth for each species was assessed by comparing pre-trial larval lengths and weights to post-trial lengths and weights of surviving larvae from each replicate. Immediately prior to beginning a trial, we randomly selected larvae from their holding tanks, euthanized them with

an overdose of tricaine methanesulfonate (MS-222), and preserved them in 10% phosphate-buffered formalin. At the end of a trial, selected larvae were similarly euthanized and preserved. Larval length was measured to the nearest 0.01 mm using a dissecting scope with image analysis capabilities. Larval weight was measured to the nearest 0.01 g using a digital scale. To prevent biasing variance in sample measurements, the same proportion of individuals was measured for IPH and 40-DPH trials. Larval mortality was determined by recording the number of living larvae in each tank at the end of the trial and subtracting that from the initial population in each tank.

Statistical Analyses

All analyses were conducted using the statistical software R (Version 2.13.1). Data were tested to determine if the assumptions of normality were met, and, when data did not meet these assumptions, a transformation of the data was performed and analyses conducted on the transformed data. Tank effects were tested for in all analyses of laboratory data.

Average length and weight (+ SE) at each substrate were calculated for each species at each age group and treatment level. Differences in length and weight among substrates, sampling day, and species/family were determined using a factorial analysis of variance (ANOVA), with each substrate type, sampling day, and species/family as the categorical predictor variables and length as the continuous response. Average mortality (+ 95% confidence interval) at each substrate was determined for each species at each age group. Substrate, age, sampling day, and species/family effects were assessed with a factorial ANOVA, with the three afore-mentioned factors as the categorical predictor variables and mortality as the continuous response. Tukey tests were used to determine differences between treatments for length, weight, and mortality.

Results

In 2011, ammonia was elevated in all trails and highly influenced mortality and any treatment effects were masked by mortality associated with elevated ammonia; thus, the 2011 data are not presented here. In 2012, water-quality parameters were similar among substrates

treatments (because we used Proline Zeolite Ammonia Removing crystals) and values remained within normal levels for sturgeon.

Growth

Overall, pre-treatment larvae were shorter than post-treatment larvae (all *P*-values = 0.00). Pre-treatment shovelnose sturgeon larvae were similar in length to pre-treatment pallid sturgeon larvae ($P > 0.05$). Shovelnose sturgeon larvae were significantly longer than pallid sturgeon larvae at the end of the 6-day exposure in the control, gravel, and sand treatments (all *P*-values ≤ 0.01; Figure 8.3). At the 40-DPH age group, shovelnose sturgeon larvae were shorter than pallid sturgeon larvae ($P = 0.00$). There was no effect of substrate type on length (all *P*-values > 0.05). At both the IPH and 40-DPH age groups, there were no significant effects of substrate type on weight (all *P*-values > 0.05).

Mortality

Larval mortality was influenced by substrate, species, and age (Figure 8.4). There were significant effects of substrate ($P = 0.00$), species ($P < 0.00$), and age ($P < 0.00$) on larval mortality, and there was a significant interaction between age and species ($P = 0.00$). Regardless of age or species, there was lower larval mortality in the sand treatment than in either the FPOM ($P = 0.03$) or cobble ($P = 0.01$) treatments. There were no significant differences in mortality among any of the other substrate treatments (*P*-values > 0.05). At the IPH age group, shovelnose sturgeon larvae experienced lower mortality than pallid sturgeon larvae ($P = 0.00$), but there was no effect of species on mortality at the 40-DPH age group ($P = 1.00$). Finally, shovelnose sturgeon and pallid sturgeon larvae experienced greater mortality at the IPH age group than at the 40-DPH age group (*P*-values = 0.00).

Discussion

At IPH, shovelnose sturgeon larvae and pallid sturgeon larvae experienced lower mortality in the sand treatment than FPOM from the headwater of Ft. Peck or cobble. This suggests that pallid sturgeon and shovelnose sturgeon larvae have higher survival over sand substrates. It is also interesting in the context of habitat currently available in the Ft. Peck Reservoir

headwater to drifting larval sturgeon. Similar to the sedimentation rate trials, IPH shovelnose sturgeon larvae experienced lower mortality than pallid sturgeon larvae. Interestingly, shovelnose sturgeon IPH survived in the sediment substrate treatments (Fuller's Earth and Muck), but there was no difference in mortality between species at the 40-DPH age stage. These results coupled with the results in Chapter 6 suggest that the physical and chemical properties of the headwater habitat (i.e., transition zone) are not conducive to pallid sturgeon survival. The differences in survival between shovelnose sturgeon and pallid sturgeon that we observed in several experiments merits further research to help explain why shovelnose sturgeon recruit in the upper Missouri River and pallid sturgeon fail to recruit.

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Figure 8.1 Tank set-up for 2012 substrate trials.

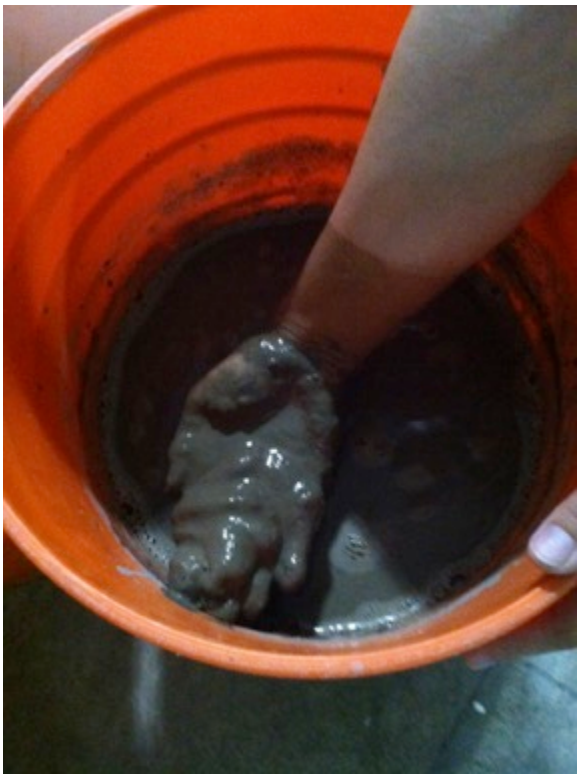

Figure 8.2 “FPOM” substrate (also termed ‘muck’) collected from the Ft. Peck Reservoir headwater.

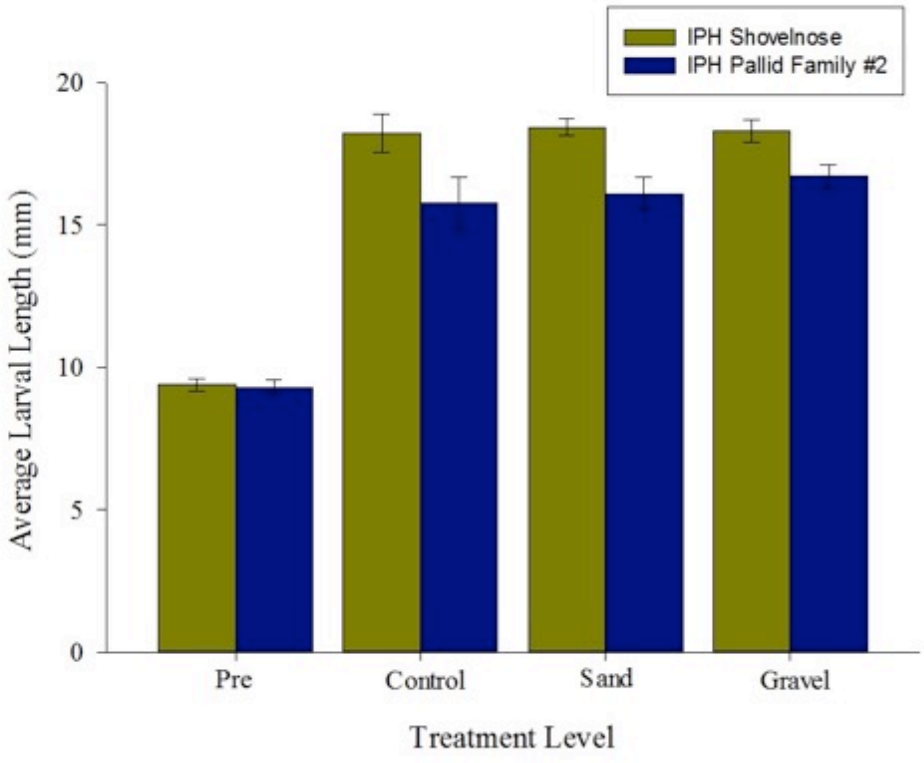

Figure 8.3. Average larval length of immediate post-hatch shovelnose sturgeon and pallid sturgeon family #2 larvae exposed to different substrate treatments. Control treatment was no substrate.

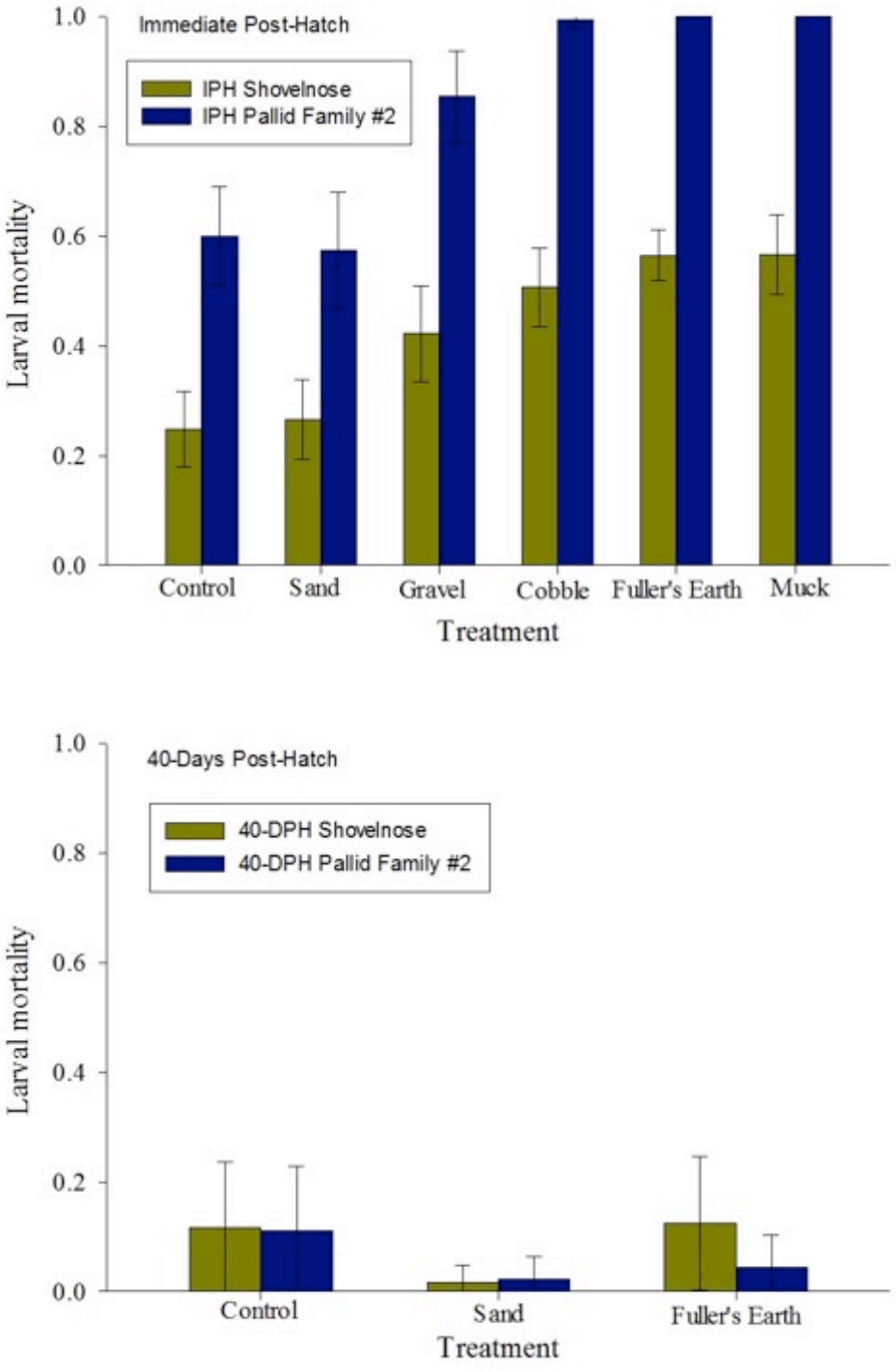

Figure 8.4. Average proportion mortality (± 95% Confidence Interval) of immediate post-hatch and 40-days post hatch shovelnose sturgeon and pallid sturgeon. As a result of technical issues, we were only able to compare mortality in the 40-DPH age group in the control, sand, and Fuller's Earth treatment levels. Control treatment was no substrate.

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