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Sturgeon *Scaphirhynchus albus* Larvae

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EFFECT OF WATER VELOCITY AND TEMPERATURE ON ENERGY USE,  
BEHAVIOR, AND MORTALITY OF PALLID STURGEON *SCAPHIRHYNCHUS*

*ALBUS* LARVAE

BY

JOSEPH THOMAS MRNAK

A thesis submitted in partial fulfillment of the requirements for

Master of Science

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Specialization in Fisheries Science

South Dakota State University

2019

EFFECT OF WATER VELOCITY AND TEMPERATURE ON ENERGY USE,  
BEHAVIOR, AND MORTALITY OF PALLID STURGEON *SCAPHIRHYNCHUS*  
*ALBUS* LARVAE

This thesis is approved as a credible and independent investigation by a candidate for the Master of Science in Wildlife and Fisheries Sciences degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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## ABSTRACT

EFFECT OF WATER VELOCITY AND TEMPERATURE ON ENERGY USE,  
BEHAVIOR, AND MORTALITY OF PALLID STURGEON *SCAPHIRHYNCHUS**ALBUS* LARVAE

JOSEPH THOMAS MRNAK

2019

Pallid Sturgeon *Scaphirhynchus albus* are a federally endangered species endemic to the Missouri River basin. Natural reproduction has been limited for decades and a recruitment bottleneck is hypothesized to occur during the larval stage of development. Understanding factors that affect survival of Pallid Sturgeon larvae is key given their critical status and ongoing recovery efforts. In this study, I evaluated the effects of water velocity and temperature on energy use, behavior, and mortality of endogenously feeding Pallid Sturgeon larvae (<25 mm TL). In the first experiment, I examined the effect of water velocity (0 – 8.3 cm s<sup>-1</sup>) on larval growth, energy density, swimming activity, and mortality. Larval growth rate at 16.3 °C was similar across treatments (0.97 mm d<sup>-1</sup>), but energy reserves of larvae reared at 0 cm s<sup>-1</sup> decreased significantly compared to larvae reared at velocities of 3.5 or 8.3 cm s<sup>-1</sup>. Larvae maintained at zero to low water velocities exhibited significantly greater activity and mortality than those maintained at greater velocities. In the second experiment, I examined the effect of water temperature (18.7, 20.4, or 23.3 °C) on larval growth,

energy density, and rheotactic behavior at water velocity averaging  $8.8 \text{ cm s}^{-1}$ . Larvae reared at  $23.3 \text{ }^{\circ}\text{C}$  exhibited a significantly greater growth rate ( $0.85 \text{ mm d}^{-1}$ ) than at  $18.7$  or  $20.4 \text{ }^{\circ}\text{C}$  ( $0.55$  and  $0.65 \text{ mm d}^{-1}$ , respectively). Energy reserves of larvae maintained at  $23.3 \text{ }^{\circ}\text{C}$  decreased significantly compared to those maintained at cooler temperatures. Additionally, larvae reared at  $23.3 \text{ }^{\circ}\text{C}$  made a behavioral transition from negative to positive rheotaxis (drifting to settling) significantly faster than larvae in other treatments. In natural environments, areas with zero to low flow (reservoir headwaters) may pose a significant source of mortality to Pallid Sturgeon larvae, owing to their negative rheotactic behavior. I recommend that water velocities  $>10 \text{ cm s}^{-1}$  be considered during the construction of shallow water habitat (current U.S. Army Corps of Engineers' definition states  $<60 \text{ cm s}^{-1}$ ). The manipulation of dam releases to increase riverine temperature may aid survival of Pallid Sturgeon larvae by decreasing the extent of spatial and temporal drift, mitigating the drift habitat deficiency caused by impoundments.

## CHAPTER 1. INTRODUCTION

### Current status and research needs

Pallid Sturgeon *Scaphirhynchus albus* are a long-lived, large-bodied fish endemic to the Missouri and the middle and lower Mississippi River basins (Keenlyne 1989; Dryer and Sandvol 1993). Pallid Sturgeon evolved for riverine life with a broad-dorsoventrally flattened snout, a protrusible inferior mouth preceded by four barbells, broad pectoral fins, a long slender caudal peduncle, and a reproductive strategy that is dependent upon river connectivity (Kallemeyn 1983; Kynard et al. 2002a; Braaten et al. 2008). However, 67% of their historic range has been lost due to anthropogenic habitat alterations (Hesse 1987). River channelization in 1927 (Schneiders 1996) and construction of six impoundments between 1937 – 1963 led to substantial changes to historic flow and thermal regimes in the Missouri River (Hesse and Mestl 1993; Pegg et al. 2003). Changes to the natural river environment resulted in reduced connectivity, hydrologic variation, thermal refugia, and critical habitat (Jacobson and Galat 2006; DeLonay et al. 2009; Erwin et al. 2018) believed at the time to be responsible for the decline of Pallid Sturgeon populations (Bergman et al. 2008; DeLonay et al. 2016). As a result, Pallid Sturgeon were listed as federally endangered under the Endangered Species Act in 1990 (U.S. Fish and Wildlife Service 1990, 55 FR 36641-36647).

Due to the endangered status of Pallid Sturgeon, recovery efforts have focused primarily on habitat restoration (long-term, Dryer and Sandvol 1993) and propagation and subsequent stocking of genetically diverse juveniles (short-term, Jordan et al. 2006).

Specifically, the U.S. Army Corps of Engineers (USACE) was mandated to restore 20,000 acres of shallow water habitat (U.S. Fish and Wildlife Service 2003). Shallow water habitat is defined as areas <1.5 m deep with mean water velocities <60 cm s<sup>-1</sup> (Gosch et al. 2013). The USACE has been restoring shallow water habitat by constructing off-channel habitats (e.g., chutes and backwaters) and(or) modifying or removing existing control structures (Gosch et al. 2013). More information is needed to better understand if these shallow water areas can provide satisfactory habitat to restore the population (Steffensen et al. 2010; DeLonay et al. 2016). Stocking of juveniles has been relatively successful in terms of survivorship and maintaining genetic diversity (DeLonay et al. 2016). However, due to the late maturation of Pallid Sturgeon (15-20 years for females, Keenlyne and Jenkins 1993), it remains unknown if stocked individuals will successfully spawn and if those larvae will survive and recruit to the adult population.

Sturgeons (family *Acipenseridae*, 4 genera, 25 *spp.*) are one of the most threatened groups of vertebrates in the world (Ludwig 2006). At present, 16 *spp.* are critically endangered, 2 *spp.* are endangered, and 5 *spp.* are currently vulnerable (IUCN 2019). The prevalence of *Acipenseridae* on endangered species lists is primarily attributed to fragmentation of riverine habitat, commercial harvest (Bettoli et al. 2009), and slow growth and late maturation (i.e., slow population replacement, Elvira 2006). Survival during the larval phase of sturgeon is critically important for population recovery because it directly relates to year-class strength and subsequent recruitment (Hjort 1914; Houde 2008). Given their r-selected strategy (MacArthur and Wilson 1967) and type III survivorship (Demetrius 1978), sturgeon populations experience high

mortality during this key development stage (Houde 2002), compounding the issues facing population recovery. In order to restore and manage these imperiled populations, it is critically important to understand factors contributing to larval mortality.

Fish reproduction is a continuous, developmental process throughout ontogeny, requiring energetic, ecological, physiological, anatomical, and biochemical adaptations (Rocha et al. 2008). All species of sturgeon reproduce in freshwater and exhibit one of three general life history strategies; (1) a potamodromous migration where fish reproduce and reside in freshwater habitats, or anadromous migrations where fish either (2) reproduce in freshwater and reside in estuary/brackish habitat, or (3) reproduce in freshwater and reside in marine habitat (Rochard et al. 1990). Regardless of the life history strategy, all sturgeon spawn in a lotic environment and after hatch, larvae go through a key drift and dispersal stage (Deng et al. 2002; Kynard and Horgan 2002; Kynard et al. 2002a, 2007; Smith and King 2005). Post hatch behaviors and drift characteristics vary broadly among sturgeon species (Gisbert and Ruban 2003; Braaten et al. 2008) and many factors that influence survival of sturgeon larvae are poorly understood (Bemis and Kynard 1997; Kynard and Horgan 2002).

### Larval drift

Pallid Sturgeon embryos hatch as endogenously feeding larvae and exhibit a negative rheotactic behavior where they orient away from flow in order to drift downriver (Kynard et al. 2002a, 2007, Braaten et al. 2008, 2012). Negative rheotaxis is a

common behavior of many lotic fish larvae that facilitates drift and dispersal (Pavlov 1994) and is commonly observed in *Acipenseridae* larvae. For example, larval White Sturgeon *A. transmontanus* (Deng et al. 2002), Siberian Sturgeon *A. baerii* (Gisbert and Ruban 2003), Kaluga Sturgeon *Huso dauricus*, Amur Sturgeon *A. schrencki*, Chinese Sturgeon *A. sinensis* (Zhuang et al. 2002, 2003), and Shovelnose Sturgeon *Scaphirhynchus platorynchus* (Kynard et al. 2002a; Braaten et al. 2008) are negatively rheotactic immediately after hatch. Conversely, larval Lake Sturgeon *A. fulvescens* (Smith and King 2005), Green Sturgeon *A. medirostris* (Deng et al. 2002), Shortnose Sturgeon *A. brevirostrum* (Richmond and Kynard 1995), and Atlantic Sturgeon *A. oxyrinchus* (Kynard and Horgan 2002) remain positively rheotactic (benthic oriented) for the first 5 – 8 days post hatch (dph) before making a behavioral transition to negative rheotaxis, thus initiating downriver drift and dispersal later in ontogeny. A number of mechanisms have been postulated to explain downriver larval drift including redistribution to suitable rearing and(or) feeding habitat (Pavlov 1994; D'Amours et al. 2001; Lechner et al. 2016), predator avoidance (Copp et al. 2002; Humphries 2005; Usvyatsov et al. 2013; Lechner et al. 2016), and(or) reduced competition during ontogenetic development (Kynard et al. 2002b; Nathan et al. 2008; Lechner et al. 2016).

During the drift stage, larvae depend exclusively on their lipid-rich yolk sac for energy that is allocated to growth and metabolism (Kamler 2008; Deslauriers et al. 2017). Pallid Sturgeon larvae typically drift downriver for 11 – 17 days until they absorb their yolk sac and make a behavioral transition from negative to positive rheotaxis (Kynard et al. 2002a, 2007; Braaten et al. 2008, 2012). This behavioral transition is

characterized by larvae settling to the benthos, and orienting towards flow and maintaining position before transitioning to the next life stage as exogenously feeding fry (Gisbert and Ruban 2003; Nguyen and Crocker 2006). Pallid Sturgeon fry will feed primarily on aquatic invertebrates until they develop into larger juveniles and begin to prey on small fishes (Gerrity et al. 2006; Wanner et al. 2007; Grohs et al. 2009).

Changes in water velocity, temperature, and river connectivity (distance between an impoundment and the next downriver reservoir) in the Missouri River have been hypothesized to reduce available drift habitat that could impede the transition from larva to fry (Kynard et al. 2007; Braaten et al. 2008, 2012). Drift models developed for Pallid Sturgeon larvae show that distances of 304 km (Kynard et al. 2007) or 245 – 530 km (Braaten et al. 2008) are required to complete this key developmental stage. However, the longest free-flowing, unaltered stretch of the Missouri River is only 340 km, located between Fort Peck Dam (Fort Peck, MT) and the headwaters of Lake Sakakawea (near Williston, ND). Further, using an advanced one-dimensional modeling framework, Erwin et al. (2018) concluded that there is insufficient drift habitat to allow for Pallid Sturgeon to transition from larva to fry. Regardless of the model, all indicate there is a high probability that drifting larvae reach low velocity environments (e.g., reservoir headwaters) before transitioning to fry where Kynard et al. (2007) assumed survival will be low because the species is adapted to rearing in riverine habitat. For endogenously feeding larvae on a fixed energy reserve, growth is explicitly linked to metabolic requirements that are driven by water temperature (Chipps and Wahl 2008; Heironimus 2014; Boltana et al. 2017) and activity (Kamler 2008). More energy used for

metabolism (e.g., activity, time and effort searching for adequate dispersal flow) results in less energy available for growth, which may have important implications for Pallid Sturgeon recruitment in the Missouri River.

Life history differences between sympatric congeners such as Shovelnose and Pallid Sturgeon are important for exploring hypotheses related to recruitment success (Kynard et al. 2002a; Braaten et al. 2008). While recruitment is negligible in the Missouri River for Pallid Sturgeon, it is not for Shovelnose Sturgeon. Studies have shown that the transition from endogenously feeding larvae (drifting, negative rheotaxis) to exogenously feeding fry (benthic oriented, positive rheotaxis) in Shovelnose Sturgeon occurs in about half the time (6 dph) and space (94 – 250 km) as that observed for Pallid Sturgeon larvae (11 – 17 dph and 245 – 530 km, Braaten et al. 2008). Moreover, drift rate of Shovelnose larvae was about  $13 \text{ cm s}^{-1}$  slower than the mean water column velocity, whereas Pallid larvae drift rate was similar to the mean water column velocity (Braaten et al. 2008, 2012). As the only notable life history difference between Shovelnose and Pallid Sturgeon, drift time and distance may have critical implications for larval survival. Given the success of Shovelnose Sturgeon in the Missouri River, this key difference in drift extent and duration may be a reason for the lack of recruitment.

Lack of natural recruitment for Pallid Sturgeon is the ultimate cause of their endangered status. In the last 35 years, larval Pallid Sturgeon catches in the Missouri River have been extremely rare (Hrabik et al. 2007) and there has been no documented recruitment spanning back 50+ years (Braaten et al. 2015). Despite advances in propagation and rearing techniques, the early life stages of Pallid Sturgeon and the

potential factors that influence their survival in the wild are poorly understood (Bergman et al. 2008; DeLonay et al. 2016). With a lack of data surrounding the early life history of Pallid Sturgeon, it is increasingly difficult to understand, let alone predict, what the future holds for this federally endangered species. Thus, gaining a better understanding of how environmental factors (e.g., water velocity and temperature) affect Pallid Sturgeon larvae is critical given the uncertainty surrounding potential recruitment bottlenecks for this endangered species.

### Research hypotheses

Given the negative rheotactic behavior of Pallid Sturgeon larvae, I hypothesize that areas with zero to low flow will result in increased activity resulting from increased time and effort searching for adequate drift and dispersal flows. Specifically, increased activity should lead to greater energy depletion and ultimately, lower survivorship. Recognizing that fish growth is directly related to temperature-dependent metabolic requirements, I hypothesize that warmer water temperatures will lead to greater growth and metabolism, resulting in a shorter time to transition from negative to positive rheotaxis (drifting to settling), which directly relates to a transition from endogenously feeding larvae to exogenously feeding fry (Gisbert and Ruban 2003; Kynard et al. 2007; Braaten et al. 2008, 2012).

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## CHAPTER 2. EFFECT OF WATER VELOCITY AND TEMPERATURE ON ENERGY USE, BEHAVIOR, AND MORTALITY OF PALLID STURGEON *SCAPHIRHYNCHUS*

### *ALBUS* LARVAE

#### Abstract

Natural reproduction of Pallid Sturgeon *Scaphirhynchus albus* has been limited for decades and a recruitment bottleneck is hypothesized to occur during the larval stage of development. In this study, I evaluated the effects of water velocity and temperature on energy use, behavior, and mortality of endogenously feeding larvae (<25 mm TL). In the first experiment, I examined the effect of water velocity (0 – 8.3 cm s<sup>-1</sup>) on larval growth, energy density, swimming activity, and mortality. Larval growth was similar across treatments, but energy reserves of larvae reared at 0 cm s<sup>-1</sup> decreased significantly compared to larvae reared at greater velocities. Larvae maintained at zero to low water velocities exhibited significantly greater activity and mortality than those maintained at greater velocities. In the second experiment, I examined the effect of water temperature (18.7, 20.4, or 23.3 °C) on larval growth, energy density, and rheotactic behavior. Larvae reared at 23.3 °C exhibited significantly greater growth than at 18.7 or 20.4 °C. Energy reserves of larvae maintained at 23.3 °C decreased significantly compared to those maintained at cooler temperatures. Additionally, larvae reared at 23.3 °C made a significant behavioral transition from negative to positive rheotaxis (drifting to settling) in the shortest time interval. In natural environments, areas with zero to low flow may pose a significant source of

mortality to larvae, owing to their negative rheotactic behavior. The manipulation of dam releases to increase riverine temperature may aid survival of Pallid Sturgeon larvae by decreasing the extent of spatial and temporal drift, mitigating the drift habitat deficiency caused by impoundments.

## Introduction

Sturgeons (family *Acipenseridae*, 4 genera, 25 spp.) are one of the most threatened groups of vertebrates in the world (Ludwig 2006) and the factors that influence their survival during early life stages are poorly understood (Bemis and Kynard 1997; Kynard and Horgan 2002). Compounding the issue, the larval phase of freshwater fishes is characterized by high mortality (Houde 2002), and sturgeons are no exception given their r-selected reproductive strategy (MacArthur and Wilson 1967). Survival during the larval phase of sturgeon is critically important for population recovery because it directly relates to year-class strength and subsequent recruitment (Hjort 1914; Gross et al. 2002; Houde 2008). In order to restore and manage these endangered populations, it is imperative to understand factors contributing to larval mortality.

Pallid Sturgeon *Scaphirhynchus albus*, endemic to the Missouri River and lower Mississippi River basins, are one of the most threatened fishes in North America (Kallemeyn 1983; Keenlyne 1989). Prior to river channelization and construction of six main-stem impoundments (1937 – 1963), the Missouri River provided a diverse range of hydrologic variation and thermal habitat (Hesse and Mestl 1993; Pegg et al. 2003; Jacobson and Galat 2006; DeLonay et al. 2009). Anthropogenic modifications to the natural river environment resulted in reduced connectivity (e.g., migration corridors) and loss of thermal refugia and shallow water habitat (Hesse 1987; Jacobson and Galat 2006; DeLonay et al. 2016; Erwin et al. 2018), believed at the time to be responsible for

the decline of Pallid Sturgeon populations (Dryer and Sandvol 1993; Bergman et al. 2008; DeLonay et al. 2016). As a result, Pallid Sturgeon were listed as federally endangered under the Endangered Species Act in 1990 (U.S. Fish and Wildlife Service 1990, 55 FR 36641-36647).

Due to the endangered status of Pallid Sturgeon, recovery efforts have focused primarily on habitat restoration (long-term, Dryer and Sandvol 1993) and propagation and subsequent stocking of genetically diverse juveniles (short-term, Jordan et al. 2006). Specifically, the U.S. Army Corps of Engineers (USACE) was mandated to restore 20,000 acres of shallow water habitat (U.S. Fish and Wildlife Service 2003), defined as areas <1.5 m deep with mean water velocities <60 cm s<sup>-1</sup> (Gosch et al. 2013). The USACE has been restoring shallow water habitat by constructing off-channel habitats (e.g., chutes and backwaters) and(or) modifying or removing existing control structures (Gosch et al. 2013). More information is needed to better understand if these shallow water areas are providing satisfactory habitat to restore the population (Steffensen et al. 2010; DeLonay et al. 2016). Stocking of juveniles has been relatively successful in terms of survivorship and maintaining genetic diversity (DeLonay et al. 2016). However, due to the late maturation of Pallid Sturgeon (15-20 years for females, Keenlyne and Jenkins 1993), it remains unknown if stocked individuals will successfully spawn and if those progeny will survive and recruit to the adult population.

Pallid Sturgeon are an obligate riverine species with a life history strategy dependent on river connectivity. Mature adults migrate upriver and form spawning aggregates when water temperatures reach 16 – 18 °C (DeLonay et al. 2016). After egg

fertilization, embryos hatch as endogenously feeding larvae (5 – 8 days post fertilization, Snyder 2002) and exhibit a negative rheotactic behavior characterized by larvae orienting away from flow to drift and disperse downriver (Kynard et al. 2002a, 2007). Negative rheotaxis is a common behavior of many lotic fish larvae that facilitates drift and dispersal (Pavlov 1994) and is commonly seen in *Acipenseridae* larvae (Gisbert and Ruban 2003; Braaten et al. 2008). A number of mechanisms have been offered to explain downriver larval drift including redistribution to suitable rearing and(or) feeding habitat (Pavlov 1994; D'Amours et al. 2001; Lechner et al. 2016), predator avoidance (Copp et al. 2002; Humphries 2005; Usvyatsov et al. 2013; Lechner et al. 2016), and(or) reduced competition with other fishes (Kynard et al. 2002b; Nathan et al. 2008; Lechner et al. 2016). Regardless of the reason, drift behavior is ultimately an adaptation to increase larval survival during ontogeny (Pavlov 1994; Lechner et al. 2016).

Pallid Sturgeon larvae require ample distance of free-flowing river for drift and ontogenetic development prior to transitioning to their next life history stage (Kynard et al. 2002a, 2007; Braaten et al. 2008, 2012). During this life history transition, larvae expel their yolk-plug and make a behavioral transition from negative to positive rheotaxis. This behavioral change is characterized by larvae settling to the benthos, orienting towards flow, and transitioning to exogenously feeding fry (Gisbert and Ruban 2003; Nguyen and Crocker 2006). Pallid Sturgeon fry will feed primarily on aquatic invertebrates, until they develop into larger juveniles and begin to prey on small fishes (Gerrity et al. 2006; Wanner et al. 2007; Grohs et al. 2009). However, changes in water velocity, temperature, and river connectivity in the Missouri River have been

hypothesized to reduce drift habitat (i.e., free-flowing river between an impoundment and the next downriver reservoir) required by larvae and impede the transition from larva to fry. Field and laboratory studies have indicated that under existing flow and thermal regimes, the amount of available drift habitat is insufficient to allow for this life stage transition (Kynard et al. 2007; Braaten et al. 2008, 2012). Drift models developed for Pallid Sturgeon larvae indicate that distances of 245 – 600 km (Kynard et al. 2007; Braaten et al. 2008, 2012) are required to complete this key developmental stage—dependent on water velocity, temperature, and larva drift characteristics. However, the longest free-flowing, unaltered stretch of the Missouri River is only 340 km, located between Fort Peck Dam (Fort Peck, MT) and the headwaters of Lake Sakakawea (near Williston, ND). Using an advanced one-dimensional modeling framework, Erwin et al. (2018) concluded that Pallid Sturgeon embryos hatching near Fort Peck Dam will likely reach Lake Sakakawea before completing the larval stage. Regardless of the model, all indicate there is a high probability that drifting larvae reach low velocity environments (e.g., reservoir headwaters) before transitioning to fry where Kynard et al. (2007) assumed survival will be low because the species is adapted to rearing in riverine habitat. Thus, there is strong support for the notion that drift habitat deficiency is a limiting factor for Pallid Sturgeon in the Missouri River.

Pallid Sturgeon are not the only member of the *Acipenseridae* family that inhabits the Missouri River. Shovelnose Sturgeon *Scaphirhynchus platyrhynchus* are thriving throughout the Missouri River basin. Age-0 Shovelnose Sturgeon are collected annually (Braaten and Fuller 2007; Braaten et al. 2007) with multiple year-classes

present in the population (Quist et al. 2002). Though these sympatric species share many commonalities, a key difference arises in their early life history, which aids in exploring hypotheses related to recruitment success (Kynard et al. 2002a; Braaten et al. 2008). Pallid Sturgeon larvae require roughly twice the temporal and spatial extent to transition from endogenously feeding larvae (drifting, negative rheotaxis) to exogenously feeding fry (benthic oriented, positive rheotaxis) than Shovelnose Sturgeon (11 – 17 days post hatch (dph) and 245 – 530 km for Pallid Sturgeon versus 6 dph and 94 – 250 km for Shovelnose Sturgeon, Braaten et al. 2008). Further, Shovelnose larvae drift about  $13 \text{ cm s}^{-1}$  slower than the mean water column velocity whereas Pallid larvae drift at rates similar to the mean water column velocity (Braaten et al. 2008, 2012). Thus, Pallid larvae are dispersed further downriver per unit time than Shovelnose larvae. As the only notable life history difference between Shovelnose and Pallid Sturgeon, drift duration and distance may have critical implications for species recovery. Given the success of Shovelnose Sturgeon in the Missouri River, this key difference in drift extent may be a reason for the lack of recruitment of Pallid Sturgeon.

During the drift stage, *Scaphirhynchus spp.* larvae rely exclusively on their lipid-rich yolk sac for growth and metabolism (Kamler 2008; Deslauriers et al. 2017a). Alterations to river connectivity and flow regime have affected Pallid Sturgeon larvae by limiting the amount of available drift habitat, which is substantially greater than that required by their successful congener, Shovelnose Sturgeon (Kynard et al. 2007, Braaten et al. 2008). For endogenously feeding larvae on a fixed energy reserve, growth is explicitly linked to metabolic requirements that are driven by water temperature

(Chipps and Wahl 2008; Heironimus 2014; Boltana et al. 2017) and activity (Kamler 2008). More energy used for activity, such as swimming/searching for adequate dispersal flows in zero to low flow environments (e.g., reservoir headwaters, back waters, side channels), results in less energy available for growth that may have important implications for Pallid Sturgeon recruitment in the Missouri River.

Similar to river connectivity, water temperature is critical for early life development and success in sturgeons (Armour 1991; Detlaff et al. 1993; Bolker 2004). For Pallid Sturgeon, increased water temperature has been associated with reduced larval drift distance and increased growth (Kynard et al. 2007; Braaten et al. 2008, 2012). However, the hypothesis that warmer temperatures can result in a quicker behavioral transition from negative rheotaxis (drifting larvae) to positive rheotaxis (benthic oriented fry) has never been explicitly tested. Further, the affect temperature has on larval Pallid Sturgeon mortality is unknown. Given the management option of river temperature manipulation via dam releases (U.S. Fish and Wildlife Service 2003; U.S. Army Corps of Engineers and U.S. Fish and Wildlife Service 2018), a temperature increase for the system may mitigate issues surrounding the drift habitat deficiency by increasing larval development rate. Increased development rates may lead to a faster behavioral transition from negative rheotaxis to positive rheotaxis, thus reducing the spatial and temporal drift extent required by Pallid Sturgeon larvae in the Missouri River.

Lack of natural recruitment for Pallid Sturgeon is the ultimate cause of their endangered status. In the last 35 years, larval Pallid Sturgeon catches in the Missouri

River have been extremely rare (Hrabik et al. 2007) and there has been no documented recruitment spanning back 50+ years (Braaten et al. 2015). Despite advances in propagation and rearing techniques, the early life stages of Pallid Sturgeon and the potential factors that influence their survival in the wild are poorly understood (Bergman et al. 2008; DeLonay et al. 2016). With a lack of data surrounding the early life history of Pallid Sturgeon, it is increasingly difficult to understand, let alone predict, what the future holds for this federally endangered species. Thus, gaining a better understanding of how environmental factors (e.g., water velocity and temperature) affect Pallid Sturgeon larvae is critical given the uncertainty surrounding potential recruitment bottlenecks.

Impoundments built on the Missouri River have resulted in reduced connectivity (Hesse 1987; Hesse and Mestl 1993; Jacobson and Galat 2006) and a cooler thermal regime (Heironimus 2014; DeLonay et al. 2016; Erwin et al. 2018). Given the negative rheotactic behavior of Pallid Sturgeon larvae, I hypothesize that areas with zero to low flow will result in increased activity resulting from increased time and effort searching for adequate drift and dispersal flows. Specifically, increased activity should lead to greater energy depletion and ultimately, lower survivorship. Because fish growth is directly related to temperature-dependent metabolic requirements (Chipps and Wahl 2008; Boltana et al. 2017), I hypothesize that warmer water temperatures will lead to greater growth and metabolism, resulting in a shorter time to transition from negative to positive rheotaxis (drifting to settling), which directly relates to a transition from endogenously feeding larvae to exogenously feeding fry (Gisbert and Ruban 2003;

Kynard et al. 2007; Braaten et al. 2008, 2012). To test these hypotheses, I conducted microcosm experiments during May – July of 2017 and 2018. The goal of my study was to assess and quantify the effects of water velocity and temperature on energy use, behavior, and mortality of endogenously feeding Pallid Sturgeon larvae. Research objectives for the first experiment were to test for differences in (1) growth rate, (2) energy depletion, (3) swimming activity, and (4) time (days post hatch, dph) to 100% mortality among three water velocity treatments. Objectives for the second experiment were to test for differences in (1) growth rate, (2) energy depletion, and (3) time (dph) for larvae to make a behavioral transition from negative to positive rheotaxis (drifting to settling).

## Methods

### *Influence of water velocity on growth, energy density, swimming activity, and mortality of Pallid Sturgeon larvae*

#### Experimental design

To simulate riverine conditions, I designed a microcosm system using 115 L oval, black polyethylene tanks (90 x 64 x 30 cm). Each tank contained an outflow standpipe (5.1 cm diameter) covered with a 7.6 cm diameter mesh screen (Figure 1A). A recirculating, biofiltration system was constructed by placing two experimental tanks inside a larger 454 L containment tank (244 x 64 x 30 cm; Figure 1B). One water pump (120 V, Aquaclear 70, Hagen Inc., Mansfield, MD) was mounted on the outside of each experimental tank and used to circulate dechlorinated water between the containment

tank and experimental tank (Figure 1B). Plastic biofilters and one aerator were placed on each end of the containment tank, near the water pumps, and water discharge into each tank was controlled using 1.9 cm metered ball valve. A PVC spray bar (1.9 cm diameter x 25.4 cm) was mounted vertically in each tank and drilled with five equally spaced 0.63 cm diameter holes to homogenize flow distribution in the tank (Figure 1A).

I used three water velocity treatments at a water temperature of  $16.3 \pm 0.04$  °C to evaluate effects on growth, energy depletion, and mortality of endogenously feeding Pallid Sturgeon larvae. Treatments included (1) zero velocity ( $0 \text{ cm s}^{-1}$ ), (2) intermediate velocity ( $1 - 8 \text{ cm s}^{-1}$ ), or (3) high velocity ( $2 - 20 \text{ cm s}^{-1}$ ) and were randomly assigned to a total of 12 tanks ( $n = 4$  replicate tanks/treatment). Water velocity was measured at six points along the minor axis of each tank (Figure 1B) using a Marsh-McBirney flow meter (Flo Mate model 2000, Marsh-McBirney Inc., Frederick, MD). Measurements were taken at mid-water column at the beginning and end of the experiment, and then averaged to estimate a mean water velocity value for each tank and treatment. Water temperature was recorded daily adjacent to the water pump for each experimental tank using a YSI portable meter (Pro10, Yellow Springs Instruments Inc., Yellow Springs, OH). The system was maintained on a natural photoperiod ( $44.3114^\circ \text{ N}$ ,  $96.7984^\circ \text{ W}$ ) using full-spectrum artificial lighting.

To quantify the influence of water velocity on larval swimming activity, I used data collected from a related study in 2013 (L.B. Heironimus, unpublished data). This laboratory experiment used a recirculating aquaculture system comprised of 120 L circular tanks (58 x 46 cm). Each tank was equipped with a screened overflow that

drained to a vertical sediment settling column before returning water to a 568 L biofiltration tank. Water entering the biofiltration tank was filtered through bio-media before passing to a second compartment containing a heating/chilling unit and water pump intake. Water was then pumped through a large UV sterilizer and returned to the experimental tanks. Water temperature in the recirculating system was held at  $17 \pm 0.5$  °C and photoperiod was set to natural conditions ( $44.3114^\circ$  N,  $96.7984^\circ$  W) using full-spectrum artificial lighting.

Two water velocity treatments, representing either static ( $0 - 1 \text{ cm s}^{-1}$ ) or flowing ( $1 - 5 \text{ cm s}^{-1}$ ) conditions were randomly assigned to a total of 10 tanks ( $n = 5$  replicate tanks/treatment). In each tank, water velocity was controlled using metered PVC ball valves attached to a capped PVC spray bar that extended down into the tank. To maximize water velocities in the flow treatment tanks, ball valves were opened approximately 85% and PVC spray bars were drilled with eight equally spaced 3.2 cm diameter holes and set parallel to tank wall. To reduce velocities in the static treatment tanks, while allowing fresh water recirculation, ball valves were opened approximately 15% and PVC spray bars were drilled with eight equally spaced 4.8 cm diameter holes on all four sides of the arm. To characterize flow conditions in static and flow tanks, water velocity was measured using a Marsh-McBirney flow meter along a transect extending from the center of the tank to the outside edge. Measurements were taken at three points along the radius that included the center of the tank, 50% radius length, and 100% radius length (i.e., outside edge). At each location, water velocity was measured

at the surface, mid-water column, and bottom of the tank. Measurements were then averaged to obtain a mean water velocity value for each tank and treatment.

#### Energy use and mortality

I obtained larval Pallid Sturgeon from the U.S. Fish & Wildlife Service, Garrison Dam National Fish Hatchery (Riverdale, ND) that were produced from wild-caught adults captured in the Upper Missouri River Basin. Pallid Sturgeon larvae from genetically unique family crosses ( $n = 7$ ) were randomly sorted into large plastic bags containing oxygenated water and transported to the Fisheries Research Laboratory at South Dakota State University (Brookings, SD) on 3 July 2017 at 1 day post hatch (dph). Upon arrival, larvae were acclimated to the water temperature in the experimental tanks (16.3 °C) and then removed from the bags in batches using a small dip net, weighed wet, and stocked into the oval experimental tanks at a mean stocking density of 2,028 (SE = 14.8) larvae per tank ( $n = 12$ ).

I collected larvae ( $n = 40$ ) at 3 or 4 dph from each tank and repeated this sampling procedure at two-day intervals (i.e., an individual tank was sampled every other day). Dead larvae were removed daily and I recorded the time (dph) for the population in each tank to reach 100% mortality. On each sample date, I measured larvae for total length (TL) to the nearest mm and obtained a total wet weight (nearest 0.0001 g) for each sample by weighing a plastic tube containing a mesh filter and the larvae, removing the larvae, and then reweighing the tube and filter. Larval samples were then placed in cleaned, pre-weighed aluminum tins, dried at 65 °C for at least 24 h,

and then cooled in a desiccator. After desiccating, the tin and larvae were weighed and that weight was subtracted from the pre-weighed tin to obtain a total dry weight (nearest 0.0001 g) for each sample. Using the ratio of dry-to-wet weight from each sample, I estimated energy density of larvae (*ED*) using a regression model developed by Deslauriers et al. (2016),

$$ED = -1,077 + DW(27,683.2),$$

where *ED* is larval energy density (J/g wet weight) and *DW* is the dry-to-wet weight ratio (expressed as a proportion) of the larvae.

### Swimming activity

Larval pallid sturgeon used in swimming activity experiments were progeny of three wild-caught, adult females spawned with male broodstock at the U.S. Fish & Wildlife Service, Gavins Point National Fish Hatchery (Yankton, SD). Larvae were randomly sorted into large plastic bags filled with oxygenated water and transported to the Fisheries Research Laboratory at South Dakota State University on 22 June 2013 at 2 dph. Upon arrival, larvae were acclimated to 17 °C and stocked into experimental tanks at a density of approximately 300 larvae per tank.

I quantified swimming activity of Pallid Sturgeon larvae using videography. At 4 dph, swimming behavior of larvae was recorded in each tank using a digital camera (GoPro HERO3, GoPro Inc., San Mateo, CA) mounted above the tank that allowed for viewing of all larvae. To quantify swimming behaviors, larvae were recorded for 2.5 minutes and the footage was used to quantify swimming duration ( $n = 5$  larvae/tank)

and time between swimming events ( $n = 5$  larvae/tank). Swimming duration ( $S_D$ ) for a randomly selected larva was quantified as,

$$S_D (s) = T_{1S} - T_{0S},$$

where  $T_{0S}$  is the initial time of a swimming event and  $T_{1S}$  is end time (nearest 0.01 s) of the same swimming event. Similarly, the time between swimming events ( $S_E$ ) for a randomly selected larva was quantified as,

$$S_E (s) = T_{2E} - T_{1E},$$

where  $T_{1E}$  is the end time (nearest 0.01 s) of a swimming event and  $T_{2E}$  is the start time of the successive swimming event. Only swimming events that lasted  $>0.5$  s were included in the analysis because brief swimming motions ( $\leq 0.5$  s) were associated with larvae correcting their body position rather than actively swimming in the water column.

### Statistical analysis

I used an analysis of covariance (ANCOVA) to evaluate the effect of water velocity on larvae total length (TL, mm) and energy density (J/g wet weight) using time (dph) as a covariate. A one-way analysis of variance was used to evaluate the effect of water velocity on mean swimming duration, mean time between swimming events, and mean time to 100% mortality. All analyses were conducted using the FSA (v.0.8.18, Ogle 2017) and ggplot2 (v.2.2.1, Wickham 2016) packages in the R<sup>TM</sup> statistical environment (v3.4.3, R Development Core Team 2017). All statistical tests used  $\alpha = 0.1$  to determine statistical significance.

*Influence of water temperature on growth, energy density, and rheotactic behavior of Pallid Sturgeon larvae*

Experimental design

I used three water temperature treatments at a water velocity averaging  $8.8 \text{ cm s}^{-1}$  ( $3 - 16 \text{ cm s}^{-1}$ ) to evaluate effects on growth and energy depletion of endogenously feeding Pallid Sturgeon larvae as well as the time (dph) for larvae to exhibit positive rheotaxis (i.e., settle). Treatments included (1) low temperature ( $18.7 \pm 0.01 \text{ }^\circ\text{C}$ ), (2) medium temperature ( $20.4 \pm 0.02 \text{ }^\circ\text{C}$ ), or (3) high temperature ( $23.3 \pm 0.02 \text{ }^\circ\text{C}$ ) and were randomly assigned to a total of 18 tanks ( $n = 6$  replicate tanks/treatment). The microcosm system depicted in Figure 1 was used as the experimental arena with the only difference being the addition of one submersible heating tube (115 V, 800 W, Finnex Titanium Heating Tube, Finnex Inc., Chicago, IL) in each of the containment tanks (Figure 1B). The heating tubes were connected to a digital controller that allowed for precise temperature control ( $\pm 0.2 \text{ }^\circ\text{C}$ ). The same protocol described earlier was followed to obtain water velocity and temperature measurements. The system was maintained on a natural photoperiod ( $44.3114^\circ \text{ N}$ ,  $96.7984^\circ \text{ W}$ ) using full-spectrum artificial lighting.

Energy use

I obtained larval Pallid Sturgeon (Gavins Point National Fish Hatchery) produced from hatchery-reared adults. Larvae from genetically unique family crosses ( $n = 6$ ) were randomly sorted into large plastic bags filled with oxygenated water and transported to

the Fisheries Research Unit Laboratory at South Dakota State University on 7 June 2018 at 1 dph. Upon arrival, larvae were acclimated, weighed wet, and stocked into the experimental tanks as described earlier. Mean stocking density was 2,042 (SE = 5.9) larvae per tank (n = 18).

I collected larvae (n = 20) at 2 dph from every tank and repeated this sampling procedure daily. The same protocol described earlier was used to obtain larval lengths, wet and dry weights, energy densities, and to assess mortality.

#### Rheotactic behavior

Unique to this experiment, time (dph) for larvae to make a behavioral transition from negative to positive rheotaxis (drifting to settling) was quantified by direct observation. Each day, two people (both experienced with larval Pallid Sturgeon) independently recorded an estimate for the percent of larvae (5% intervals) exhibiting positive rheotaxis in each experimental tank. Larvae were considered as being positively rheotactic when they were on the bottom of the tank, oriented towards the flow and maintaining position (Gisbert and Ruban 2003; Nguyen and Crocker 2006). The day that observers agreed  $\geq 95\%$  of larvae were exhibiting positive rheotaxis was recorded as the time (dph) for the behavioral transition from negative to positive rheotaxis for larvae in that tank. Digital photographs (Nikon D60, Nikon Inc., Melville, NY) were taken of larvae each day to corroborate observations.

#### Statistical analysis

I used an ANCOVA to evaluate the effect of water temperature on larvae total length (TL, mm) and energy density (J/g wet weight) using time (dph) as a covariate. In cases where data were not normally distributed, I  $\log_{10}$ -transformed the response variable for analysis. I then estimated slopes and associated confidence intervals using non-linear regression analyses of TL or energy density modeled against dph to compare larval growth and energy depletion rate among water temperatures. Regression analysis was used to model the effect of water temperature on settling time (dph) of Pallid Sturgeon larvae (i.e., amount of time for  $\geq 95\%$  of larvae to exhibit positive rheotaxis). All analyses were conducted using the FSA (v.0.8.18, Ogle 2017) and ggplot2 (v.2.2.1, Wickham 2016) packages in the R<sup>TM</sup> statistical environment (v3.4.3, R Development Core Team 2017). All statistical tests used  $\alpha = 0.1$  to determine statistical significance.

## Results

### *Influence of water velocity on growth, energy density, swimming activity, and mortality of Pallid Sturgeon larvae*

Larval Pallid Sturgeon total length (TL, mm) increased linearly during the experiment ( $F_{1, 179} = 100, P < 0.001$ ) and was similar among velocity treatments ( $F_{2, 179} = 0.18, P = 0.83$ ). Mean TL was 12.6 mm (95% CI, 12.3 to 12.9) for larvae reared at velocities averaging 0 – 8.3  $\text{cm s}^{-1}$ . I found no significant interaction effect between water velocity and dph on larvae TL among water velocity treatments ( $F_{2, 179} = 0.35, P =$

0.7). Mean growth rate was  $0.97 \text{ mm d}^{-1}$  for larvae reared at  $0 - 8.3 \text{ cm s}^{-1}$ . Larval TL as a function of time can be estimated as,

$$TL = 7.1102 + dph(0.968),$$

where  $TL$  is total length (mm) and  $dph$  is the age (days post hatch) of endogenously feeding Pallid Sturgeon larvae (linear regression;  $F_{1, 18} = 804.5$ ,  $P < 0.001$ ,  $r^2 = 0.98$ ; Figure 2).

Energy density (J/g wet weight) of Pallid Sturgeon larvae reared at velocities of  $0 - 8.3 \text{ cm s}^{-1}$  decreased linearly during the experiment ( $F_{1, 31} = 69.2$ ,  $P < 0.001$ ) and varied among treatments ( $F_{2, 31} = 2.64$ ,  $P = 0.08$ ). Mean energy density was  $5,739 \text{ J/g wet weight}$  (95% CI,  $4,629$  to  $6,849$ ),  $5,276 \text{ J/g wet weight}$  ( $4,447$  to  $6,105$ ) and  $4,766 \text{ J/g wet weight}$  ( $3,907$  to  $5,625$ ) at water velocities averaging  $0$ ,  $3.5$ , or  $8.3 \text{ cm s}^{-1}$ , respectively. I found a significant interaction effect of water velocity and  $dph$  on larvae energy density ( $F_{2, 31} = 2.62$ ,  $P = 0.08$ ), implying that rate of energy depletion varied among water velocities. Tests of homogeneity of slopes revealed that energy depletion was similar for larvae reared at  $3.5$  to  $8.3 \text{ cm s}^{-1}$  ( $F_{1, 31} = 0.05$ ,  $P = 0.82$ ), but was significantly greater among larvae reared at  $0 \text{ cm s}^{-1}$  ( $F_{1, 31} = 5.25$ ,  $P = 0.02$ ; Figure 3). Average energy depletion rate for larvae reared at  $0 \text{ cm s}^{-1}$  was  $-938.82 \text{ J g}^{-1} \text{ d}^{-1}$  (95% CI,  $-608.37$  to  $-1,267.27$ ) while larvae reared at  $3.5$  or  $8.3 \text{ cm s}^{-1}$  had a mean energy depletion rate of  $-653.11 \text{ J g}^{-1} \text{ d}^{-1}$  ( $-538.07$  to  $-759.21$ ). Energy depletion as a function of time for larvae maintained at  $0 \text{ cm s}^{-1}$  can be estimated as (linear regression;  $F_{1, 3} = 31$ ,  $P = 0.01$ ,  $r^2 = 0.91$ ),

$$ED = 10,433.6 + dph(-938.8).$$

Energy depletion as a function of time for larvae maintained at 3.5 or 8.3 cm s<sup>-1</sup> can be estimated as (linear regression;  $F_{1,13} = 330.3$ ,  $P < 0.001$ ,  $r^2 = 0.96$ ),

$$ED = 8,895.74 + dph(-653.11),$$

where *ED* represents energy density (J/g wet weight) and *dph* is the age (days post hatch) of endogenously feeding Pallid Sturgeon.

Swimming behaviors of Pallid Sturgeon larvae maintained at lower water velocities (0.08 cm s<sup>-1</sup>) differed from larvae reared at higher velocities (2.20 cm s<sup>-1</sup>). I found significant effects of water velocity on average swimming duration ( $F_{1,8} = 93.4$ ,  $P < 0.001$ ) and average time between swimming events for larvae maintained at 0.08 cm s<sup>-1</sup> or 2.20 cm s<sup>-1</sup> ( $F_{1,8} = 215.8$ ,  $P < 0.001$ ; Table 1). Larvae maintained at 0.08 cm s<sup>-1</sup> exhibited significantly longer swimming durations (range, 4.62 – 15.96 s) and significantly shorter time between swimming events (0.88 – 3.20 s) compared to larvae reared at 2.20 cm s<sup>-1</sup> (1.59 – 7.53 and 3.55 – 9.02 s, respectively).

Similar to findings for energy depletion and swimming activity, I found a significant effect of water velocity on time (dph) to 100% mortality for Pallid Sturgeon larvae ( $F_{2,9} = 22.5$ ,  $P < 0.001$ ). Average time to 100% mortality ranged from 7.3 – 9.8 dph and was statistically different among treatments ( $P < 0.02$ ; Table 2). Larval mortality was inversely related to mean water velocity in the experimental tanks.

*Influence of water temperature on growth, energy density, and rheotactic behavior of Pallid Sturgeon larvae*

Total length (TL) of Pallid Sturgeon larvae, expressed as  $\log_{10}$  mm, increased during the experiment ( $F_{1, 1,499} = 1,772, P < 0.001$ ) and varied among temperature treatments ( $F_{2, 1,499} = 7.5, P < 0.001$ ). Mean  $\log_{10}$  TL was back-transformed to be 18.0 mm (95% CI, 17.7 to 18.4), 18.2 mm (17.9 to 18.6) and 19.2 mm (18.8 to 19.6) at water temperatures averaging 18.7, 20.4 or 23.3 °C, respectively. I found a significant interaction effect of water temperature and dph on larval  $\log_{10}$  TL ( $F_{2, 1,499} = 24.62, P < 0.001$ ), implying that growth rate varied among treatments. Slopes and associated confidence intervals from non-linear regression analyses of mean larval TL modeled against dph revealed that larval growth rate was significantly greater at 23.3 °C compared to 18.7 or 20.4 °C, but did not differ between larvae maintained at 18.7 or 20.4 °C (Table 3). Larvae reared at 23.3 °C exhibited a mean growth rate of 0.85 mm  $d^{-1}$ , compared to growth rates of 0.55 and 0.65 mm  $d^{-1}$  at 18.7 or 20.4 °C, with most growth occurring between 2 – 10 dph in each treatment (Figure 4). Larval TL can be estimated as a function of time for each water temperature ( $TL_i$ ) as,

$$TL_{18.7} = 22.334 - 17.542 e^{-0.17146(dph)},$$

$$TL_{20.4} = 23.066 - 18.114 e^{-0.17828(dph)},$$

or

$$TL_{23.3} = 22.999 - 22.008 e^{-0.29087(dph)},$$

where  $TL_i$  is total length (mm) at temperature  $i$  for 18.7 °C ( $F_{2, 18} = 1,026, P < 0.001, r^2 = 0.99$ ), 20.4 °C ( $F_{2, 16} = 895.24, P < 0.001, r^2 = 0.99$ ), or 23.3 °C ( $F_{2, 12} = 778.22, P < 0.001, r^2 =$

= 0.99) and *dph* is the age (days post hatch) of endogenously feeding Pallid Sturgeon larvae (Figure 4).

Energy density of Pallid Sturgeon larvae, expressed as  $\log_{10}$  J/g wet weight, decreased with time ( $F_{1, 295} = 2,464$ ,  $P < 0.001$ ) and was similar among water temperatures ( $F_{2, 295} = 0.68$ ,  $P = 0.5$ ). Mean  $\log_{10}$  energy density was back-transformed to be 2,265 J/g wet weight (95% CI, 1,990 to 2,578), 2,317 J/g wet weight (2,013 to 2,667) and 2,118 J/g wet weight (1,810 to 2,478) at water temperatures averaging 18.7, 20.4 or 23.3 °C, respectively. However, I documented a significant interaction effect of water temperature and *dph* on larval  $\log_{10}$  energy density ( $F_{2, 295} = 61.71$ ,  $P < 0.001$ ), implying that rate of energy depletion varied inconsistently among treatments. Non-linear regression analyses of mean larval energy density versus *dph* revealed that energy depletion rate varied significantly among water temperature treatments (Table 4). Energy depletion rate was lower for larvae reared at 18.7 °C (mean =  $-375.08 \text{ J g}^{-1} \text{ d}^{-1}$ ) compared to larvae reared at 20.4 °C ( $-412.38 \text{ J g}^{-1} \text{ d}^{-1}$ ) and was greatest for larvae reared at 23.3 °C ( $-527.24 \text{ J g}^{-1} \text{ d}^{-1}$ ). Energy depletion as a function of time for larvae reared at each water temperature ( $ED_i$ ) could be estimated as,

$$ED_{18.7} = 567.4 + 11,355e^{-0.1796(dph)},$$

$$ED_{20.4} = 687.93 + 12,266e^{-0.22157(dph)},$$

or

$$ED_{23.3} = 624.02 + 13,367e^{-0.28083(dph)},$$

where  $ED_i$  represents energy density (J/g wet weight) at temperature  $i$  of 18.7 °C ( $F_{2,18} = 2,380$ ,  $P < 0.001$ ,  $r^2 = 0.99$ ), 20.4 °C ( $F_{2,16} = 2,697$ ,  $P < 0.001$ ,  $r^2 = 0.99$ ), or 23.3 °C ( $F_{2,12} = 4,332$ ,  $P < 0.001$ ,  $r^2 = 0.99$ ), and dph is the age (days post hatch) of endogenously feeding Pallid Sturgeon larvae (Figure 5).

The amount of time (dph) for Pallid Sturgeon larvae to make a behavioral transition from negative to positive rheotaxis (drifting to settling) was inversely related to water temperature. I found a significant relationship between water temperature and time for larvae to become positively rheotactic (linear regression;  $F_{1,16} = 76.24$ ,  $P < 0.001$ ,  $r^2 = 0.83$ ; Figure 6). Larvae made this behavioral transition significantly faster at 23.3 °C and took the longest amount of time at 18.7 °C. Time to positive rheotaxis ( $PR_t$ , in dph) for endogenously feeding Pallid Sturgeon larvae reared at 8.8 cm s<sup>-1</sup> can be estimated as,

$$PR_t = 24.53 + T(-0.85),$$

where  $T$  is water temperature (°C). The mean (SE) dph for larvae to exhibit positive rheotaxis was 8.7 (0.4), 6.8 (0.3), and 4.7 (0.2) at water temperatures of 18.7, 20.4, or 23.3 °C, respectively. Related, due to no additional food sources provided in the microcosm, the mean (SE) dph for larvae to reach 100% mortality was 21.7 (0.4), 20.3 (0.3), and 16.0 (0.3) at water temperatures of 18.7, 20.4, or 23.3 °C, respectively.

## Discussion

*Influence of water velocity on energy use, behavior, and mortality of Pallid Sturgeon larvae*

The deficiency of drift habitat available to Pallid Sturgeon larvae in the Missouri River is a result of the river being fragmented by impoundments (Kynard et al. 2007; Braaten et al. 2008, 2012; Erwin et al. 2018) that has resulted in more lentic habitat than what was present during historic conditions (Hesse 1987; Hesse and Mestl 1993; Jacobson and Galat 2006). Further, the USACE's mandate to restore 20,000 acres of shallow water habitat has the potential to be creating more areas of zero or low velocity in the Missouri River because the definition of shallow water habitat includes a maximum mean velocity ( $<60 \text{ cm s}^{-1}$ ), but no minimum velocity (U.S. Fish and Wildlife Service 2003; Gosch et al. 2013). This is problematic because results from this study show that Pallid Sturgeon larvae exhibit greater mortality in zero to low flow environments than in flowing environments. Indeed, I found that activity level, endogenous energy use, and mortality were greatest at low water velocities. Because larvae are negatively rheotactic, areas without adequate drift habitat will result in increased activity and energy consumption, leading to reduced survivorship before larvae can transition to benthic oriented, exogenously feeding fry. I recommend that water velocities  $>10 \text{ cm s}^{-1}$  be considered during the construction of shallow water habitat.

A common assumption that there are less energetic demands at lower water velocities is true for riverine species that swim into or maintain their position within a lotic environment (positive rheotaxis, e.g., fry, juvenile, and adult *Acipenseridae*), but is not the case for drifting larvae exhibiting negative rheotaxis. That assumption could be argued by examining growth data alone, but growth is only one component of a fish's

energy budget. Energy consumed by a fish (e.g., depletion of endogenous energy reserves such as a yolk sac) is balanced by energy expended for total metabolism (respiration + active metabolism + specific dynamic action), waste losses (egestion + excretion), and growth (somatic + gonadal, Deslauriers et al. 2017a). Even though there was no difference in growth in length among velocity treatments, results show greater energy depletion rates and levels of activity for larvae rearing in systems with zero to low flow. Assuming that variation in specific dynamic action (energy required to digest food) and waste loss are negligible due to the same source of energy being used (endogenous yolk sac), the differences in rates of energy depletion are likely attributable to elevated metabolism, specifically active metabolism associated with increased larval activity (Fry 1947; Kamler 2008). Given a fish's energy budget, increases in activity require more energy to be consumed (Fry 1947; Deslauriers et al. 2017a). During this life stage, Pallid Sturgeon larvae are endogenous feeders relying on a fixed energy reserve, when reserves are depleted, the need for more energy consumption can only be met by transitioning to exogenously feeding fry.

Reduced survivorship of larvae maintained at  $0 \text{ cm s}^{-1}$  was attributed to greater energy depletion caused by increased activity associated with their negative rheotactic behavior (i.e., time and effort searching for drift flow). In contrast, larvae that are actively drifting can divert more energy towards ontogenetic development rather than active metabolism, resulting in a more efficient use of limited energy reserves. The difference in time to 100% mortality between larvae reared at  $3.5$  or  $8.3 \text{ cm s}^{-1}$  likely occurred because at lower velocities, larvae have a greater potential to drop out of the

flow, requiring an energy expenditure to reenter the drift. Ultimately, increases in activity within zero to low flow systems caused Pallid Sturgeon larvae to deplete their energy reserves at a faster rate, leading to greater mortality.

Pallid Sturgeon larvae reared at  $0 \text{ cm s}^{-1}$  may have experienced an initial period, within the first 3 – 4 dph, of low activity as evidenced by greater energy density (mean =  $7,838 \text{ J/g}$  wet weight) compared to larvae reared at  $3.5 \text{ cm s}^{-1}$  ( $7,103 \text{ J/g}$  wet weight) or  $8.3 \text{ cm s}^{-1}$  ( $7,317 \text{ J/g}$  wet weight). This provides evidence that the species' negative rheotactic behavior is not initiated immediately after hatch, as concluded by Kynard et al. (2002a, 2007). Negative rheotaxis in systems without adequate dispersal flows would have resulted in increased activity and energy consumption. If this behavior was evident, the 24 hours (2 – 3 dph) before first sampling should have resulted in a lower initial energy density for larvae reared at  $0 \text{ cm s}^{-1}$ . If negative rheotaxis is not initiated immediately following hatch, larvae would conserve energy by spending less time being active (e.g., swimming/searching for flows). More support for delayed drift can be observed by examining cumulative energy loss over time. Larvae reared at  $0 \text{ cm s}^{-1}$  had a unique pattern of energy loss that occurred between 4 – 5 dph where they lost an average of  $-1,771 \text{ J g}^{-1} \text{ d}^{-1}$  (Figure 7). This was greater than the largest two-day energy depletion event for larvae reared at  $3.5 \text{ cm s}^{-1}$  ( $-1,393 \text{ J g}^{-1} \text{ d}^{-1}$ , 4 – 5 dph) or  $8.3 \text{ cm s}^{-1}$  ( $-1,107 \text{ J g}^{-1} \text{ d}^{-1}$ , 3 – 4 dph; Figure 7). The large energy expenditure is likely correlated to greater swimming activity documented for 4 dph larvae at very low velocities ( $0.08 \text{ cm s}^{-1}$ ). Longer swimming durations and shorter time between swimming events for larvae in zero to low flow systems suggests a negative rheotactic behavior whereby they are

attempting to initiate drift and dispersal (Gisbert and Ruban 2003; Nguyen and Crocker 2006).

Negative rheotaxis after hatch is a common behavior found in endogenously feeding *Acipenseridae* larvae. For example, larval White Sturgeon *A. transmontanus* (Deng et al. 2002), Siberian Sturgeon *A. baerii* (Gisbert and Ruban 2003), Kaluga Sturgeon *Huso dauricus*, Amur Sturgeon *A. schrencki*, Chinese Sturgeon *A. sinensis* (Zhuang et al. 2002, 2003), and Shovelnose Sturgeon (Kynard et al. 2002a; Braaten et al. 2008) all exhibit downstream drift and dispersal shortly after hatching. Conversely, delayed drift has been documented for some *Acipenseridae* species, but is less common. For example, larval Lake Sturgeon *A. fulvescens* (Smith and King 2005), Green Sturgeon *A. medirostris* (Deng et al. 2002), Shortnose Sturgeon *A. brevirostrum* (Richmond and Kynard 1995), and Atlantic Sturgeon *A. oxyrinchus* (Kynard and Horgan 2002) are positively rheotactic for the first 5 – 8 dph and then make a behavioral transition to negative rheotaxis, initiating downriver drift and dispersal later in ontogeny. Although a paucity of literature hypothesizing potential reasons can be found, delayed drift may be a mechanism during ontogenetic development to shorten the required drift extent and(or) to conserve energy during early life (as hypothesized in this study). Delayed drift may also indicate a low predation risk at hatch sites because natural selection would favor immediate drift and dispersal when predation is high (Kynard and Horgan 2002). Regardless, this behavior is an adaptation to increase larval survival during ontogeny (Pavlov 1994; Lechner et al. 2016). The delayed drifting behavior observed for Pallid Sturgeon larvae in this study provides evidence for an adaptation to conserve

energy when adequate drift flows are not present. However, when Pallid Sturgeon spend the entire larval phase in zero to low flow environments, this adaptation becomes a detriment that results in greater mortality.

*Influence of water temperature on energy use and behavior of Pallid Sturgeon larvae*

Impoundment of the Missouri River has resulted in a colder thermal regime (Braaten et al. 2012; Heironimus 2014; Figure 8) where effects of cold water (hypolimnetic) releases can suppress riverine temperatures for over 300 km downriver (Sherman et al. 2007; DeLonay et al. 2016; Erwin et al. 2018). A cooler thermal regime is believed to increase the drift extent requirements of Pallid Sturgeon larvae by slowing down metabolic processes and associated development rates (Kynard et al. 2007; Braaten et al. 2008, 2012; Deslauriers et al. 2017b; this study). Larvae had greater energy use and growth in length at warmer water temperatures, which resulted in a faster behavioral transition from negative to positive rheotaxis (drifting to settling). This behavioral transition directly relates to a life history transition from endogenously feeding larvae to exogenously feeding fry (Gisbert and Ruban 2003; Kynard et al. 2007; Braaten et al. 2008, 2012). Less time spent drifting also means less drift space required in natural environments because larval drift rates are mediated by water velocities (Braaten et al. 2008, 2012). Therefore, the deficiency of drift habitat is also a result of the contemporary thermal regime being cooler than during historic conditions in which the species evolved. Pallid Sturgeon now require more time and space to transition from larvae to fry. This study provides evidence that the manipulation of dam releases to increase riverine temperatures can reduce these extents and alleviate some of the

drift habitat deficiency caused by river fragmentation. For example, using my predictive model to estimate time for larvae to exhibit positive rheotaxis (settle), a 5 °C increase in water temperature (18 to 23°C) decreased drift time by 54%.

Studies modeling the drift distance required by Pallid Sturgeon larvae in relation to water velocity, temperature, and available riverine drift habitat found that larvae may reach low velocity environments (e.g., reservoir headwaters) prior to transitioning to fry (Kynard et al. 2007; Braaten et al. 2008, 2012), where mortality will be high (this study). Though true that the amount of available drift habitat is insufficient (Erwin et al. 2018), drift distance estimates have traditionally been based on physical rather than behavioral assumptions. For example, Kynard et al. (2007) described the initiation of the fry life stage (i.e., settling) as the time (dph) characterized by expulsion of the yolk-plug and presence of food in the stomach. They then related this to temperature-dependent development by calculating the daily cumulative thermal units (CTUs, = 200) required to initiate benthic settling. Using this model, larval drift distance at a mean water temperature of 17.8 °C and water velocity of 32 cm s<sup>-1</sup> was estimated to be 304 km (Kynard et al. 2007). The presence of food in larvae stomachs, while related to initiation of exogenous feeding (fry life stage), may not directly relate to when larvae settle out from the drift to the benthos. The behavioral transition from negative to positive rheotaxis likely occurs before first feeding because larvae settle to the benthos before beginning to forage as fry (Gisbert and Ruban 2003; Nguyen and Crocker 2006). Thus, the model proposed by Kynard et al. (2007) may overestimate drift distance required during the larval life stage. In a related study using 200 CTUs to define settling time

( $dph = CTU / ^\circ C$ ), Braaten et al. (2012) reported that larvae would settle out in 8.6 days at 23.3 °C with a cumulative drift distance of 668 km at a water velocity of 90  $cm\ s^{-1}$ . However, using behavioral data reported in this study, I found that larvae would settle out in 4.8 days at 23.3 °C (112 CTUs); when applied to the drift model reported in Braaten et al. (2012), cumulative drift distance would be 373 km at 90  $cm\ s^{-1}$ , approximately 295 km (56%) less (Table 5).

Large variation in drift distance can arise from fundamental differences in how settling time for Pallid Sturgeon larvae is defined. The transition from negative to positive rheotaxis reported in this study was observed at an average water velocity of 8.8  $cm\ s^{-1}$ . Given the negative rheotactic behavior Pallid Sturgeon larvae exhibit, it could be argued that a lower water velocity might lead to greater energy use (e.g., swimming activity), and thus faster development and settling time. However, I didn't observe differences in growth or energy expenditure between larvae reared at 3.5 or 8.3  $cm\ s^{-1}$ , potentially due to the minimal differences between velocities examined and(or) the length of the experiment (i.e., larval survival duration) not providing adequate resolution. If lower water velocities do result in a faster behavioral transition from negative to positive rheotaxis at the ecological (river) level, velocity reductions in the Missouri River could have important implications for Pallid Sturgeon by reducing drift extents and mitigating some of the drift habitat deficiency.

The relatively long drift distance requirements seen in Pallid Sturgeon larvae are common among other *Acipenseridae* where larvae exhibit negative rheotaxis. For example, White Sturgeon larvae are thought to drift about 150 km (McCabe Jr. and

Tracy 1994) while larval Siberian Sturgeon may drift 300 – 440 km downriver before transitioning to fry and thus, becoming positively rheotactic and settling to the benthos (Gisbert et al. 1999; Gisbert and Ruban 2003). Required drift distances become less substantial when *Acipenseridae* larvae hatch and initially exhibit positive rheotaxis. For example, larval Atlantic Sturgeon may only drift for 38 – 84 km (Kynard and Horgan 2002) and larval Shortnose Sturgeon drift for no more than 150 km before settling in rearing areas just upriver of saline water (Buckley and Kynard 1985; Kynard 1997). Further, larval Lake Sturgeon do not initiate drift (exhibit negative rheotaxis) until at least 5 dph and require <60 km of riverine drift habitat to transition to fry (Auer and Baker 2002; Smith and King 2005). A number of mechanisms have been proposed to explain drift distance of sturgeon larvae (Pavlov 1994; Gisbert and Ruban 2003; Braaten et al. 2008; Lechner et al. 2016). Two leading arguments supporting negative rheotaxis in Pallid Sturgeon larvae include reduced predation risk at hatch sites (Kynard et al. 2002a, 2007) and(or) the transport of larvae from coarse substrate found at hatch sites (DeLonay et al. 2016) to downriver habitats that are sand dominated, providing suitable foraging habitat for fry (Kynard et al. 2002a; Braaten et al. 2012). Regardless, Pallid Sturgeon larvae require great distances to drift before transitioning to exogenously feeding fry. These distances have become even greater given the cooler, contemporary thermal regime, making the drift habitat deficiency resulting from river fragmentation to become more pronounced.

Anthropogenic modifications on the Missouri River reduced Pallid Sturgeon migration corridors as well as the availability of thermal and lotic habits (Jacobson and

Galat 2006; DeLonay et al. 2009; Erwin et al. 2018). This study provides evidence that river fragmentation and cooler water temperatures associated with impoundments may negatively affect larval development and thus, drift requirements of Pallid Sturgeon. The velocity experiment indicates that a bottleneck for the species may arise if larvae are dispersed to zero to low flow environments prior to transitioning to fry, providing evidence that recruitment failure may be related to a mismatch between early life stage drift requirements and habitat conditions in the river (Kynard et al. 2007; Braaten et al. 2008, 2012; Erwin et al. 2018). More energy will be partitioned towards active metabolism rather than towards ontogenetic development, increasing larval mortality rates. The temperature experiment shows that increased water temperatures result in a faster transition from negative to positive rheotaxis (drifting to settling). Therefore, manipulation of dam releases to increase riverine temperatures may aid survival of Pallid Sturgeon larvae by shortening the temporal and spatial drift requirements during this critical life stage.

The larvae used in this study were produced from wild or hatchery-reared adults. Kynard et al. (2002a, 2007) found strong evidence for innate ontogenetic and drift behavior in Pallid Sturgeon, regardless of parental source. Even though the larvae used in this study were produced in a hatchery setting, it is unlikely to have had an influence on life history behaviors (Kynard et al. 2002a). Based on that consideration, these manipulative laboratory experiments may be indicative of flow and temperature effects on naturally produced Pallid Sturgeon within the Missouri River. Though there are obvious disconnects between microcosms and natural systems, results drawn from

laboratory experiments provide support and direction for more ecologically based research and management endeavors (Carpenter 1996; Drenner and Mazumder 1999).

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## Tables and figures

Table 1. Mean swimming duration and time between swimming events (seconds, s) of Pallid Sturgeon larvae reared at two water velocities; 0.08 or 2.20  $\text{cm s}^{-1}$ . For each response variable, values with the same letter are not significantly different (Tukey HSD;  $P > 0.05$ ). Values in parentheses represent 1 SE. Mean water temperature was 17 °C.

Velocity treatment ( $\text{cm s}^{-1}$ )	Velocity range ( $\text{cm s}^{-1}$ )	n	Swimming duration	Time between swimming events
			mean (s)	mean (s)
0.08	0 – 1	5	9.74 (0.45) <sup>a</sup>	2.01 (0.15) <sup>a</sup>
2.20	1 – 5	5	4.72 (0.25) <sup>b</sup>	5.72 (0.20) <sup>b</sup>

Table 2. Mean time (days post hatch, dph) to 100% mortality of Pallid Sturgeon larvae reared at three water velocities; 0, 3.5, or 8.3  $\text{cm s}^{-1}$ . Values with the same letter are not significantly different (Tukey HSD;  $P > 0.05$ ). Values in parentheses represent 1 SE.

Mean water temperature was 16.3 °C.

Velocity treatment ( $\text{cm s}^{-1}$ )	Velocity range ( $\text{cm s}^{-1}$ )	n	Time to 100% mortality	
			mean (dph)	range (dph)
0	0	4	7.3 (0.3) <sup>a</sup>	7 – 8
3.5	1 – 8	4	8.5 (0.3) <sup>b</sup>	8 – 9
8.3	2 – 20	4	9.8 (0.3) <sup>c</sup>	9 – 10

Table 3. Slope values ( $\beta$ ) and associated 95% confidence intervals estimated from non-linear regression analyses of larval Pallid Sturgeon mean total length (mm) modeled against days post hatch reared at water temperatures of 18.7, 20.4, or 23.3 °C. Slope values with the same letter are not significantly different ( $P > 0.05$ ).

Water temperature (°C)	$\beta$	95% confidence interval		t-value	P
		lower	upper		
18.7	0.1714 <sup>a</sup>	0.1507	0.1922	16.16	<0.001
20.4	0.1782 <sup>a</sup>	0.1542	0.2022	14.54	<0.001
23.3	0.2908 <sup>b</sup>	0.2536	0.3279	15.36	<0.001

Table 4. Slope values ( $\beta$ ) and associated 95% confidence intervals estimated from non-linear regression analyses of larval Pallid Sturgeon mean energy density (J/g wet weight) modeled against days post hatch reared at water temperatures of 18.7, 20.4, or 23.3 °C. Slope values with the same letter are not significantly different ( $P > 0.05$ ).

Water temperature (°C)	$\beta$	95% confidence interval		t-value	P
		lower	upper		
18.7	-0.1796 <sup>a</sup>	-0.1936	-0.1657	25.26	<0.001
20.4	-0.2215 <sup>b</sup>	-0.2368	-0.2062	28.36	<0.001
23.3	-0.2808 <sup>c</sup>	-0.2962	-0.2654	35.69	<0.001

Table 5. Larval Pallid Sturgeon cumulative drift distance estimates (kilometers, km) using either the day that larvae exhibit positive rheotaxis (this study) or the day that larvae accumulate 200 cumulative thermal units (CTUs; Braaten et al. 2012, Environmental Biology of Fishes 93:337-392) as the time (days post hatch, DPH) for larvae to settle (transition to fry).

Temperature (°C)	Time to larva settling		Passive, cumulative drift distance at 50 cm s <sup>-1</sup>		Passive, cumulative drift distance at 90 cm s <sup>-1</sup>	
	This study (dph)	Braaten et al. (2012) (dph)	This study (km)	Braaten et al. (2012) (km)	This study (km)	Braaten et al. (2012) (km)
18.7	8.6	10.7	372	462	669	832
20.4	6.8	9.8	294	424	529	762
23.3	4.8	8.6	207	371	373	668

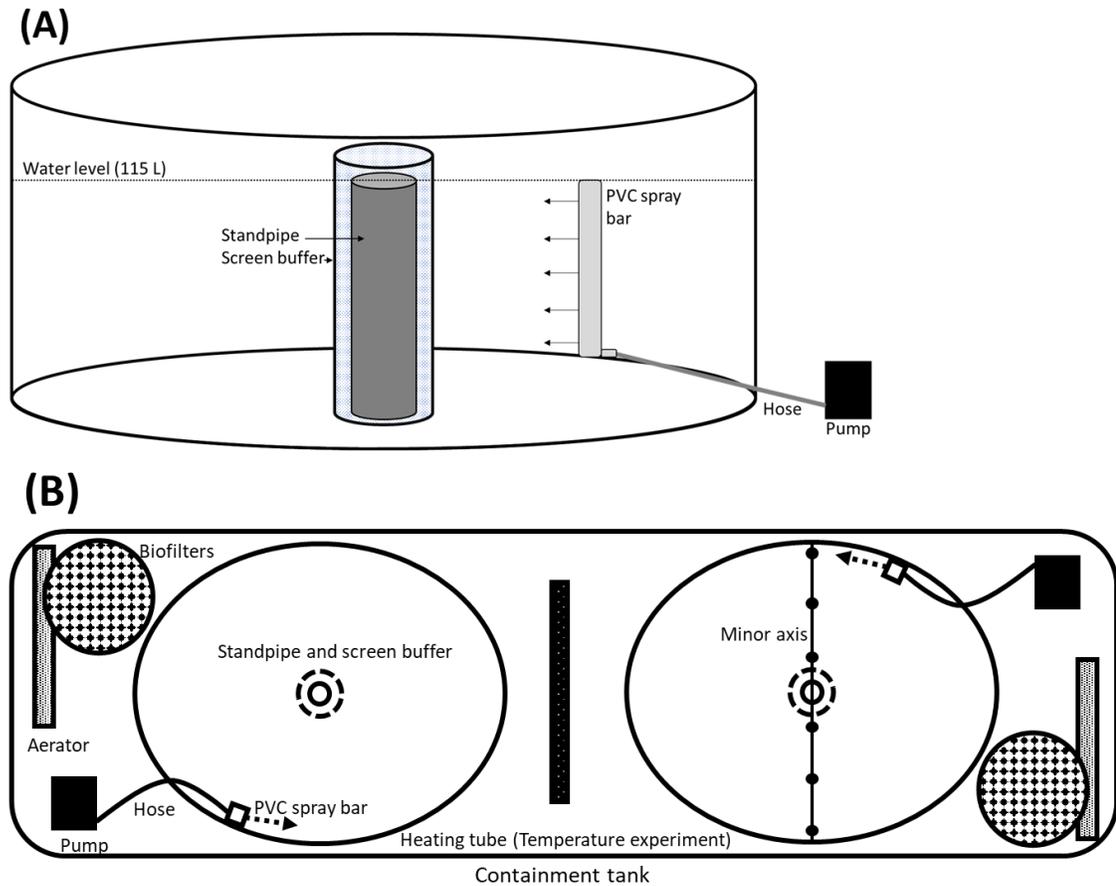


Figure 1. Side view (A) and top view (B) of the microcosm system used to assess the effects of water velocity and temperature on Pallid Sturgeon larvae. Depicted is the containment tank (244 x 64 x 30 cm, 454 L) containing two oval experimental tanks (90 x 64 x 30 cm, 115 L) with external pumps and hoses, PVC spray bars, standpipes with screen buffers, biofilters, aerators, and a heating tube.

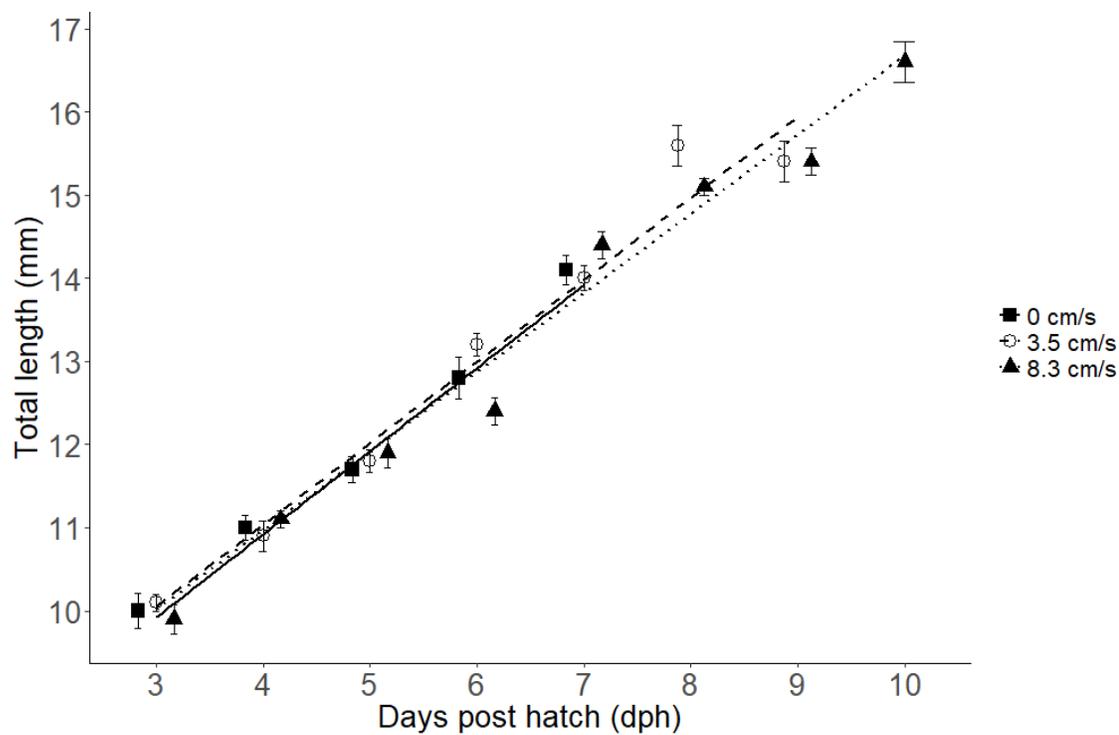


Figure 2. Mean total length (mm) of Pallid Sturgeon larvae reared at three water velocities; 0, 3.5, or 8.3  $\text{cm s}^{-1}$ . Mean water temperature during growth trials was 16.3 °C. Values along the x-axis are offset for clarity; error bars represent 1 SE.

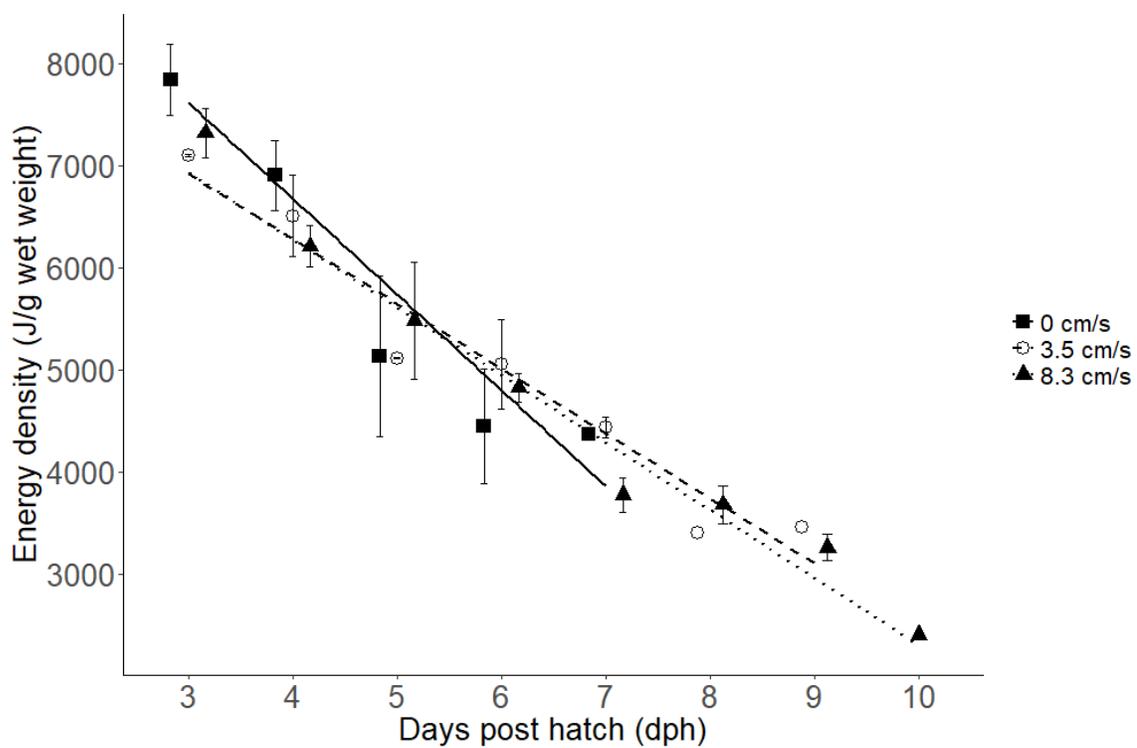


Figure 3. Mean energy density (J/g wet weight) of Pallid Sturgeon larvae reared at three water velocities; 0, 3.5, or 8.3 cm s<sup>-1</sup>. Mean water temperature during energy depletion trials was 16.3 °C. Values along the x-axis are offset for clarity; error bars represent 1 SE.

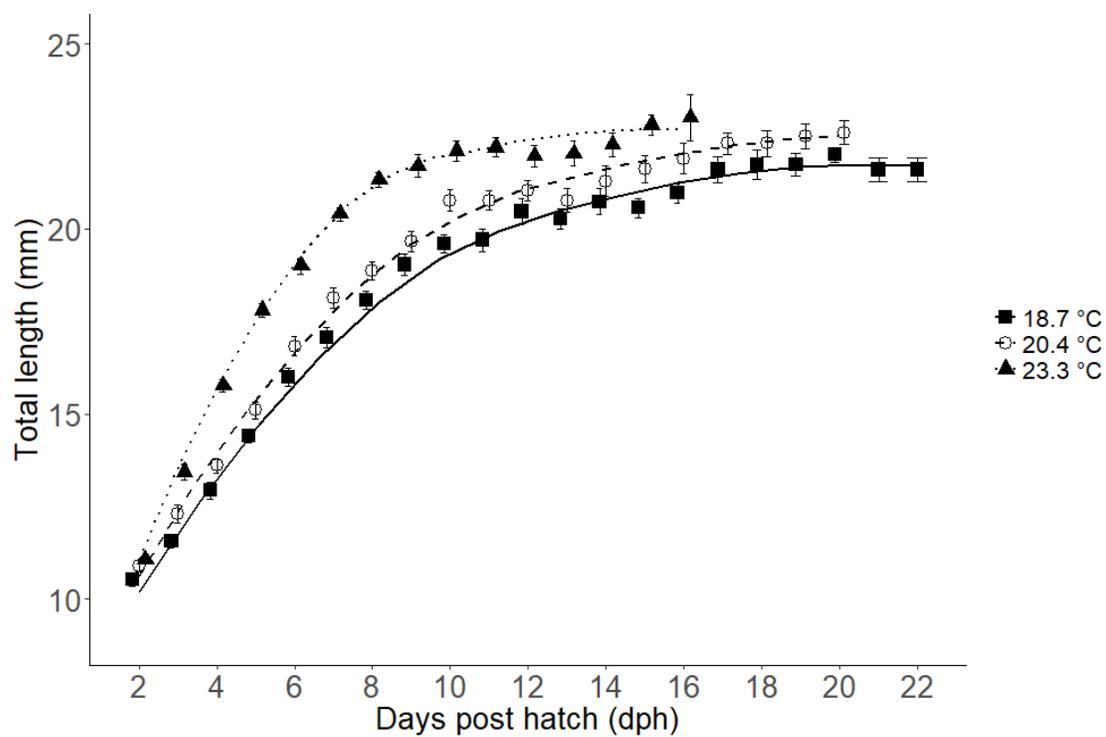


Figure 4. Mean total length (mm) of Pallid Sturgeon larvae reared at water temperatures of 18.7, 20.4, or 23.3 °C. Values along the x-axis are offset for clarity; error bars represent 2 SE.

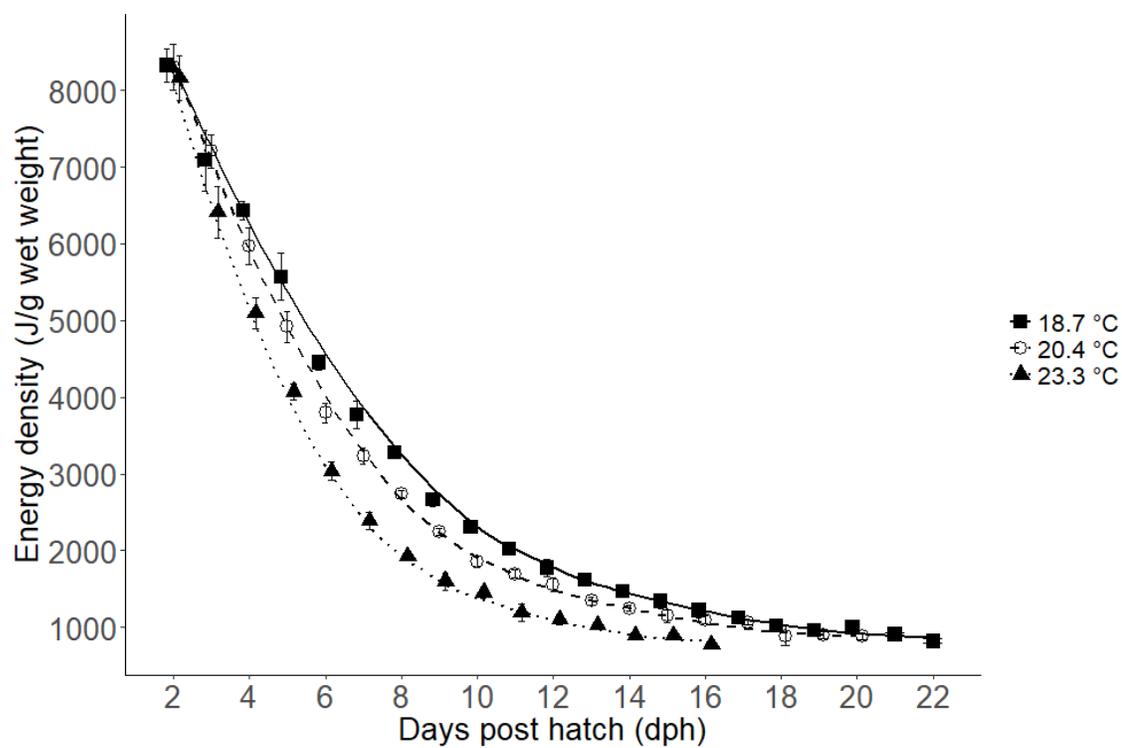


Figure 5. Mean energy density (J/g wet weight) of Pallid Sturgeon larvae reared at water temperatures of 18.7, 20.4, or 23.3 °C. Values along the x-axis are offset for clarity; error bars represent 2 SE.

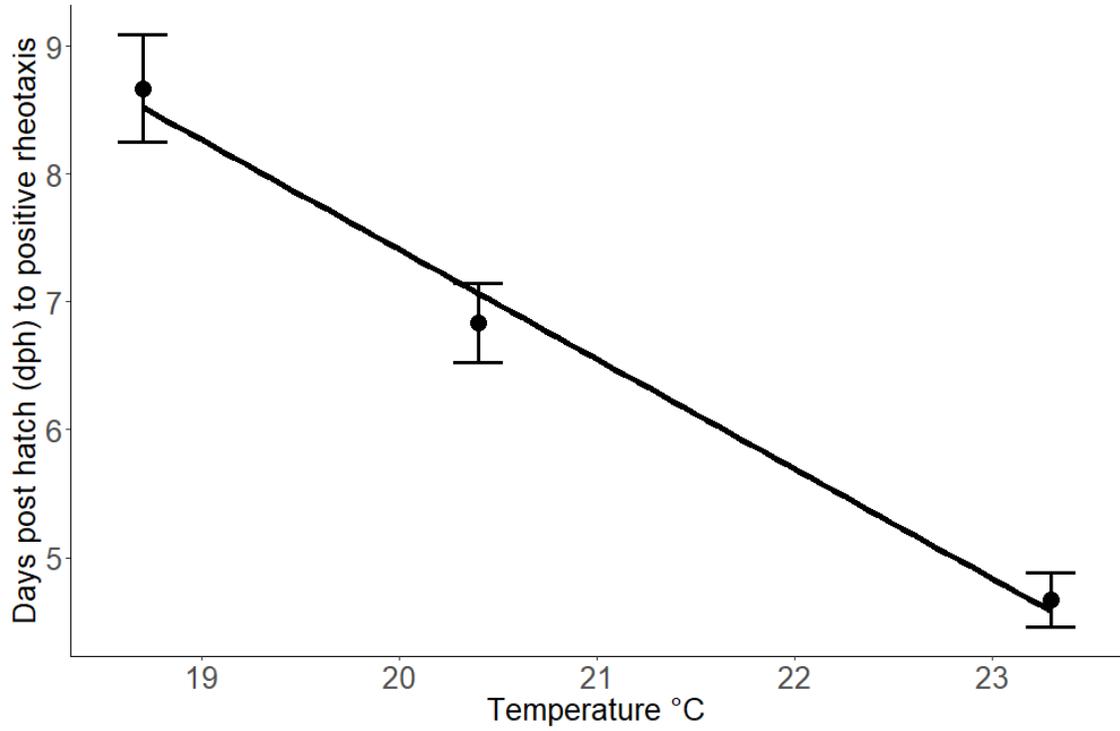


Figure 6. Mean days post hatch (dph) for Pallid Sturgeon larvae reared at water temperatures of 18.7, 20.4, or 23.3 °C to make a behavioral transition from negative to positive rheotaxis (drifting to settling). Error bars represent 1 SE.

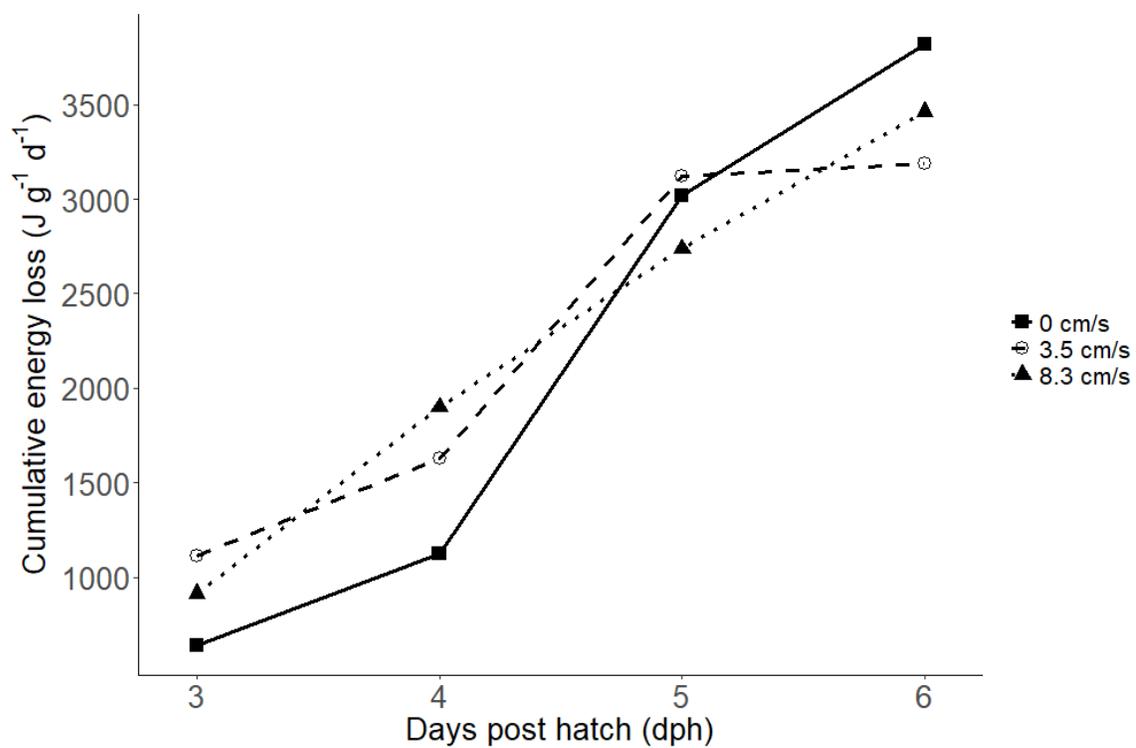


Figure 7. Cumulative energy loss ( $\text{J g wet weight}^{-1} \text{d}^{-1}$ ) of Pallid Sturgeon larvae reared at three water velocities; 0, 3.5, or  $8.3 \text{ cm s}^{-1}$ . Mean water temperature during energy depletion trials was  $16.3 \text{ }^{\circ}\text{C}$ .

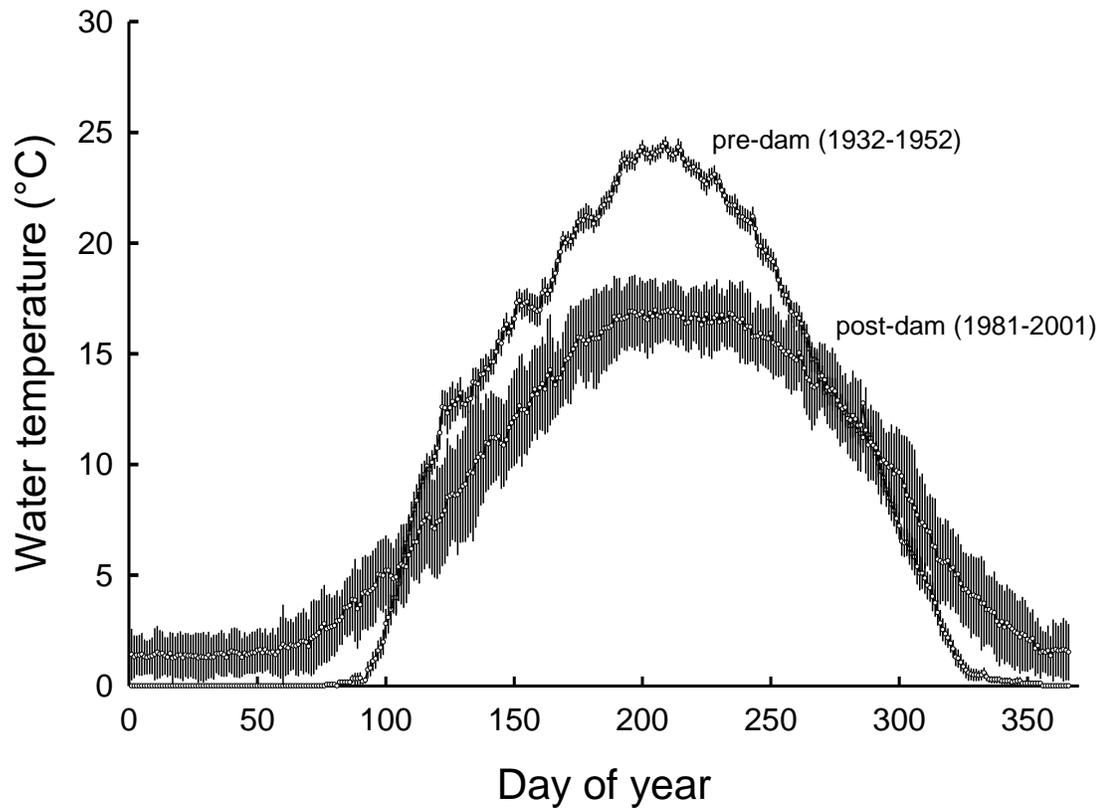


Figure 8. Mean, daily water temperature for the Missouri River 110 km below Garrison Dam (Riverdale, North Dakota). Data were collected from the water treatment plant in Bismarck, North Dakota. Time periods represent two 20-year intervals; pre-dam (1932 – 1952) and post-dam (1981 – 2001). Error bars represent 1 SE. Adapted from Heironimus (2014).

### CHAPTER 3. SUMMARY AND MANAGEMENT IMPLICATIONS

My study has key implications given the current status of the Missouri River and the ongoing recovery efforts for Pallid Sturgeon. The Missouri River has been greatly modified (i.e., channelized and impounded) which has led to substantial changes in natural flow and thermal regimes, reduced river connectivity (e.g., migration corridors), and loss of shallow water habitat (Dryer and Sandvol 1993; Pegg et al. 2003; DeLonay et al. 2016; Erwin et al. 2018). The U.S. Army Corps of Engineers was mandated to restore approximately 20,000 acres of shallow water habitat. This restoration has the potential to create more areas of zero to low velocity habitat in the Missouri River because the definition of shallow water habitat includes a maximum mean velocity, but no minimum velocity (U.S. Fish and Wildlife Service 2003; Gosch et al. 2013). As a result, there are more areas of lentic habitat today than during historic conditions in which this species evolved, with the potential of more arising in the future. My findings show that Pallid Sturgeon larvae balance their energy budget with greater efficiency and survive better in lotic environments. In contrast, areas with zero to low flow (e.g., reservoir headwaters, back waters, side channels) could pose as an important source of mortality to Pallid larvae, owing to increase activity when trying to enter the drift. I recommend that water velocities  $>10 \text{ cm s}^{-1}$  be considered during the construction of shallow water habitat.

Restoration actions that may benefit the success of Pallid Sturgeon are difficult to conceptualize. The first thought being fish passage advancements, which would

allow adults access to further upriver spawning grounds and provide a greater drift distance for larvae. However, this is assuming that fish passages would also provide a method for larval transport (drift) downriver. The enhancement of river connectivity and flow regime throughout the Missouri River basin would also benefit the success of Pallid Sturgeon. The goal should be restoration that is characteristic of historic (i.e., pre-1937) conditions. However, due to the permanence and the economic and cultural value these impoundments and associated reservoirs provide (e.g., hydroelectric power, flood control, navigation, municipal water, irrigation, and recreation), the enhancement and restoration of river connectivity and flow regime in the Missouri River is unrealistic. The temperature experiment elucidates an alternative restoration action that may be more palatable by stakeholders, managers, and state and federal agencies associated with the Missouri River system. Pallid Sturgeon transition from negative to positive rheotaxis (drifting to settling) faster at warmer temperatures, which relates to a reduction in the temporal and spatial drift extent required to transition from larvae to fry. The manipulation of dam releases to increase riverine temperature may be a more plausible restoration action that may aid survival of Pallid Sturgeon larvae, mitigating some of the drift habitat deficiency caused by impoundments.

Due to their negative rheotactic behavior, Pallid Sturgeon larvae that were unable to drift (velocity =  $0 \text{ cm s}^{-1}$ ) experienced significantly greater mortality than larvae maintained in tanks with flowing water. Areas without adequate drift velocities lead to greater activity (i.e., time and effort searching for flow) and energy use. Further, I found that in warmer water, Pallid Sturgeon larvae transitioned to fry in less time,

resulting from faster development and behavioral transitioning from negative to positive rheotaxis (drifting to settling), which has direct implications for drift extent during this critical life stage (Gisbert and Ruban 2003; Kynard et al. 2007; Braaten et al. 2008, 2012).

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