

REPRODUCTIVE ECOLOGY OF HATCHERY-ORIGIN PALLID STURGEON UPSTREAM
OF FORT PECK RESERVOIR, MONTANA

by

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ABSTRACT

Conservation propagation of pallid sturgeon above Fort Peck Reservoir has successfully recruited a new generation of spawning-capable pallid sturgeon to a location that would otherwise have fewer than 30 remaining. Successful recovery of pallid sturgeon will now rely on spawning in locations that provide adequate drift distance for larvae to recruit. Prior to this study, all reproductively-active female pallid sturgeon underwent ovarian follicular atresia. The reasons for and prevalence of ovarian follicular atresia were unclear, spawning periodicity of females remained undescribed, and remaining prepubescent fish indicated that age- and size-at-first maturity would vary more than currently described. Furthermore, spawning location, spawning-related interactions among conspecifics, and substrate composition at spawning locations remained undescribed. We used data on reproductive status and location to describe age- and size-at-first maturity, the prevalence of ovarian follicular atresia during first gametogenic cycles, spawning periodicity for female and male pallid sturgeon, where spawning occurs, if spawning locations are related to discharge, if substrate characteristics at the spawning locations were similar to other river reaches, and if female, male, and atretic female pallid sturgeon use the river similarly. Pallid sturgeon matured at older ages and larger sizes than described for other populations with females maturing at 18 years old and males at 15; however, prepubescent pallid sturgeon as old as 20 were documented. During the presumed-first gametogenic cycle, 62.5% of female pallid sturgeon underwent follicular atresia. Females had biennial reproductive cycles, and males had annual and biennial reproductive cycles. Reproductively-active male and female pallid sturgeon were found in similar locations, while locations of atretic female pallid sturgeon varied. The furthest upstream locations of pallid sturgeon including locations in the Marias River occurred during 2018 when discharge was at an unprecedented high. Spawning occurred in locations less than 131 km from the river-reservoir transition zone, which does not provide adequate drift-distance for larvae. Altering discharge and water temperature at Tiber Dam to mimic observed values in 2018 may increase use of the Marias River by pallid sturgeon during spawning, which would provide adequate drift distance to larvae.

CHAPTER ONE

FIRST MATURITY, SPAWNING PERIODICITY, AND FOLLICULAR ATRESIA
OF HATCHERY-ORIGIN PALLID STURGEON IN THE MISSOURI RIVER
ABOVE FORT PECK RESERVOIR, MONTANA

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ABSTRACT

The Pallid Sturgeon Conservation Propagation and Stocking Program began stocking pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir in 1998 with 1997-year-class pallid sturgeon. Thirteen of the hatchery-origin pallid sturgeon had become reproductively mature by 2016. However, ten hatchery-origin pallid sturgeon were still prepubescent in 2016, and no mature females had successfully spawned as all females underwent ovarian follicular atresia. The prevalence of ovarian follicular atresia was unknown and mechanism(s) causing follicular atresia were not well understood. Spawning periodicity of females could not previously be characterized because the multi-year spawning periodicity of pallid sturgeon prevented observations of females completing more than one gametogenic cycle. We combined historical data and contemporary data to describe the spawning periodicity for female and male pallid sturgeon, characterize age- and size-at-first maturity, and evaluate what proportion of pallid sturgeon experience ovarian follicular atresia during their presumed-first gametogenic cycle. Furthermore, we attempted to determine if ovarian follicular atresia was experienced as a component of puberty or the result of environmental conditions or interactions among conspecifics. Pallid sturgeon matured at older ages and larger sizes than described for other populations. Females matured at 18 years and males at 15 years; however, prepubescent pallid sturgeon as old as 20 were documented. We found that 62.5% of female pallid sturgeon underwent ovarian follicular atresia during the presumed-first gametogenic cycle suggesting ovarian follicular atresia is associated with puberty. During a successive gametogenic cycle, some females but not all ovulated suggesting environmental conditions or interactions among conspecifics contribute to ovarian follicular atresia. Females had biennial reproductive cycles, and males had annual and biennial reproductive cycles. Hatchery-origin pallid sturgeon becoming reproductively mature and successfully spawning indicates that this population can be recovered if limitations to recruitment are addressed. Therefore, management actions should focus on resolving recruitment limitations including inadequate larval drift distance.

Introduction

Conservation propagation of endangered species has been used to prevent the extirpation of many taxa such as plants (e.g., Scott et al., 2011), insects (e.g., Amaral et al., 1997), birds (e.g., Walters et al., 2010), mammals (e.g., Jachowski & Lockhart, 2009), amphibians (e.g., Griffiths & Pavajeau, 2008), and fishes (e.g., Schooley & Marsh, 2007). However, individuals from a conservation propagation program may not perform such that recovery of the population may occur. Success of the conservation propagation program for white sturgeon (*Acipenser transmontanus*) in the Kootenai River relies in part on successful adaptation of hatchery fish released into the wild, survival of hatchery fish to sexual maturity, and retention of wild sturgeon life history characteristics and genetics in the hatchery-reared population (Ireland et al., 2002). Unfortunately, successfully propagating a species does not ensure that these milestones will be met. For example, reintroduction of Yarkon bleak (*Acanthobrama telavivensis*) failed when fish were unable to breed due to habitat quality in the wild; however, through continued research and management actions, successful spawning occurred allowing for successful reintroduction (Goren, 2008). Therefore, it is imperative to study the behavior of individuals from conservation propagation programs after they are placed in the natural environment to assess performance as it relates to recovery objectives, where performance is defined as the vital rates, such as survival and reproductive output that result in population persistence.

Conservation propagation of fishes has increased abundance and prevented extirpation of multiple fish species (e.g., cutthroat trout [*Oncorhynchus clarkia*; Al-Chokhachy et al., 2020], lake sturgeon [*Acipenser fulvescens*; Bezold & Peterson, 2008], and razorback sucker [*Xyrauchen texanus*; Marsh et al., 2015]). Conservation propagation has become a necessity for

conservation of sturgeon and paddlefish species. According to the IUCN red list, 70.4% of species in the order Acipenseriformes are endangered or critically endangered (IUCN, 2020), and some species are already extinct (e.g., Chinese paddlefish [*Psephurus gladius*; Zhang et al., 2020]. Of the three *Scaphirhynchus* species, two are endangered—Alabama sturgeon (*Scaphirhynchus suttkusi*), which has gone undetected since 2009 besides eDNA detections in the Mobile River Basin [Pfleger et al., 2016] and pallid sturgeon [*Scaphirhynchus albus*; U.S. Federal Register, 1990]). The Pallid Sturgeon Conservation Propagation and Stocking Program was initiated to prevent extirpation of the species and preserve genetic diversity (USFWS 2014). Stocking pallid sturgeon upstream of Fort Peck Reservoir, Montana, began in 1998 with 1997 year-class (hereafter, referred to as hatchery-origin) pallid sturgeon stocked as yearlings. In the Great Plains Management Unit (i.e., the upper Missouri River and tributaries of the upper Missouri River), there has been a lack of recruitment since the species has been monitored (USFWS, 2014).

Previous research successfully documented reproductive maturity of hatchery-origin pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir, Montana, and spawning periodicity in males (Holmquist et al., 2019) using plasma sex steroids, gonadal biopsies, or both (see Webb et al., 2019). There were 13 hatchery-origin reproductively-mature pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir at the conclusion of the 2016 spawning season—10 reproductively active males and 3 reproductively-active females (Holmquist et al., 2019). Hatchery-origin pallid sturgeon experiencing different environmental conditions reach first maturity at different ages and sizes, and in the Missouri River upstream of Fort Peck Reservoir, hatchery-origin pallid sturgeon reach puberty when they are older and larger than

captive hatchery-origin pallid sturgeon (Jordan et al., 2016; Holmquist et al., 2019).

Contemporary age and size estimates of hatchery-origin pallid sturgeon upstream of Fort Peck Reservoir at their presumed-first maturity vary from 15–19 years, 1,011–1,198 mm fork length, and 4,848–8,700 g for males and 18–19 years, 1,070–1,140 mm fork length, and 6,400–6,940 g for females (Holmquist et al., 2019). However, ten hatchery-origin pallid sturgeon (1997 year class) that were sampled in 2016 had not reached puberty (Holmquist et al., 2019) indicating that the variation of age and size at first maturity may be greater than currently described. Younger and smaller hatchery-origin pallid sturgeon that had reached puberty have been sampled downstream of Fort Peck Reservoir in the Missouri and Yellowstone rivers—the youngest and smallest male was captured in the fall at 10.5 years of age, 773 mm, and 1,775 g and was presumed to be spawning capable at 11 years of age, and the youngest and smallest female was 14 years of age, 1,078 mm, and 5,900 g (M. A. H. Webb, U.S. Fish and Wildlife Service, unpublished data). Spawning periodicity has not been described for any female hatchery-origin pallid sturgeon upstream of Fort Peck Reservoir, Montana as the data has not yet been available. That is, no hatchery-origin females have been documented completing more than one reproductive cycle. Three male pallid sturgeon have been found to spawn annually and two appear to have biennial cycles (Holmquist et al., 2019). With individuals becoming reproductively mature, conservation-propagation efforts have been successful to this point. However, captive-origin individuals often experience reproductive challenges. Therefore, investigating reproduction (e.g., when puberty is reached, how frequently reproduction occurs, and if there are any unexpected limitations on reproductive potential) of released individuals remains an important step in recovering (i.e., achieving a self-sustaining genetically diverse

population) the species as outlined in the revised recovery plan for pallid sturgeon (USFWS, 2014).

One apparent limitation on the reproductive potential of pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir is ovarian follicular atresia. All hatchery-origin female pallid sturgeon upstream of Fort Peck Reservoir, Montana that completed reproductive cycles during 2015 ($n = 1$) and 2016 ($n = 2$) underwent follicular atresia (Holmquist, 2017). Though the number of females is low, the high-incidence of follicular atresia experienced by hatchery-origin female pallid sturgeon may be a component of puberty. A spawning dummy run has been described in teleosts and entails pre-pubertal fish exhibiting activation of the brain-pituitary axis, but the gonads do not yet have the capacity to respond to the chemical signals during the first attempted gametogenic cycle (Okuzawa, 2002; Newman et al., 2008). Therefore, vitellogenesis (tertiary oocyte growth) does not occur. In contrast, female hatchery-origin pallid sturgeon that underwent follicular atresia in their presumed-first gametogenic cycle (Holmquist et al., 2019) underwent late vitellogenesis in 2015 and 2016 but failed to undergo oocyte maturation. Pallid sturgeon experiencing ovarian follicular atresia during their first gametogenic cycle may be experiencing something like a spawning dummy run observed in teleosts with a break in the brain-pituitary-gonadal axis occurring at a later stage of development than in teleosts (i.e., after vitellogenesis has started but before ovulation). It is unknown if the hatchery-origin pallid sturgeon that underwent ovarian follicular atresia during their presumed-first gametogenic cycle will successfully ovulate during their successive gametogenic cycles. Furthermore, it is unknown what proportion of females fail to complete gametogenesis during their first cycle. Not

accounting for ovarian follicular atresia could result in overestimation of the spawning population size.

We designed this study to answer key questions about the reproductive physiology of hatchery-origin pallid sturgeon. Specifically, 1) what is the spawning periodicity for female and male pallid sturgeon, 2) at what age and size do pallid sturgeon complete puberty, and 3) what proportion of pallid sturgeon experience ovarian follicular atresia during their presumed first reproductive event? Furthermore, we pose the question, is follicular atresia experienced as a component of puberty (i.e., experienced during only the first gametogenic cycle) or the result of environmental conditions or interactions among conspecifics? Answering these questions will provide a more accurate assessment of the reproductive potential for the population of hatchery-origin pallid sturgeon upstream of Fort Peck Reservoir.

Study Area

The study area is within the Great Plains Management Unit described in USFWS (2014) and consists of the Missouri River from the upstream end of Fort Peck Reservoir to Morony Dam (river kilometer [rkm] 3,010 to rkm 3,388) and the Marias River from the confluence with the Missouri River to Tiber Dam (rkm 0 to rkm 126; Figure 1.1). The study area described here represents the northernmost distribution of pallid sturgeon in the Missouri River basin (USFWS, 2014).

The Missouri River upstream of Fort Peck Reservoir and the Marias River downstream of Tiber Dam have been influenced by anthropogenic factors including discharge regulation on the mainstem and on some tributaries (Hesse, 1987; Bovee & Scott, 2002). The mainstem Missouri River has nine impoundments upstream of the study area described here. Eight of the upstream

impoundments maintain outflow roughly equal to inflow and have little effect on discharge of the Missouri River (DNRC, 2014; NWE, 2016). Canyon Ferry Dam (rkm 3,626) influences discharge of the Missouri River (Bovee & Scott, 2002) and Tiber Dam on the Marias River influences discharge in the Marias River and in the Missouri River downstream from the mouth of the Marias River (rkm3,303, Bovee & Scott, 2002). Data recorded at the Landusky USGS (06115200) gauging station in the Missouri River indicates peak spring discharge occurs between late May and late June. Upstream impoundments have little effect on water temperature in the study area other than dampening daily variation of water temperature (Leathe, 2018). Water temperature in the Marias River is influenced by hypolimnetic water released from Tiber Dam (Stober, 1962).

Methods

Fish Sampling

Pallid sturgeon were sampled during this study between early May and late July of 2018 and 2019. Additionally, pallid sturgeon were sampled by Montana Fish, Wildlife and Parks and collaborators from 2011 through 2017 using similar methods. Pallid sturgeon included in this study were previously radio telemetered as part of a concurrent study conducted by Montana Fish, Wildlife and Parks and past research conducted by Holmquist et al. (2019). Pallid sturgeon were located using telemetry and trammel nets 45.7-m long, 1.8-m deep with 10.16-cm inner bar mesh, and 25.4-cm or 20.32-cm outer bar mesh were used to capture relocated pallid sturgeon. Smaller mesh trammel nets 45.7-m long, 1.8-m deep with 5.08-cm inner bar mesh, and 25.4-cm outer bar mesh were occasionally used if the larger mesh trammel nets were ineffective at capturing an individual. During 2018 and 2019, known reproductively-active females were

resampled as water temperature neared 24°C to determine ovulatory outcome. Similar to Holmquist et al. (2019), discharge and water temperature were used to define spawning season because spawning of pallid sturgeon occurs in late spring to early summer on the descending limb of the hydrograph (Fuller et al., 2008; DeLonay et al., 2016), and optimal water temperatures during spawning are estimated to be from 12°C to 24°C based on larval survival (Kappenman et al., 2013). Individuals for capture were prioritized using reproductive assessment records beginning in 2011. Known female pallid sturgeon that experienced reproductive activity in the past were considered high priority and were targeted first. After capturing high-priority pallid sturgeon, other known females were targeted. Male pallid sturgeon and pallid sturgeon of unknown sex were lower priority and were captured opportunistically. Pallid sturgeon without active transmitters were occasionally captured as bycatch (i.e., unintentionally captured) when trammel netting for targeted pallid sturgeon. Hatchery-origin pallid sturgeon captured as bycatch during this study were included in the study.

Biological samples and metrics were collected from all captured pallid sturgeon. Handling and sampling procedures conformed to protocols developed for pallid sturgeon (USFWS, 2012). Passive Integrated Transponder (PIT) tag codes were recorded and used to identify individual pallid sturgeon. Pallid sturgeon were measured in fork length (± 1 mm) and weighed (± 1 g). Blood was collected from the caudal vasculature using a 3-ml syringe, immediately transferred to a 7-ml lithium heparinized vacutainer, and stored in a cool environment until transported to the field station. At the field station, blood was centrifuged at 1,228 x g for 5 minutes, and plasma was transferred to 1.5-ml vials and stored at -20 to -80°C until analyzed at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center.

Gonadal tissue was collected from all pallid sturgeon of unknown sex, and ovarian follicles were collected from all females that were known or thought to be reproductively active. A small abdominal incision (1-2 cm) was made just off the midline between the third and fourth scutes anterior to the urogenital pore. An otoscope was used to identify the gonad, and gonadal tissue was collected through the otoscope specula using a Miltex biopsy cup (Webb et al., 2019). The otoscope speculum was angled to collect gonad from three different locations to account for gonadal heterogeneity. All tools used for collection of gonadal tissue were disinfected with 70% isopropyl alcohol and were rinsed with sterile saline prior to use. Ovarian follicles and gonadal tissue were preserved in 10% phosphate-buffered formalin.

Hatchery-origin pallid sturgeon that were captured and did not have an active radio transmitter were implanted with a radio transmitter (Lotek MCFT2-3L) and a PIT tag if the PIT tag was missing. The radio transmitter was inserted through the same incision used to collect gonadal tissue, and the external transmitter antenna was threaded through the abdominal cavity and made to exit between the pelvic fins using a large suture needle guarded by flexible polyethylene tubing. Incisions were closed with evenly spaced size 0 absorbable suture material attached to a CP-1 suture needle (Ethicon PDS*II).

Reproductive Assessment

Blood, ovarian follicles, and gonadal tissue were analyzed at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center. Sex steroids (testosterone [T] and estradiol-17 β [E2]) were extracted from blood plasma using methods described in Fitzpatrick et al. (1987). An extraction solvent (2 mL of diethyl ether; extracted twice) was added to tubes with 100 μ L of the plasma and vortexed. The aqueous phase was removed by snap-freezing with liquid nitrogen,

and ether was allowed to evaporate overnight in a chemical hood. The extract was reconstituted in 1 mL of phosphate-buffered saline with gelatin (PBSG). Ten or 50 μ L of reconstituted steroid extract was analyzed by radioimmunoassay as described in Fitzpatrick et al. (1986) and modified by Feist et al. (1990). A slightly more concentrated charcoal solution (6.25 g charcoal and 4.0 g dextran/L PBSG) was used for all assays. Testosterone and E2 concentrations were validated by verifying that serial dilutions were parallel to standard curves. Recovery efficiency was determined by adding tritiated steroids to tubes containing plasma (n=4), which were extracted as described above. Recovery efficiencies were 91–95% for T and 83–93% for E2. All steroid assay results were corrected for recovery. Non-detectable plasma sex steroid concentrations (i.e., not quantifiable) were assigned half of the minimum quantifiable concentration for statistical purposes (0.10 ng/mL for T and 0.05 ng/mL for E2; Croghan & Egeghy, 2003). The intra- and inter-assay coefficients of variation for all assays were less than 5% and 10%, respectively.

Blood plasma sex steroid concentrations were used to assign sex and stage of maturity to pallid sturgeon. Concentrations of T greater than 38 ng/mL and E2 less than 0.30 ng/mL were used to assign a reproductively-active male pallid sturgeon. Concentrations of T greater than 10 ng/mL and E2 greater than 0.30 ng/mL were used to assign a reproductively-active female pallid sturgeon. Pallid sturgeon that were not reproductively active were assigned as a non-reproductively-active male or non-reproductively-active female if the respective sex of the fish was known from prior capture. Pallid sturgeon were assigned as non-reproductively active and unknown sex if sex had not been previously determined and sex steroid concentrations were not indicative of a reproductively-active pallid sturgeon. A reproductively-active male pallid sturgeon based on steroid concentrations would have testicular cysts with germ cells that had

initiated meiosis or contain spermatozoa, and a reproductively-active female pallid sturgeon based on steroid concentrations would have initiated vitellogenesis or have post-vitellogenic ovarian follicles. A non-reproductively-active male pallid sturgeon would have germ cells that had not initiated meiosis in testicular cysts. A non-reproductively-active female pallid sturgeon would not have initiated vitellogenesis. Pallid sturgeon determined to not be reproductively active were not expected to spawn during the spawning season in which they were sampled. For three hatchery-origin male pallid sturgeon with T concentrations within 2 ng/ml of the concentration indicative of reproductive activity (36–40 ng/ml), behavior was used to indicate and validate reproductive state. Male pallid sturgeon with T concentrations near the indicative concentration of reproductive activity that remained sedentary and did not interact with other reproductively-active pallid sturgeon were not considered reproductively active. Pallid sturgeon that had completed puberty were considered reproductively mature irrespective of reproductive status at any given time.

When possible, histological analysis of gonadal tissue verified sex and stage of maturity in fish that were assigned using plasma sex steroids. Additionally, histological analysis provided detail about whether a female was vitellogenic or ripe (i.e., whether the female would spawn during the year she was sampled) and ovulatory outcome of all ripe females. Post-ovulatory ovarian follicles indicated ovulatory success, and ovulatory failure was indicated by atretic ovarian follicles. Gonadal tissue from pallid sturgeon were microscopically examined to determine sex and stage of maturity (Webb et al., 2019).

First Maturity

Reproductive histories of hatchery-origin pallid sturgeon were used to further characterize age and size at first maturity and update the characterization of age and size at first maturity by Holmquist et al. (2019). Pallid sturgeon were considered to have reached presumed-first maturity the first time they were determined to be experiencing a gametogenic cycle. Some pallid sturgeon at presumed-first maturity were known to be experiencing a confirmed-first gametogenic cycle (i.e., reproductive history indicated the fish had not previously experienced a gametogenic cycle). Pallid sturgeon could become reproductively-active the year prior to spawning (see Webb & Droshov, 2011), and ages of pallid sturgeon that became reproductively-active in the fall were rounded up to represent the age that the individual would be the following spring. Age, length, and weight for all hatchery-origin pallid sturgeon, including those characterized by Holmquist et al. (2019), were described and summarized for males and females. Additionally, age, length, and weight of hatchery-origin pallid sturgeon that were prepubescent were compared to those of male and female hatchery-origin pallid sturgeon at presumed-first maturity. A linear model was created to illustrate the length-weight relationship of pallid sturgeon in all categories. Five-hundred draws from the joint posterior distribution for the intercept and slope values associated with the length-weight regression were used to indicate uncertainty of the intercept and slope of the regression. Additionally, a 95% interval of the simulated predictive distribution was created by 50,000 simulations and random draws from the posterior predictive distribution to indicate where future observations are expected to fall with a probability of 0.95.

Ovarian Follicular Atresia

The percent of pallid sturgeon that experienced ovarian follicular atresia during the confirmed-first or presumed-first reproductive cycle and mechanism(s) for incidence of follicular atresia were evaluated. Historical data on reproductive status of individual female pallid sturgeon were used to determine if reproductively-active females were having their first reproductive cycle or a successive reproductive cycle. Ovarian follicular atresia during the confirmed-first or presumed-first gametogenic cycle was considered evidence of atresia related to puberty as wild pallid sturgeon are known to spawn successfully in the upper Missouri River (i.e., genetically verified larval pallid sturgeon have been collected downstream of suspected spawning locations; Eichelberger et al., 2014). However, ovarian follicular atresia experienced during a successive spawning event was considered evidence that factors besides puberty were contributing to atresia (e.g., inadequate environmental conditions or insufficient interactions among conspecifics).

Spawning Periodicity

Reproductive histories of individual hatchery-origin pallid sturgeon that were captured in separate years were used to describe spawning periodicity. Pallid sturgeon that were reproductively active in consecutive years were defined as having an annual reproductive cycle. Pallid sturgeon that were reproductively active every other year were defined as having a biennial reproductive cycle. Annual reproductive cycles were not considered for female pallid sturgeon because sturgeon species require at least 18 months to complete vitellogenesis (i.e., an annual reproductive cycle would be an anomaly; Doroshov et al., 1997; Zhang et al., 2011).

Results

First Maturity

Age at first maturity varied between 18 and 22 years old for hatchery-origin females and 15 and 22 years old for hatchery-origin males (Table 1.1). Ages of hatchery-origin prepubescent pallid sturgeon overlapped those of mature females and males (Table 1.1). Length of female pallid sturgeon at presumed-first maturity varied from 1,070 mm to 1,233 mm and weight varied from 6,150 g to 8,980 g (Table 1.1). Length and weight for male pallid sturgeon was less and varied from 917 mm to 1214 mm and 4,760 g to 8,765 g (Table 1.1). Similar to the age results, length and weight values for prepubescent pallid sturgeon overlapped values for mature female and male pallid sturgeon. The overlap in length and weight among categories were at the ends of the distributions, that is, smaller mature female and mature male pallid sturgeon overlapped with larger prepubescent pallid sturgeon (Figure 1.2).

Ovarian Follicular Atresia

Five hatchery-origin female pallid sturgeon underwent ovarian follicular atresia during their first or presumed-first gametogenic cycle—two during their confirmed-first gametogenic cycle (IDs 7B17 and 2E17) and three during their presumed-first gametogenic cycle (IDs 0E09, 3B15, and 4033). Two of the three hatchery-origin female pallid sturgeon that underwent ovarian follicular atresia during their presumed-first gametogenic cycle (IDs 0E09 and 4033) ovulated during a successive cycle, while one (ID 3B15) underwent follicular atresia for a second time. The two pallid sturgeon that underwent follicular atresia during their confirmed-first gametogenic cycle have not yet been observed during a successive cycle, and it is not known if they will successfully ovulate in the future. Three additional hatchery-origin female pallid

sturgeon (IDs 5A67, 7272, and 6862) experienced their presumed-first gametogenic cycle and successfully ovulated. Assuming all fish at their presumed-first gametogenic cycle were truly having their first gametogenic cycle, five of eight females (62.5 %) underwent follicular atresia during their first gametogenic cycle.

Spawning Periodicity

Spawning periodicity was determined for three hatchery-origin female pallid sturgeon that were observed completing more than one reproductive cycle—all had biennial reproductive cycles (Table 1.2). Female hatchery-origin pallid sturgeon ID 0E09 was reproductively active in 2015 and underwent ovarian follicular atresia. Pallid sturgeon ID 0E09 was vitellogenic in 2016 and reproductively active in 2019 suggesting a biennial reproductive cycle. Female hatchery-origin pallid sturgeon ID 3B15 and 4033 were reproductively active in 2016 and 2018 indicating biennial reproductive cycles (Table 1.2). In addition to the three female hatchery-origin pallid sturgeon described above, pallid sturgeon ID 6862 was reproductively active in 2018 and not reproductively active in 2019 indicating a biennial or longer reproductive cycle. The most reproductively-active hatchery-origin female pallid sturgeon documented in a spawning season occurred in 2018 with six reproductively-active females—the prior highest was two reproductively-active females in 2016.

Spawning periodicity was determined for six male hatchery-origin pallid sturgeon that were observed completing more than one reproductive cycle—four (IDs 0907, 3165, 2518, and 7E42) had annual reproductive cycles and two (IDs 4E58 and 2A09) had biennial reproductive cycles (Table 1.3). One of the male pallid sturgeon that had an annual reproductive cycle (2015 and 2016, ID 2518) did not maintain an annual cycle because the fish was not reproductively

active in 2019. One additional male (ID 503F) was reproductively active in 2018 (determined by elevated plasma sex steroids in the fall of 2017) and not reproductively active in 2019 indicating a biennial or longer reproductive cycle.

Discussion

Age- and size-at-first maturity and spawning periodicity was further evaluated than previously possible as additional hatchery-origin pallid sturgeon have become reproductively mature. Female pallid sturgeon in this study matured between ages 18 and 22 and females appeared to have a biennial spawning periodicity. Male pallid sturgeon matured between ages 15 and 22 and appeared to have annual and biennial reproductive cycles. The spawning periodicities of pallid sturgeon in this study are similar to pallid sturgeon in other portions of the upper Missouri River (Jordan et al., 2016) and to shovelnose sturgeon (*Scaphirhynchus platorynchus*; Tripp et al., 2009). Additionally, male pallid sturgeon maturing at younger ages and smaller sizes than female pallid sturgeon is consistent with pallid sturgeon in other locations (Jordan et al., 2016); however, age- and size-at-first maturity of pallid sturgeon upstream of Fort Peck Reservoir continue to exceed those of hatchery-origin pallid sturgeon in other downstream locations (DeLonay et al., 2009; Jordan et al., 2016; Holmquist et al., 2019). Therefore, location-specific characterization of age- and size-at-first maturity is imperative to providing accurate information to stock-assessments.

Overlap between the age and size of prepubescent pallid sturgeon and mature pallid sturgeon upstream of Fort Peck Reservoir suggested that age and size alone did not indicate when a hatchery-origin pallid sturgeon would reach maturity. Two other metrics that may be related to maturity of pallid sturgeon included lipid stores and feed ration (Webb & Doroshov,

2011; Szczepkowski et al., 2015). Providing feed with high lipid content to Atlantic salmon (*Salmo salar*) decreases age and size at first maturity (Jonsson et al., 2013). Therefore, age and size at first maturity is not only affected by feed ration but also by feed quality. That is, a pallid sturgeon that was successful at foraging may have delayed maturity if forage quality was poor. Future studies should consider if maturation may be associated with metrics other than age and size (e.g., feed ration or feed quality). At the population level, pallid sturgeon upstream of Fort Peck Reservoir appeared to mature at older ages and larger sizes than pallid sturgeon in other locations. Mature hatchery-origin female and male pallid sturgeon from 9–14 years old were documented in the lower Missouri River (DeLonay et al., 2009), and we documented age at first maturity ranging from 15 to 22 years old in the upper Missouri River upstream of Fort Peck Reservoir. Pallid sturgeon upstream of Fort Peck Reservoir maturing at older ages and larger sizes than other locations is explained by recent research, which found that phenotypic plasticity allows pallid sturgeon in the upper Missouri River to grow slower but grow larger and reach maturity at older ages than pallid sturgeon in other locations (Hamel et al., 2020). Furthermore, pallid sturgeon upstream of Fort Peck Reservoir are the northernmost distributed population of pallid sturgeon, and maturing at older ages and larger sizes than more southerly populations may be related to geographic latitude, which has been described in other species such as Atlantic Cod (*Gadus morhus*; Thorsen et al., 2010).

In general, ovarian follicular atresia experienced by hatchery-origin female pallid sturgeon appears to be associated with puberty, and hatchery-origin female pallid sturgeon underwent follicular atresia during the presumed-first gametogenic cycle 62.5% of the time. Notably, two females (IDs 2E17 and 7B17) included in this analysis were known to have their

first gametogenic cycle and both underwent follicular atresia. One female (ID 3B15) underwent ovarian follicular atresia during her second gametogenic cycle, and therefore, not all instances of ovarian follicular atresia were associated with puberty. Furthermore, two wild pallid sturgeon have been documented undergoing follicular atresia for reasons not associated with puberty—one in 2014 and one in 2015 (Holmquist et al., 2017). Incidences of ovarian follicular atresia not related to puberty may have been caused by various environmental factors or interactions among conspecifics. Environmental conditions such as water temperature have been shown to result in ovarian follicular atresia in other sturgeon species (Webb et al., 1999). Current rarity of reproductively-mature individuals upstream of Fort Peck Reservoir could result in interactions among conspecifics that do not favor spawning (e.g., skewed sex ratio or lack of suitable mates). Skewed sex ratio has been associated with ovarian follicular atresia in white suckers (*Catostomus commersonii*; Trippel & Harvey, 1990) and could contribute to ovarian follicular atresia in pallid sturgeon. Additionally, a lack of suitable mates has been shown to prevent mating in other taxa. For example, Australian field crickets (*Teleogryllus oceanicus*) with similar cuticular hydrocarbon profiles, which are associated with genetic relatedness, will forego mating (Thomas & Simmons, 2011). Furthermore, oocytes of a batch spawning fish, gilthead seabream (*Sparus aurata*), will undergo atresia if male fish are removed from the spawning population (Meiri et al., 2002). Reproductively-active female pallid sturgeon unable to find a suitable mate would likely not spawn and undergo ovarian follicular atresia. Future research should determine how prevalent ovarian follicular atresia is in pallid sturgeon following the first gametogenic cycle and consider how environmental conditions and interactions among conspecifics might contribute to the incidence of atresia.

Multi-year spawning periodicities of individuals within a population can influence reproductive output and alter population growth rate. Thus, fully characterizing reproductive periodicity is necessary for stock assessments. Interestingly, we did not detect any female pallid sturgeon having a reproductive cycle longer than two years. However, triennial reproductive cycles have been documented in pallid sturgeon in other locations (Jordan et al., 2016), and reproductive cycles longer than biennial are common in other sturgeon species particularly in females (Auer, 1999; Erickson & Webb, 2007). Given the rarity of reproductively-active female pallid sturgeon, continued sampling that complements the current historical data would be useful to further evaluate the proportion of females with a two- or three-year spawning periodicity. Interestingly, one hatchery-origin male pallid sturgeon had varying lengths in the reproductive cycle by having an annual periodicity, but later remaining non-reproductively-active during a spawning season. Therefore, assuming a constant and consistent spawning periodicity for male pallid sturgeon would be a flawed assumption. Variation in duration of reproductive cycles has been documented in other sturgeon species (e.g., white sturgeon [Webb, 2011; Webb, 2012] and lake sturgeon [*Acipenser fulvescens*; Forsythe et al., 2012]). Although, individual-based variation in spawning periodicity appears to be relatively uncommon in lake sturgeon (Forsythe et al., 2012), the prevalence in pallid sturgeon is not known, and we only detected one male with a change in spawning periodicity. Furthermore, the mechanism(s) for individual variation in spawning periodicity of sturgeon are not known. Physiological factors determining the spawning periodicity for teleosts (e.g., skipped spawning) are often correlated with feed ration or nutritional deficiencies (Rideout & Tomkiewicz, 2010), and a non-reproductive state can be induced in winter flounder (*Pseudopleuronectes americanus*) by withholding food (Burton &

Idler, 1987). In species that regularly experience multi-year reproductive cycles such as sturgeon, a change to longer or shorter spawning periodicities may be associated with feed ration, nutritional quality of feed, or both.

Sex-steroid concentrations are dynamic with short-term variation despite predictable trends associated with gametogenesis (Zohar & Billard, 1984). Therefore, a single sampling event may occasionally misrepresent the reproductive status of an individual. One hatchery-origin female pallid sturgeon (ID 6862) at presumed-first maturity with ripe ovarian follicles had an E2 concentration below the level used to indicate a reproductively-active female. This was the first study to document a reproductively-active female with an E2 concentration below 0.30 ng/mL, and we suggest lowering the discriminating E2 concentration to 0.28 ng/mL. Additionally, one hatchery-origin male pallid sturgeon (ID 2A09) with a biennial reproductive cycle had slightly elevated testosterone concentration of 39.10 ng/mL but was determined to be non-reproductively active by Holmquist et al. (2019) based on sedentary behavior and a lack of interaction with other reproductively-active pallid sturgeon during the spawning season. The male and female pallid sturgeon that were miscategorized by plasma sex steroid concentrations had concentrations near the discriminating values used to indicate reproductive activity. Discriminating sex-steroid concentrations should be continually adapted to represent the best available knowledge, and short-term variation in sex-steroid concentrations should be recognized as blood-plasma samples represent sex-steroid concentrations only during the sampling event, which may not always be representative of sex-steroid concentrations over the course of a spawning season.

We found that pallid sturgeon upstream of Fort Peck Reservoir do successfully spawn, and some incidences of ovarian follicular atresia were associated with puberty. With successful spawning occurring, the population of pallid sturgeon upstream of Fort Peck Reservoir will be capable of recovering if recruitment limitations are adequately addressed. Therefore, future research and management actions should focus on procuring spawning in locations that provide adequate drift distance to larvae.

References

- Al-Chokhachy, R., Heki, L., Loux, T., & Peka, R. (2020). Return of a giant: Coordinated conservation leads to the first wild reproduction of Lahontan cutthroat trout in the Truckee River in nearly a century. *Fisheries*, *45*, 63–73. <https://doi.org/10.1002/fsh.10350>
- Amaral, M., Kozol, A., & French, T. (1997). Conservation status and reintroduction of the endangered American burying beetle. *Northeastern Naturalist*, *4*, 121–132. DOI 10.2307/3858707
- Auer, N. A. (1999). Population characteristics and movements of lake sturgeon in the Sturgeon River and Lake Superior. *Journal of Great Lakes Research*, *25*, 282–293. [https://doi.org/10.1016/S0380-1330\(99\)70737-9](https://doi.org/10.1016/S0380-1330(99)70737-9)
- Bezold, J., & PETERSON, D. L. (2008). Assessment of lake sturgeon reintroduction in the Coosa River system, Georgia–Alabama. In *American Fisheries Society Symposium*, *62*.
- Bovee, K. D., & Scott, M. L. (2002). Implications of flood pulse restoration for Populus regeneration on the upper Missouri River. *River Research and Applications*, *18*, 287–298. <https://doi.org/10.1002/rra.672>
- Burton, M. P., & Idler, D. R. (1987). An experimental investigation of the non-reproductive, post-mature state in winter flounder. *Journal of Fish Biology*, *30*, 643–650. <https://doi.org/10.1111/j.1095-8649.1987.tb05793.x>
- Croghan, C., & Egeghy, P. P. (2003). Methods of dealing with values below the limit of detection using SAS. Southeastern SAS User Group, St. Petersburg, FL.
- DeLonay, A. J., Jacobson, R. B., Chojnacki, K. A., Braaten, P. J., Buhl, K. J., Eder, B. L., Elliott, C. M., Erwin, S. O., Fuller, D. B., Haddix, T. M., Ladd, H. L. A., Mestl, G. E., Papoulias, D. M., Rhoten, J. C., Wesolek, C. J., & Wildhaber, M. L. (2016). Ecological requirements for pallid sturgeon reproduction and recruitment in the Missouri River—annual report 2013. U.S. Geological Survey, Open-File Report 2015–1197. Reston, VA.
- DeLonay, A. J., Jacobson, R. B., Papoulias, D. M., Simpkins, D.G., Wildhaber, M. L., Reuter, J. M., Bonnot, T. W., Chojnacki, K. A., Korschgen, C. E., Mestl, G. E., & Mac, M. J. (2009). Ecological requirements for pallid sturgeon reproduction and recruitment in the Lower Missouri River – A research synthesis 2005-08. U.S. Geological Survey Scientific Investigations Report 2009-5201. Reston, VA.
- DNRC (Montana Department of Natural Resources and Conservation). (2014). Toston Dam (Broadwater-Missouri). Montana Department of Natural Resources and Conservation, Helena, MT.

- Doroshov, S. I., Moberg, G. P., & Van Eenennaam, J. P. (1997). Observations on the reproductive cycle of cultured white sturgeon, *Acipenser transmontanus*. *Environmental Biology of Fishes*, 48, 265–278.
- Eichelberger, J. S., Braaten, P. J., Fuller, D. B., Krampe, M. S., & Heist, E. J. (2014). Novel single-nucleotide polymorphism markers confirm successful spawning of endangered pallid sturgeon in the upper Missouri River basin. *Transactions of the American Fisheries Society*, 143, 1373–1385. <https://doi.org/10.1080/00028487.2014.935479>
- Erickson, D. L., & Webb, M. A. (2007). Spawning periodicity, spawning migration, and size at maturity of green sturgeon, *Acipenser medirostris*, in the Rogue River, Oregon. *Environmental Biology of Fishes*, 79, 255–268.
- Feist, G., Schreck, C. B., Fitzpatrick, M. S., & Redding, J. M. (1990). Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. *General and Comparative Endocrinology*, 80, 299–313.
- Fitzpatrick, M. S., Redding, J. M., Ratti, F. D., & Schreck, C. B. (1987). Plasma testosterone concentration predicts the ovulatory response of coho salmon (*Oncorhynchus kisutch*) to gonadotropin-releasing hormone analog. *Canadian Journal of Fisheries and Aquatic Sciences*, 44, 1351–1357.
- Fitzpatrick, M. S., Van Der Kraak, G., & Schreck, C. B. (1986). Profiles of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch*, during final maturation. *General and Comparative Endocrinology*, 62, 437–451.
- Forsythe, P. S., Crossman, J. A., Bello, N. M., Baker, E. A., & Scribner, K. T. (2012). Individual-based analyses reveal high repeatability in timing and location of reproduction in lake sturgeon (*Acipenser fulvescens*). *Canadian Journal of Fisheries and Aquatic Sciences*, 69, 60–72. <https://doi.org/10.1139/f2011-132>
- Fuller, D. A., Jaeger, M. E., and Webb M. A. H. (2008). *Spawning and associated movement patterns of pallid sturgeon in the lower Yellowstone River. Report prepared for the Upper Missouri River Basin pallid sturgeon Workgroup, Western Area Power Administration, and U.S. Army Corps of Engineers*. Helena, MT: Montana Fish, Wildlife and Parks.
- Goren, M. (2009). Saving critically endangered fish species—utopia or a practical idea? The story of the Yarqon bleak—*Acanthobrama telavivensis* (Cyprinidae) as a test case. *Aqua*, 15, 1–12.
- Griffiths, R. A., & Pavajeau, L. (2008). Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, 22, 852–861. <https://doi.org/10.1111/j.1523-1739.2008.00967.x>

- Hamel, M. J., Spurgeon, J. J., Steffensen, K. D., & Pegg, M. A. (2020). Uncovering unique plasticity in life history of an endangered centenarian fish. *Scientific Reports*, *10*, 12866. <https://doi.org/10.1038/s41598-020-69911-1>
- Hesse, L. W. (1987). Taming the wild Missouri River: what has it cost?. *Fisheries*, *12*(2), 2–9. [https://doi.org/10.1577/1548-8446\(1987\)012<0002:TTWMRW>2.0.CO;2](https://doi.org/10.1577/1548-8446(1987)012<0002:TTWMRW>2.0.CO;2)
- Holmquist, L. M., (2017). Reproductive ecology of hatchery-origin and wild pallid sturgeon in the Missouri River above Fort Peck Reservoir, Montana. Master's thesis, Montana State University, Bozeman.
- Holmquist, L. M., Guy, C. S., Tews, A., & Webb, M. A. (2019). First maturity and spawning periodicity of hatchery-origin pallid sturgeon in the upper Missouri River above Fort Peck Reservoir, Montana. *Journal of Applied Ichthyology*, *35*, 138–148. <https://doi.org/10.1111/jai.13751>
- Ireland, S. C., Beamesderfer, R. C. P., Paragamian, V. L., Wakkinen, V. D., & Siple, J. T. (2002). Success of hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) following release in the Kootenai River, Idaho, USA. *Journal of Applied Ichthyology*, *18*, 642–650. DOI: 10.1046/j.1439-0426.2002.00364.x
- IUCN (International Union for Conservation of Nature and Natural Resources). (2020). *The IUCN redlist of threatened species. IUCN Global Species Programme Red List Unit, Cambridge, United Kingdom*. Retrieved October 10, 2020, from www.iucnredlist.org
- Jachowski, D. S., & Lockhart, J. M. (2009). Reintroducing the black-footed ferret *Mustela nigripes* to the Great Plains of North America. *Small Carnivore Conservation*, *41*, 58–64.
- Jonsson, B., Jonsson, N., & Finstad, A. G. (2013). Effects of temperature and food quality on age and size at maturity in ectotherms: an experimental test with Atlantic salmon. *Journal of Animal Ecology*, *82*, 201–210. <https://doi.org/10.1111/j.1365-2656.2012.02022.x>
- Jordan, G. R., Heist, E. J., Braaten, P. J., DeLonay, A. J., Hartfield, P., Herzog, D. P., Kappenman, K. M., & Webb, M. A. H. (2016). Status of knowledge of the pallid sturgeon (*Scaphirhynchus albus* Forbes and Richardson, 1905). *Journal of Applied Ichthyology*, *32*, 191–207. <https://doi.org/10.1111/jai.13239>
- Kappenman, K. M., Webb, M. A. H., & Greenwood, M. (2013). The effect of temperature on embryo survival and development in pallid sturgeon *Scaphirhynchus albus* (Forbes & Richardson 1905) and shovelnose sturgeon *S. platyrhynchus* (Rafinesque, 1820). *Journal of Applied Ichthyology*, *29*, 1193–1203. <https://doi.org/10.1111/jai.12301>
- Leathe, S. (2018). Effects of Great Falls reservoirs on Missouri River water temperature. North Western Energy, Sioux Falls, SD.

- Marsh, P. C., Dowling, T. E., Kesner, B. R., Turner, T. F., & Minckley, W. L. (2015). Conservation to stem imminent extinction: the fight to save razorback sucker *Xyrauchen texanus* in Lake Mohave and its implications for species recovery. *Copeia*, 2015, 141–156. <https://doi.org/10.1643/OT-14-030>
- Meiri, I., Gothilf, Y., Zohar, Y., & Elizur, A. (2002). Physiological changes in the spawning gilthead seabream, *Sparus aurata*, succeeding the removal of males. *Journal of Experimental Zoology*, 292, 555–564. <https://doi.org/10.1002/jez.10072>
- Newman, D. M., Jones, P. L., & Ingram, B. A. (2008). Age-related changes in ovarian characteristics, plasma sex steroids and fertility during pubertal development in captive female Murray cod *Maccullochella peelii peelii*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 150, 444–451. <https://doi.org/10.1016/j.cbpa.2008.05.005>
- NWE (Northwestern Energy). (2016). Hydro operational requirements from FERC license articles and SOP agreements with agencies for NWE's 11 hydropower dams (3 FERC licenses). North Western Energy, Sioux Falls, SD.
- Okuzawa, K. (2002). Puberty in teleosts. *Fish Physiology and Biochemistry*, 26, 31–41. <https://doi.org/10.1023/A:1023395025374>
- Pfleger, M. O., Rider, S. J., Johnston, C. E., & Janosik, A. M. (2016). Saving the doomed: Using eDNA to aid in detection of rare sturgeon for conservation (Acipenseridae). *Global ecology and conservation*, 8, 99–107. <https://doi.org/10.1016/j.gecco.2016.08.008>
- Rideout, R. M., & Tomkiewicz, J. (2011). Skipped spawning in fishes: more common than you might think. *Marine and Coastal Fisheries*, 3, 176–189. <https://doi.org/10.1080/19425120.2011.556943>
- Schooley, J. D., & Marsh, P. C. (2007). Stocking of endangered razorback suckers in the lower Colorado River basin over three decades: 1974–2004. *North American Journal of Fisheries Management*, 27, 43–51.
- Scott, G. L., McCaughey, W. W., & Izlar, K. (2011). Guidelines for whitebark pine planting prescriptions. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. 63, 362–364.
- Stober, Q. J. (1962). Some limnological effects of Tiber Reservoir on the Marias River. Master's thesis, Montana State University, Bozeman.

- Szczepkowski, M., Kolman, R., & Szczepkowska, B. (2015). Impact of feed ration on growth and the results of sterlet, *Acipenser ruthenus* L., artificial reproduction. *Aquaculture Research*, 46, 2147–2152. <https://doi.org/10.1111/are.12370>
- Thomas, M. L., & Simmons, L. W. (2011). Crickets detect the genetic similarity of mating partners via cuticular hydrocarbons. *Journal of evolutionary biology*, 24, 1793–1800. <https://doi.org/10.1111/j.1420-9101.2011.02319.x>
- Thorsen, A., Witthames, P. R., Marteinsdóttir, G., Nash, R. D., & Kjesbu, O. S. (2010). Fecundity and growth of Atlantic cod (*Gadus morhua* L.) along a latitudinal gradient. *Fisheries Research*, 104, 45–55. <https://doi.org/10.1016/j.fishres.2010.03.020>
- Tripp, S. J., Phelps, Q. E., Colombo, R. E., Garvey, J. E., Burr, B. M., Herzog, D. P., & Hrabik, R. A. (2009). Maturation and reproduction of shovelnose sturgeon in the middle Mississippi River. *North American Journal of Fisheries Management*, 29, 730–738. <https://doi.org/10.1577/M08-056.1>
- Trippel, E. A., & Harvey, H. H. (1990). Ovarian atresia and sex ratio imbalance in white sucker, *Catostomus commersoni*. *Journal of Fish Biology*, 36, 231–239. <https://doi.org/10.1111/j.1095-8649.1990.tb05598.x>
- U.S. Office of the Federal Register (1990). Endangered and threatened wildlife and plants; determination of endangered status for the pallid sturgeon. *Federal Register* 55:173(September 6, 1990):36641–36647.
- USFWS (United State Fish and Wildlife Service). (2012). *Biological procedure and protocols for researchers and managers handling pallid sturgeon*. (40 pp.). Denver, CO: Fish and Wildlife Service.
- USFWS (United States Fish and Wildlife Service). (2014). *Revised recovery plan for the pallid sturgeon (Scaphirhynchus albus)*. (115 pp.). Denver, CO: Fish and Wildlife Service.
- Walters, J. R., Derrickson, S. R., Michael Fry, D., Haig, S. M., Marzluff, J. M., & Wunderle Jr, J. M. (2010). Status of the California Condor (*Gymnogyps californianus*) and efforts to achieve its recovery. *The Auk*, 127, 969–1001. <https://doi.org/10.1525/auk.2010.127.4.969>
- Webb, M. A. H. (2010). Determine spawning interval of white sturgeon in the Columbia River. White sturgeon mitigation and restoration in the Colombia and Snake rivers upstream from Bonneville Dam. Annual Progress Report, April 2010-March 2011.
- Webb, M. A. H. (2011). Determine spawning interval of white sturgeon in the Columbia River. White sturgeon mitigation and restoration in the Colombia and Snake rivers upstream from Bonneville Dam. Annual Progress Report, April 2011-March 2012.

- Webb, M. A. H., Van Eenennaam, J. P., Crossman, J. A., & Chapman, F. A. (2019). A practical guide for assigning sex and stage of maturity in sturgeons and paddlefish. *Journal of Applied Ichthyology*, *35*, 169–186. <https://doi.org/10.1111/jai.13582>
- Webb, M. A., & Doroshov, S. I. (2011). Importance of environmental endocrinology in fisheries management and aquaculture of sturgeons. *General and Comparative Endocrinology*, *170*, 313–321. <https://doi.org/10.1016/j.ygcen.2010.11.024>
- Webb, M. A., Van Eenennaam, J. P., Doroshov, S. I., & Moberg, G. P. (1999). Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. *Aquaculture*, *176*, 315–329. [https://doi.org/10.1016/S0044-8486\(99\)00108-8](https://doi.org/10.1016/S0044-8486(99)00108-8)
- Zhang, H., Jarić, I., Roberts, D. L., He, Y., Du, H., Wu, J., Wang, C., & Wei, Q. (2020). Extinction of one of the world's largest freshwater fishes: Lessons for conserving the endangered Yangtze fauna. *Science of the Total Environment*, *710*, 136242. <https://doi.org/10.1016/j.scitotenv.2019.136242>
- Zhang, Y., Qu, Q., Sun, D., Liu, X., Suo, L., & Zhang, Y. (2011). Vitellogenin in Amur sturgeon (*Acipenser schrenckii*): induction, purification and changes during the reproductive cycle. *Journal of applied ichthyology*, *27*, 660–665. <https://doi.org/10.1111/j.1439-0426.2011.01698.x>
- Zohar, Y., & Billard, R. (1984). Annual and daily changes in plasma gonadotropin and sex steroids in relation to teleost gonad cycles. *Transactions of the American Fisheries Society*, *113*, 444–451. [https://doi.org/10.1577/1548-8659\(1984\)113<444:AADCIP>2.0.CO;2](https://doi.org/10.1577/1548-8659(1984)113<444:AADCIP>2.0.CO;2)

Tables

Table 1.1. Summary statistics by reproductive status for age (years), fork length (mm), and weight (g) of 1997 year-class hatchery-origin pallid sturgeon at presumed-first maturity and 1997 year-class hatchery-origin pallid sturgeon known to be prepubescent.

Variable	Summary statistic	Status		
		Female	Male	Prepubescent
Age	N	8	15	11
	Mean (SE)	20.25 (0.49)	18.33 (0.59)	19 (0.13)
	Median	21	19	19
	Minimum	18	15	18
	Maximum	22	22	20
Length	N	8	15	11
	Mean (SE)	1148.88 (19.28)	1098.87 (20.27)	1032.55 (25.65)
	Median	1155	1097	1046
	Minimum	1070	917	873
	Maximum	1233	1215	1144
Weight	N	8	15	11
	Mean (SE)	7443.75 (370.87)	6354.93 (369.47)	5030.91 (462.11)
	Median	7300	5750	5060
	Minimum	6150	4760	2190
	Maximum	8980	8765	7060

Table 1.2. Sex steroid concentrations (testosterone (T) ng/ml and estradiol (E2) ng/ml) by year for eight female 1997 year-class hatchery-origin pallid sturgeon that were documented reproductively active between 2015 and 2019.

ID	Steroid	Reproductive year				
		2015	2016	2017	2018	2019
0E09 [†]	T	62.18^b	29.73 ^c			33.71
	E2	0.67^b	ND ^c			4.32
3B15 [†]	T		52.42^b	24.71	0.19^{ab}	
	E2		1.83^b	0.47	ND^{ab}	
4033 [†]	T		32.08^b		53.49	
	E2		2.55^b		1.13	
2E17	T	6.16	2.73		74.49^b	
	E2	ND	ND		0.64^b	
7B17	T	3.03	9.12		56.71^b	
	E2	ND	ND		ND^b	
6862	T		7.40		25.63	27.3
	E2		ND		0.28	ND
7272	T		8.31		0.16^a	
	E2		ND		ND^a	
5A67	T					65.05
	E2					0.43

Bold font denotes reproductively-active status of an individual.

ND denotes steroid concentrations below detectable levels.

^a Sampled post-spawn; histological analysis used to determine reproductive state.

^b Reproductive cycle concluded by follicular atresia.

^c Resampled 6/27/2016 after vitellogenesis had begun (T = 25.45, E2 = 1.51).

[†] Pallid sturgeon data from 2015 to 2016 included in Holmquist et al. (2019).

Table 1.3. Sex steroid concentrations (testosterone (T) ng/ml and estradiol (E2) ng/ml) by year for 15 male 1997 year-class hatchery-origin pallid sturgeon that were documented reproductively active between 2011 and 2019.

ID	Steroid	Reproductive year								
		2011	2012	2013	2014	2015	2016	2017	2018	2019
0907†	T	153.02^a		224.68		104.02	73.67			
	E2	ND^a		ND		ND	ND			
3165†	T				38.10	98.87	146.18			
	E2				ND	ND	ND			
2518†	T					195.76	168.24			11.82
	E2					ND	ND			ND
7E42	T							138.10	49.13	
	E2							ND	ND	
4E58†	T				139.44	37.61	177.21			
	E2				0.14	ND	ND			
2A09†	T				82.71	39.10	105.05			122.14
	E2				ND	ND	ND			ND
7D4A†	T		374.49							
	E2		ND							
6104†	T		128.84							
	E2		ND							
4F03†	T			6.15	4.73		114.71			
	E2			ND	ND		ND			
5870†	T						121.28			93.35
	E2						ND			ND
6C21†	T						105.49			
	E2						ND			
503F	T							314.70^a		1.62
	E2							ND^a		ND
321F	T							173.60^a		
	E2							ND^a		
4402	T							132.2		
	E2							ND		
644D	T									103.55
	E2									ND

Bold font denotes reproductively-active status of an individual.

ND denotes steroid concentrations below detectable levels.

^a Sample collected in the fall and assumed reproductively active the following spring.

† Pallid sturgeon data from 2011 to 2016 included in Holmquist et al. (2019).

Figures

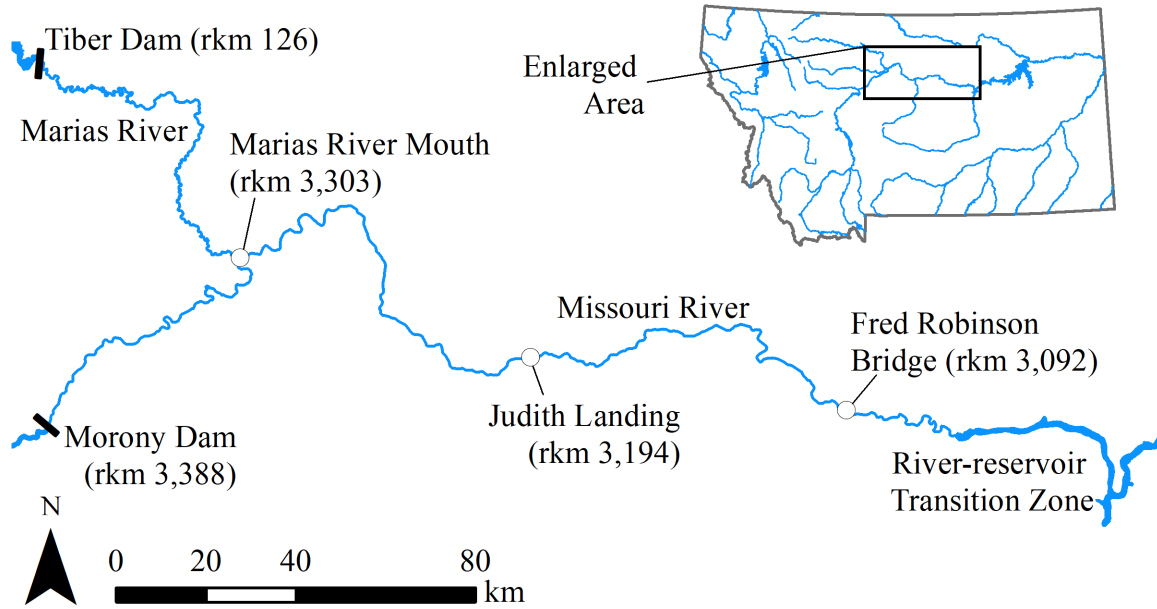


Figure 1.1. Map of the Missouri River from the river-reservoir transition zone at Fort Peck Reservoir, Montana to Morony Dam, Montana (river kilometer [rkm] 3,010–rkm 3,388) and the Marias River from the confluence with the Missouri River to Tiber Dam, Montana (rkm 0–rkm 126). Dams denoted by —, and points of reference denoted by ○.

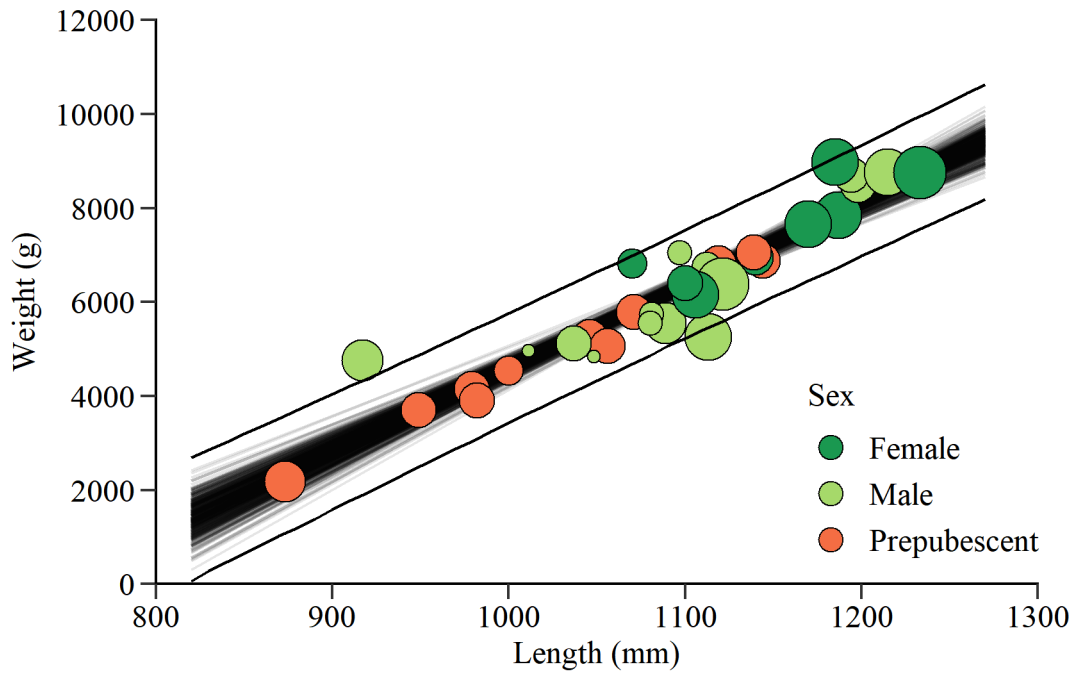


Figure 1.2. Plot of length (mm) and weight (g) of prepubescent, male at presumed-first maturity, and female at presumed-first maturity hatchery-origin 1997 year-class pallid sturgeon. Size of point represents age of fish varying from the youngest (15 years) to the oldest (22 years). Translucent lines represent 500 draws from the joint posterior distribution for the intercept and slope values associated with the length-weight regression and solid lines indicate the 95% interval of the simulated predictive distribution (50,000 simulations).

CHAPTER TWO

REPRODUCTIVE ECOLOGY AND BEHAVIOR OF HATCHERY-ORIGIN
PALLID STURGEON IN THE MISSOURI RIVER ABOVE FORT PECK
RESERVOIR, MONTANA

Contributions of Authors and Co-authors

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ABSTRACT

Conservation propagation of pallid sturgeon upstream of Fort Peck Reservoir has successfully recruited a new generation of spawning-capable pallid sturgeon to a location that would otherwise have fewer than 30 remaining wild reproductively-mature pallid sturgeon. Successful recovery of pallid sturgeon will now rely on behavior of pallid sturgeon including successful spawning in locations that provide adequate drift distance for larvae to successfully recruit. Prior to this study, no female pallid sturgeon had been documented successfully ovulating. Therefore, spawning location, spawning-related interactions among conspecifics, and substrate composition at spawning locations remained undescribed. We used radio-telemetry data of pallid sturgeon during four putative spawning seasons to answer the following questions: where do pallid sturgeon spawn; are spawning locations related to discharge; are substrate characteristics at the spawning locations similar to other river reaches; and do female, male, and atretic female pallid sturgeon use the river similarly? Additionally, we consider if spawning locations are far enough from the river-reservoir transition zone such that there would be adequate drift distance for larvae to recruit. Here, for the first time, we document spawning locations for pallid sturgeon in the Missouri River above Fort Peck Reservoir. Unfortunately, the spawning locations are not far enough upstream to prevent larvae from entering the transition zone of Fort Peck Reservoir. Four reproductively active pallid sturgeon were located in the Marias River briefly during the spawning season in 2018 when discharge was at an unprecedented high. All reproductive classifications made similar median movements, but reproductively-active females and some atretic females moved much more than reproductively-active males did during the spawning season. Furthermore, males stayed relatively close to spawning locations while females moving further up and down the river was common. Management actions will be necessary to facilitate spawning further upstream than where we documented. Management of discharge and water temperature at Tiber Dam to mimic 2018 conditions observed here may increase use of the Marias River by pallid sturgeon during the spawning season, which would provide adequate drift distance to larvae.

Introduction

Conservation propagation of endangered species has been used to prevent the extirpation or extinction of many taxon such as plants (e.g., Scott et al., 2011), insects (e.g., Amaral et al., 1997), birds (e.g., Walters et al., 2010), mammals (e.g., Jachowski & Lockhart, 2009), amphibians (e.g., Griffiths & Pavajeau, 2008), and fish (e.g., Schooley & Marsh, 2007). However, individuals from a conservation propagation program may not perform well in the wild such that recovery of the population is never achieved. One textbook example of this disconnect was for the Trumpeter Swan *Cygnus buccinator* (Lumsden & Drever, 2002). Conservation propagation of the Trumpeter Swan increased abundance; however, young swans from the captive breeding program also had to be trained to effectively migrate to wintering areas or they would have perished during the winter (Lumsden & Drever, 2002). Therefore, it is imperative to study the behavior of individuals from conservation propagation programs after they are placed in the natural environment to assess performance as it relates to recovery objectives, where performance is defined as reproductive output and survival that result in population persistence.

Conservation propagation of fishes has increased abundance and prevented extirpation of several species (e.g., cutthroat trout [*Oncorhynchus clarkia*; Al-Chokhachy et al., 2020], lake sturgeon [*Acipenser fulvescens*; Bezold & Peterson, 2008], and razorback sucker [*Xyrauchen texanus*; Marsh et al., 2015]). However, not all conservation propagation of fishes has been successful and an analysis of case studies on endangered fish reintroductions (i.e., conservation propagation and translocations from existing populations) found 42% were unsuccessful (Cochran-Biederman et al., 2015). Unforeseen behavioral deficits associated with hatchery-origin fish such as lack of predator-avoidance (Alvarez & Nicieza, 2003; Jackson & Brown,

2011), limited migratory behavior (Serrano et al., 2009; Hagelin et al., 2016), and reduced foraging ability (Brown & Laland, 2002) may result in poor performance of an augmented population (e.g., in stocked populations). Therefore, the success of conservation propagation programs for species recovery requires an understanding of the behavior of the augmented population to ensure that behavior results in adequate performance as it relates to achieving recovery objectives.

Conservation propagation is used to conserve pallid sturgeon because recruitment failure has reduced the abundance of wild pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir (USFWS, 2014), such that there are probably less than 30 individual wild pallid sturgeon (L. M. Holmquist, Montana Fish, Wildlife & Parks, personal communication). Pallid sturgeon were first stocked upstream of Fort Peck Reservoir in 1998 with pallid sturgeon hatched in 1997 (USFWS, 2014). As the abundance of wild pallid sturgeon continues to decline, recovery of pallid sturgeon upstream of Fort Peck Reservoir starts with hatchery-origin pallid sturgeon becoming reproductively mature, successfully spawning, and adding recruits to the population.

Recruitment failure of pallid sturgeon in the upper basin of the Missouri River is related to spawning location juxtaposed with the location of the river-reservoir transition zone (Braaten et al., 2008; Guy et al., 2015). Thus, behavior of hatchery-origin pallid sturgeon upstream of Fort Peck Reservoir regarding spawning movements and location will determine if successful recruitment is plausible. In the past, a lack of successfully ovulating hatchery-origin female pallid sturgeon has prevented spawning locations and spawning-related movements from being characterized (Holmquist et al., 2019). However, mature hatchery-origin female pallid sturgeon are becoming more common, which allows for gaining more information on their behavior.

If hatchery-origin pallid sturgeon spawn in upstream locations of the upper Missouri River or Marias River, drift distance available to larvae will be optimized. However, spawning in downstream locations will result in inadequate drift distance, which could necessitate management actions for successful recruitment to occur. Increasing discharge has been suggested as a potential method to instigate upstream migration prior to spawning. However, it is unknown if discharge is correlated with spawning location of pallid sturgeon. Discharge in the Marias River was shown to be associated with spawning of shovelnose sturgeon (*Scaphirhynchus platorynchus*) in the Marias River—spawning occurred when discharge was greater than 28 m³/s (Goodman et al., 2013). Furthermore, spawning location could be limited by substrate composition, which has been associated with spawning location of other sturgeon species (Chiotti et al., 2008).

Recovery of pallid sturgeon upstream of Fort Peck Reservoir was initiated by successfully augmenting the population using conservation propagation. However, successful recovery of endangered species using conservation propagation requires augmented populations to perform such that recovery criteria are eventually achieved. For pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir, reaching reproductive maturity and spawning in locations that provide adequate drift distance for larvae are the necessary first steps if the population is going to be self-sustaining as outlined in the Revised Recovery Plan for Pallid Sturgeon (USFWS, 2014). However, current knowledge of hatchery-origin pallid sturgeon upstream of Fort Peck Reservoir lacks adequate characterizations of spawning locations and abiotic conditions related to spawning locations.

We designed this study to answer the following questions about the hatchery-origin pallid sturgeon: do reproductively-active female, reproductively-active male, and atretic female pallid sturgeon use the river similarly; where do pallid sturgeon spawn; and are substrate characteristics at the spawning locations similar to other river reaches? Additionally, we consider if spawning locations are far enough from the river-reservoir transition zone such that there would be adequate drift distance for larvae to survive and recruit, and we consider if discharge is related to spawning locations. Answering these questions will inform management decisions such as how to manage discharge and reservoir water-surface levels to promote successful recruitment of progeny from hatchery-origin pallid sturgeon and thereby increase the likelihood of recovery.

Study Area

The study area is located in the Great Plains Management Unit described in USFWS (2014) and consists of the Missouri River from the upstream end of Fort Peck Reservoir to Morony Dam (river kilometer [rkm] 3,010 to rkm 3,388) and the Marias River from the confluence with the Missouri River to Tiber Dam (rkm 0 to rkm 126, Figure 2.1). The study area described here represents the northernmost distribution of pallid sturgeon in the Missouri River basin (USFWS, 2014).

The population of pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir is bound by upstream and downstream barriers. Upstream movement beyond river kilometer 3,388 is prevented by Morony Dam. Before construction of Morony Dam, upstream movement was naturally prevented by the Great Falls of the Missouri River less than 7-km upstream of Morony Dam (USFWS, 2014). Downstream movement of pallid sturgeon is limited by the transition to lacustrine conditions at the headwaters of Fort Peck Reservoir near river kilometer

3,010. The location of the Fort Peck Reservoir river-reservoir transition zone varies depending on reservoir elevation.

Discharge in the Missouri River upstream of Fort Peck Reservoir is influenced by unregulated tributaries (i.e., Smith, Teton and Judith rivers, and Belt, Arrow, Dog, and Cow creeks; Scott et al., 1997), impounded tributaries, and mainstem impoundments. Upstream of Fort Peck Reservoir, the mainstem Missouri River contains nine dams. Eight of the nine dams have negligible influence on the hydrograph because outflows are maintained roughly equal to inflows (NWE, 2016; DNRC, 2014). Canyon Ferry Dam (rkm 3,626) is the exception and is used to store water and regulate discharge in the Missouri River (Bovee & Scott, 2002). Tiber Dam on the Marias River and Gibson Dam on the Sun River are also used to store water and regulate discharge, which can reduce discharge in the Missouri River during the spring and summer (Bovee & Scott, 2002). Peak discharge typically occurs between late May and late June in the Missouri River.

The first five mainstem impoundments upstream of Fort Peck Reservoir have little effect on downstream water temperature other than reducing daily variation in water temperature (Leathe, 2018). The effects of Canyon Ferry, Houser, and Holter reservoirs have not been rigorously evaluated. Water temperature in the Marias River can be decreased by hypolimnetic water releases from Tiber Dam (Stober, 1962). However, use of an auxiliary water outlet completed at Tiber Dam in 1969 and use of the spillway could counteract cold-water temperatures from the hypolimnetic release.

Substrate within the Missouri River upstream of Fort Peck Reservoir transitions from larger substrate in the upstream reaches to smaller substrate in downstream reaches. Substrate is

primarily cobble from river kilometer 3,340 to 3,130 (Richards, 2011). At river kilometer 3,130, composition shifts to mostly gravel for several kilometers before transitioning into fine and sandy substrate (Richards, 2011).

Methods

Fish Sampling

Pallid sturgeon were sampled between early May and late July of 2018 and 2019. Pallid sturgeon targeted for sampling had been previously radio telemetered by Montana Fish, Wildlife and Parks as part of a concurrent study and by Holmquist et al. (2019). Pallid sturgeon were captured during pre-spawning season (i.e., prior to the peak of the hydrograph) to determine reproductive sex and stage of maturity, and reproductively-active females were recaptured at the end of spawning season (i.e., when water temperatures neared 24°C) to determine ovulatory outcome. Discharge and water temperature were used to define spawning season because spawning of pallid sturgeon occurs in late spring to early summer on the descending limb of the hydrograph (Fuller et al., 2008; DeLonay et al., 2016b), and optimal water temperatures during spawning are estimated to be 12°C–24°C based on larval survival (Kappenman et al., 2013).

Pre-spawning-season sampling was prioritized using reproductive assessment from 2011 through 2017. Pallid sturgeon known to be female that experienced reproductive activity in the past were considered high priority and were targeted first. After sampling high-priority pallid sturgeon, other known females were targeted. Male pallid sturgeon and pallid sturgeon of unknown sex were lower priority and were sampled opportunistically. Pallid sturgeon without active transmitters were occasionally captured as bycatch when trammel netting for targeted

pallid sturgeon, and hatchery-origin pallid sturgeon captured as bycatch were included in this study.

Pallid sturgeon were located using telemetry and trammel nets 45.7-m long, 1.8-m deep with 10.16-cm inner bar mesh, and 25.4-cm or 20.32-cm outer bar mesh were used to capture relocated pallid sturgeon. Smaller mesh trammel nets 45.7-m long, 1.8-m deep with 5.08-cm inner bar mesh, and 25.4-cm outer bar mesh were occasionally used if the larger mesh trammel nets were ineffective at capturing an individual. Biological samples were collected from all captured pallid sturgeon. Handling and sampling procedures conformed to protocols developed for pallid sturgeon (USFWS, 2012). Blood was sampled from the caudal vasculature of each pallid sturgeon using a 3-ml syringe. Blood samples were immediately transferred to a 7-ml lithium heparinized vacutainer, stored in a cool environment, and transported to the field station the same day. Once at the field station, blood samples were centrifuged at 1,228 x g (relative centrifugal force) for 5 minutes to separate blood plasma from red and white blood cells. Blood plasma was transferred to 1.5-ml vials and stored at -20 to -80°C until the sample could be delivered to the U.S. Fish and Wildlife Service Bozeman Fish Technology Center for analysis.

Gonadal tissue was sampled from all pallid sturgeon of unknown sex, and ovarian follicles were sampled from all females that were known or expected to be reproductively active. A small abdominal incision (1-2 cm) was made just off the midline between the third and fourth scutes anterior to the urogenital pore. An otoscope was used to identify the gonad, and gonadal tissue samples were taken through the otoscope specula using a Miltex biopsy cup (Webb et al., 2019). The otoscope speculum was angled to collect gonad from three different locations to account for gonadal heterogeneity. All tools used for collection of gonadal tissue were

disinfected with 70% isopropyl alcohol and were rinsed with sterile saline prior to use. Incisions were closed with evenly spaced size 0 absorbable suture material attached to a CP-1 suture needle (Ethicon PDS*II). Ovarian follicles and gonadal tissue were preserved in 10% phosphate-buffered formalin.

Reproductive Assessment

Blood, ovarian follicles, and gonadal tissue were analyzed at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center. Sex steroids (testosterone [T] and estradiol-17 β [E2]) were extracted from blood plasma using methods described in Fitzpatrick et al. (1987). An extraction solvent (2 mL of diethyl ether, extracted twice) was added to tubes with 100 μ L of the plasma and vortexed. The aqueous phase was removed by snap-freezing with liquid nitrogen, and ether was allowed to evaporate overnight in a chemical hood. The extract was reconstituted in 1 mL of phosphate-buffered saline with gelatin (PBSG). Ten or 50 μ L of reconstituted steroid extract was analyzed by radioimmunoassay as described in Fitzpatrick et al. (1986) and modified by Feist et al. (1990). A slightly more concentrated charcoal solution (6.25 g charcoal and 4.0 g dextran/L PBSG) was used for all assays. Testosterone and E2 concentrations were validated by verifying that serial dilutions were parallel to standard curves. Recovery efficiency was determined by adding tritiated steroids to tubes containing plasma (n=4), which were extracted as described above. Recovery efficiencies were 91–95% for T and 83–93% for E2. All steroid assay results were corrected for recovery. Non-detectable plasma sex steroid concentrations (i.e., not quantifiable) were assigned half of the minimum quantifiable concentration for statistical purposes (0.10 ng/mL for T and 0.05 ng/mL for E2, Croghan & Egeghy, 2003). The intra- and inter-assay coefficients of variation for all assays were less than 5% and 10%, respectively.

Blood-plasma sex-steroid concentrations were used to assign sex and stage of maturity to pallid sturgeon prior to spawning. Concentrations of T greater than 38 ng/mL and E2 less than 0.3 ng/mL were used to assign a reproductively-active male pallid sturgeon. Concentrations of T greater than 10 ng/mL and E2 greater than 0.3 ng/mL were used to assign a reproductively-active female pallid sturgeon. A reproductively-active male pallid sturgeon based on steroid concentrations would have testicular cysts with germ cells that had initiated meiosis or contain spermatozoa, and a reproductively-active female pallid sturgeon based on steroid concentrations would have initiated vitellogenesis or have post-vitellogenic ovarian follicles. For three hatchery-origin male pallid sturgeon with T concentrations within 2 ng/ml of the concentration indicative of reproductive activity (36–40 ng/ml), behavior was used to indicate and validate reproductive state. Male pallid sturgeon with T concentrations near the indicative concentration of reproductive activity that remained sedentary and did not interact with other reproductively-active pallid sturgeon were not considered reproductively active. Pallid sturgeon that had completed puberty were considered reproductively mature irrespective of reproductive status at any given time.

When possible, histological analysis of gonadal tissue verified sex and stage of maturity in fish that were assigned using plasma sex steroids (Webb et al., 2019). Additionally, histological analysis provided detail about whether a female was vitellogenic or ripe (i.e., whether the female would spawn during the year it was sampled) and ovulatory outcome of all ripe females. Post-ovulatory ovarian follicles indicated ovulatory success, and ovulatory failure was indicated by atretic ovarian follicles (Webb et al., 2019). Female pallid sturgeon that were reproductively active and underwent follicular atresia were further classified as atretic females.

Telemetry

Pallid sturgeon were tracked for the duration of the spawning season using radio-telemetry equipment. Tracking began in the fifth week of May in 2018 and the fourth week of May in 2019 before peak discharge. Tracking ended in the first week of July in 2018 and the second week of July in 2019 after all reproductively-active female pallid sturgeon had been determined to have experienced ovulatory success or failure. Tracking equipment included boat-mounted, handheld, and land-based receiver systems with three- or four-element Yagi antennas. Each system used Lotek SRX 400 telemetry receivers (Lotek Wireless, Inc., Newmarket ON, Canada) to receive and decode radio-transmitter signals. Boat-mounted and handheld systems were used manually and provided real-time information about transmitters in range. Land-based systems were autonomous and recorded data for each transmitter that passed through the field of detection. Transmitters had a unique code allowing each pallid sturgeon to be individually identified.

Signal directionality and signal strength were used to maximize accuracy when locating a pallid sturgeon. Direction of a pallid sturgeon was determined by rotating the handheld antenna until the bearing that resulted in maximum signal strength was established. The boat was moved in the direction of the pallid sturgeon until passing over the fish, which was indicated by a sudden decrease in signal strength. When the boat was positioned over a pallid sturgeon a GPS location was recorded.

Pallid sturgeon were systematically tracked based on priority given sex and stage of maturity. Relocation of reproductively-active female pallid sturgeon and atretic pallid sturgeon was attempted every two days. Relocation of reproductively-mature male pallid sturgeon was

attempted once per week. Land-based telemetry stations were used to aid in searching for undetected pallid sturgeon by documenting if a fish left the last known location.

In addition to this study, location data collected by Holmquist et al. (2019) was combined with these data to produce larger sample sizes and better answer the questions posed. Holmquist et al. (2019) used similar methods to this study and located pallid sturgeon weekly. Individual pallid sturgeon located less than half the weeks tracked in a year were excluded from analyses because minimal data on individual fish may not accurately represent movement or location of the individual.

Movement

Locations of pallid sturgeon collected during this study and by Holmquist et al. (2019) were used to estimate movement rates of pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir. Movement rates were quantified for each relocation of individual pallid sturgeon as net movement per day (km/day), and the median movement rate was calculated for each individual. A negative median net movement rate indicated a downstream movement and a positive median net movement rate indicated an upstream movement. Total movement by individual pallid sturgeon was calculated as the sum of distances between locations throughout a putative spawning season. Total movement for pallid sturgeon tracked during two putative spawning seasons was determined by calculating total movement for each season and averaging the values. Median net movement rate and total movement were calculated using minimum distance moved between relocations because pallid sturgeon may have made undetected movements between relocations. Median net movement rates and total movements of pallid sturgeon were summarized for atretic females, reproductively-active females, and

reproductively-active males. Summarized data were visualized in box plots for comparison, and the interquartile range (IQR) was used to describe variation within classifications. Interquartile ranges were calculated as the difference between the 25th and 75th quantiles (i.e., the difference between the lower and upper quartiles).

Location and Spawning Location

Location data collected in this study and by Holmquist et al. (2019) were used to summarize and compare locations among reproductive classifications. Median location was calculated as the median river kilometer among all recorded locations of an individual pallid sturgeon. Maximum upstream location was calculated as the most upstream location among all recorded locations of an individual pallid sturgeon. Median location and maximum upstream locations of pallid sturgeon were summarized for atretic females, reproductively-active females, and reproductively-active males. Summarized data was visualized in box plots for comparison, and the IQR was used to describe variation within classifications.

Putative spawning reaches of pallid sturgeon that successfully ovulated were estimated using kernel densities. A kernel-density estimate map was created from locations of individual reproductively-active females after the initial peak of the hydrograph. The cell values were scaled to represent continuous relative density between one and zero, where a value of one was the maximum relative density of locations of the individual and zero represented no locations. Reaches scoring relative density values approaching one and the area between reaches scoring relative density values approaching one were included in the estimated putative spawning reach. Locations of mature male pallid sturgeon were overlaid on the kernel density map to verify mature males had occupied the delineated spawning reaches.

Spawning Substrate

Substrate imagery (Figure 2.2) was collected in 2019 in three suspected spawning locations of pallid sturgeon—one in 2018 and two in 2019. Locations were considered suspected spawning locations when reproductively-active female pallid sturgeon were observed within 0.25 km of mature male pallid sturgeon locations. All suspected spawning locations that were mapped were within putative spawning reaches delineated by kernel density analyses. Substrate was mapped for the full width of the river and ~0.5 km above and below suspected spawning locations. Substrate mapping was completed by adapting methods described in Kaeser & Litts (2010). A sonar and GPS unit (Humminbird HELIX 7 CHIRP MEGA SI GPS G3) was used to record side-scan sonar images of the riverbed and record GPS coordinates. Image recordings were georeferenced using SonarTRX (SonarTRX Honolulu Hawaii) and imported to ArcMap 10.5.1 (ESRI, Redlands, California). Substrate types were manually delineated as polygons representing sand, gravel, and cobble (Figure 2.2). Substrate type determined from the SonarTRX was verified at opportunistic locations within each by collecting substrate samples. After delineating polygons, results of physical assessments were compared to delineated areas and no discrepancies were found. Surface area of the polygons at each site were calculated and the relative proportion of each substrate type was estimated. Central tendency and variation of the proportional substrate types were characterized by the median and IQR.

Discharge and Temperature

Mean daily discharge data from U.S. Geological Survey stream gages were used to characterize discharge in the Missouri and Maris rivers during April through July. Mean daily discharge in the Missouri River near Landusky, Montana (stream gage 06115200) from 1956

through 2019 were summarized as median monthly discharge by calculating the median of daily mean discharge values for each month of each year. Mean daily discharge in the Marias River near Loma, Montana (stream gage 06102050) from 1960 through 2019, excluding 1973 through 2001 when discharge data were not recorded, was summarized as historical median monthly discharge by calculating the median of daily discharge values for each month of each year. Historical monthly discharge values were summarized for each river by calculating the 10th, 25th, median, 75th, and 90th quantiles for each month, and the summary of historical discharge data was compared with discharge when tracking occurred.

Mean daily temperature data from U.S. Geological Survey stream gages were used to characterize temperature in the Missouri and Maris rivers during April through July during years that tracking of pallid sturgeon occurred. Mean daily temperature data for the Missouri River near Landusky, Montana (stream gage 06115200) and the Marias River near Loma, Montana (stream gage 06102050) were summarized as median monthly temperature for April through July of 2015, 2016, 2018, and 2019 by calculating the median of daily temperature values for each month of each year.

Results

In general, median discharge of the Missouri River declined from the mid 1960s to 2019, and discharge during the spring was particularly low during the early 2000s (Figure 2.3). Median monthly discharge in the Missouri River in 2015 and 2016 was below the historical median during April through July and was below the historical 25th quantile in May 2015, June 2016, and July 2016 (Table 2.1). Conversely, median monthly discharge in the Missouri River in 2018 exceeded the historical 90th quantile in April and May, exceeded the historical 75th quantile in

June, and exceeded the historical median in July (Table 2.1). In 2019, median monthly discharge in the Missouri River exceeded the historical 75th quantile in April and May and was between the 25th and 75th quantiles in June and July (Table 2.1). During the April, May, and June, median monthly water temperature in the Missouri River was slightly warmer in 2015 and 2016 than in 2018 and 2019, but in July, median monthly water temperature was coldest in 2015 and 2019 and warmest in 2016 and 2018 (Table 2.2).

In general, spring discharge in the Marias River has been relatively low throughout the 2000s as compared to discharge during the 1960s and 1970s (Figure 2.4). Median monthly discharge in the Marias River was between the historical 25th and 75th quantiles during April through July of 2015 and was at or below the 25th quantile from April through June of 2016 (Table 2.3). Conversely, median monthly discharge in the Marias River reached an unprecedented high in 2018 exceeding the historical 90th quantile during April and May and exceeding the median in June and July (Table 2.3). Median monthly discharge in the Marias River in 2019 remained between the historical 25th and 75th quantiles from April through June of 2019 but rose to above the 90th quantile in July of 2019 (Table 2.3). Median monthly temperature in the Marias River in 2015 was within the range of temperature in other years of the study. However, during April through July of 2016, median monthly temperature was the higher than in 2015, 2018, and 2019 (Table 2.4). In 2018, median monthly temperature in the Marias River was lower for April through June than during other years of the study but reached temperatures similar to other years during July (Table 2.4). In 2019, median monthly temperature was within the range of other years from April through June but was colder than in other years of the study during July (Table 2.4).

Twenty reproductively-active or atretic hatchery-origin pallid sturgeon were tracked during this study. Of the 20 pallid sturgeon tracked, two females were tracked during different reproductive classifications (i.e., atretic and reproductively active) resulting in 22 individual classifications with tracking data—12 were classified as reproductively-active males, five reproductively-active females, and five atretic females.

Median of median net movement rate was similar among reproductive classifications and consisted of slight upstream movements (Figure 2.5). The median net movement rates had little variation within classifications and all movements were between -3.3 and 4.1 km/day (Figure 2.5). The largest variation in median net movement rate was for reproductively-active males, which had an IQR of 1.7 km/day. In contrast to median net movement rates, median of total movement varied considerably among reproductive classifications. Reproductively-active female pallid sturgeon had the highest median total movement, which was 269.9 km and was 176.3 km more than that of reproductively-active males (Figure 2.6). Atretic females had a median total movement of 142.3 km—less than that of reproductively-active females but 48.8 km more than reproductively-active males (Figure 2.6). Total movement of atretic females had large variation with the 25th and 75th quantiles varying from 101.3 km to 221.6 km (IQR = 120.3, Figure 2.6).

The median of median locations of reproductively-active females was within one km of reproductively-active males (Figure 2.7). The median locations of atretic females was highly variable (IQR = 203.0) compared to reproductively-active females (IQR 13.4) and reproductively active males (IQR = 19.7), and the median locations of atretic females overlapped the other classifications (Figure 2.7). Median locations were in lower reaches of the study area, and nineteen of the 22 median locations were downstream of Judith Landing (rkm 3,194); however,

three individual pallid sturgeon had median locations upstream of Judith Landing including one atretic female in the Missouri River, one atretic female in the Marias River, and one reproductively-active male in the Marias River (Figure 2.7)—all locations in the Marias River occurred in 2018 when discharge was high (Table 2.3; Figure 2.4). Interestingly, no pallid sturgeon had median locations between rkm 3,126 and rkm 3,293. Atretic females had the highest median maximum upstream location (median = 3,292.6), reproductively-active males had the lowest (median = 3,162.3), and reproductively-active females were between the other classifications (median = 3,207.0, Figure 2.8). In 2018, maximum locations were recorded for four pallid sturgeon in the Marias River—one reproductively-active female, two atretic females, and one reproductively-active male (Figure 2.8).

Putative spawning reaches for five reproductively-active females were between rkm 3,072 and rkm 3,141 (Figures 2.9 and 2.10). The lower bound of spawning reaches were further downstream during 2019 than in 2018. Substrate was similar among mapped reaches and was composed mostly of gravel and sand with a small amount of cobble (Table 2.5). The furthest upstream mapped reach (rkm 3,117.5–3,119.0) contained the largest proportion of cobble (Table 2.5).

Discussion

We characterized movement and identified spawning locations of successfully-ovulating pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir for the first time. In general, reproductively-active females made large movements over the course of the putative spawning season, but had median net movement rates that were less than 1 km/day and upstream. Reproductively-active males and atretic females had similar median movement rates to

reproductively-active females. However, total movement by reproductively-active males and atretic females was less than that of reproductively-active females. Interestingly, atretic females had large variation in total movement rate with some individuals moving similar distances as reproductively-active females and others moving much shorter distances. River locations used were similar between reproductively-active females and males, which is the result of both classifications being located near spawning locations. However, maximum river location differed, where reproductively-active females had further upstream locations than reproductively-active males. The disparity in maximum upstream location between reproductively-active females and reproductively-active males indicates that reproductively-active male pallid sturgeon do not move as far upstream as reproductively-active females during the putative spawning season. We documented spawning reaches of reproductively-active female pallid sturgeon upstream of Fort Peck Reservoir for the first time. Distance from putative spawning reaches to the transition zone at the upstream end of Fort Peck Reservoir were less than the estimated drift distance required by pallid sturgeon larvae. Downstream of Fort Peck Reservoir, minimum required drift distance has been estimated as 245 km and is positively correlated with water velocity (Braaten et al., 2008). Therefore, required drift distance upstream of Fort Peck Reservoir, may be further than 245 km. Pallid sturgeon spawning reaches described here were between 62 km and 131 km from the transition zone indicating that even the most upstream spawning locations provided far less than the required drift distance for successful recruitment of pallid sturgeon. Substrate at suspected spawning locations within spawning reaches was a gravel-sand mosaic with little to no cobble or larger substrate. Substrate in the Missouri River transitions from smaller substrate at downstream reaches to larger substrate

moving upstream (Richards, 2011). Therefore, the substrate types we observed at spawning reaches are not common at further upstream locations in the Missouri River. The Marias River may contain substrate compositions similar to what was documented in spawning reaches, but substrate in the Marias River has not been characterized.

Pallid sturgeon were located in the Marias River and upstream of Judith Landing in the Missouri River. Upstream locations and locations in the Marias River occurred during the 2018 putative spawning season, which coincided with uncharacteristically high discharge. Unfortunately, pallid sturgeon did not spawn while at upstream locations, and instead spawned in lower portions of the Missouri River. A lack of reproductively-active male locations at upstream reproductively-active female locations may limit spawning opportunities for female pallid sturgeon that do move further upstream. In other words, the scarcity of male pallid sturgeon in upstream portions of the Missouri River or in the Marias River may preclude upstream spawning opportunities even if female pallid sturgeon could spawn at those locations. Determining why reproductively-active male pallid sturgeon are apparently less likely to be located in upstream reaches during the putative spawning season could help elucidate ways to encourage spawning further upstream. Spawning in the Marias River or upstream of Judith Landing in the Missouri River would optimize larval-drift distance, and given the apparent association between discharge and upstream location, future research and management efforts should consider discharge as a way to increase use of upstream reaches and tributaries. Discharge positively influenced spawning of shovelnose sturgeon in the Marias River (Goodman et al., 2013). When reproductively-active female and male pallid sturgeon were located in the Marias River in 2018, the Marias River had historic discharge, which peaked at $157.2 \text{ m}^3/\text{s}$ on June 1, 2018. After

peaking, discharge was rapidly reduced to aid in flood control in the Missouri River and reached 20.3 m³/s by June 28, 2018. During the rapid decrease in discharge, all reproductively-active pallid sturgeon exited the Marias River.

Pallid sturgeon in the Missouri River upstream of Fort Peck Reservoir spawned in locations where the substrate is a combination of sand and gravel, which is similar to other populations of pallid sturgeon (Elliot et al., 2020; DeLonay et al., 2016a). Upstream of where we observed spawning, spawning substrate transitions to larger substrate types (e.g., cobble and gravel, Richards, 2011). If sand is an important substrate for spawning pallid sturgeon, the lack of sand further upstream could limit the likelihood of spawning in locations with adequate drift distance, regardless of discharge. Although, the Marias River may contain more preferable spawning substrate. Future research should characterize substrate in the Marias River. If spawning substrate is present in the Marias River, discharge and temperature manipulation at Tiber Dam may be a viable management action to promote upstream spawning.

Pallid sturgeon like other sturgeon species (e.g., white sturgeon [*Acipenser transmontanus*; Cherr & Clark, 1984]) have highly adhesive eggs that probably evolved to stick to hard substrates, and hard substrate such as the large proportion of gravel observed in spawning reaches during this study may be preferred spawning habitat, but why pallid sturgeon spawn in areas with high proportions of sand remains unknown. In the lower Missouri River, pallid sturgeon selected for hard substrates (i.e., gravel, cobble, boulder, or bedrock) within spawning reaches (Elliott et al., 2020). However, the dominant substrate type within the spawning reaches was sand (Elliott et al., 2020). Spawning of pallid sturgeon in the Yellowstone River has occurred in reaches that are mostly sand (DeLonay et al., 2016a), and white sturgeon have been

documented spawning over sand; although, it was hypothesized that white sturgeon spawning over sand may be due to an absence of preferred habitat (Paragamian et al., 2001). Perhaps, a gravel-sand mosaic substrate is preferable habitat for reasons other than spawning (e.g., diet items of adult shovelnose sturgeon in the lower Platte River and age-zero shovelnose sturgeon and pallid sturgeon are associated with sand substrate [Rapp et al., 2011; Gosch et al., 2018], and age-zero pallid sturgeon have been shown to use alluvial sand dunes as velocity refugia [Porreca et al., 2017]).

Variation of movement by atretic females are likely associated with the timing of ovarian follicular atresia. Ovarian follicular atresia is associated with a drop in sex-steroid concentrations (Talbot et al., 2011), and sex steroids (e.g., testosterone or estradiol) are closely associated with endocrine signaling that drives spawning related behavior. For example, upstream migration can be induced in immature landlocked sockeye salmon (*Oncorhynchus nerka*) by implanting individuals with testosterone (Munakata et al., 2012). In pallid sturgeon, a decrease in sex steroids at the onset of follicular atresia would result in decreased drive to undergo spawning related movements. Therefore, pallid sturgeon that initiate ovarian follicular atresia early in the spawning season would behave as though they were non-reproductively-active while pallid sturgeon that initiate atresia late in the spawning season would behave more similarly to reproductively-active females. Movement of non-reproductively-active pallid sturgeon consists of slower movement and shorter distances compared to that of reproductively-active pallid sturgeon (Holmquist et al., 2019). The large variation in total movement by atretic females and the difference in total movement between atretic females and reproductively-active females

indicates that grouping atretic females with reproductively-active females should be avoided when observing behavior of pallid sturgeon.

Here we report the first observations of hatchery-origin pallid sturgeon spawning in the Missouri River upstream of Fort Peck Reservoir. In addition, we found that reproductively-active pallid sturgeon will use the Marias River, but only observed this during an unprecedented discharge event. Recovery of pallid sturgeon as defined in the Recovery Plan will only be possible if pallid sturgeon spawn further upstream than the spawning locations identified here. Management actions such as modified water releases from upstream dams to encourage use of upstream locations, lowering Fort Peck Reservoir to provide more drift distance, or both may be necessary to promote successful recruitment as outlined in the Recovery Plan. Furthermore, we found that pallid sturgeon spawned in locations with gravel-sand mosaic substrate, which is negatively associated with distance upstream in the Missouri River. The Marias River may contain more suitable spawning habitat provided that discharge and temperature regimes are managed to construct suitable spawning conditions. Future research should focus on elucidating ways to encourage pallid sturgeon to spawn in upstream locations and to determine if suitable spawning habitat is available in locations that would provide adequate drift distance.

Management actions such as modifying discharge at Tiber Dam, altering reservoir-level management at Fort Peck Reservoir, or constructing spawning habitat in desired spawning locations may be necessary to ensure spawning occurs in locations with adequate larval drift distance.

References

- Al-Chokhachy, R., Heki, L., Loux, T., & Peka, R. (2020). Return of a giant: Coordinated conservation leads to the first wild reproduction of Lahontan cutthroat trout in the Truckee River in nearly a century. *Fisheries*, *45*, 63–73. <https://doi.org/10.1002/fsh.10350>
- Alvarez, D., & Nieceza, A. G. (2003). Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. *Journal of Fish Biology*, *63*, 1565–1577. <https://doi.org/10.1111/j.1095-8649.2003.00267.x>
- Amaral, M., Kozol, A., & French, T. (1997). Conservation status and reintroduction of the endangered American burying beetle. *Northeastern Naturalist*, *4*, 121–132. DOI 10.2307/3858707
- Bezold, J., & PETERSON, D. L. (2008). Assessment of lake sturgeon reintroduction in the Coosa River system, Georgia–Alabama. In *American Fisheries Society Symposium*, *62*.
- Bovee, K. D., & Scott, M. L. (2002). Implications of flood pulse restoration for Populus regeneration on the upper Missouri River. *River Research and Applications*, *18*, 287–298. <https://doi.org/10.1002/rra.672>
- Braaten, P. J., Fuller, D. B., Holte, L. D., Lott, R. D., Viste, W., Brandt, T. F., & Legare, R. G. (2008). Drift dynamics of larval pallid sturgeon and shovelnose sturgeon in a natural side channel of the upper Missouri River, Montana. *North American Journal of Fisheries Management*, *28*, 808–826. <https://doi.org/10.1577/M06-285.1>
- Brown, C., & Laland, K. (2002). Social enhancement and social inhibition of foraging behaviour in hatchery-reared Atlantic salmon. *Journal of Fish Biology*, *61*, 987–998. <https://doi.org/10.1111/j.1095-8649.2002.tb01857.x>
- Cherr, G. N., & Clark Jr, W. H. (1984). Jelly release in the eggs of the white sturgeon, *Acipenser transmontanus*: an enzymatically mediated event. *Journal of Experimental Zoology*, *230*, 145–149. <https://doi.org/10.1002/jez.1402300120>
- Chiotti, J. A., Holtgren, J. M., Auer, N. A., & Ogren, S. A. (2008). Lake sturgeon spawning habitat in the Big Manistee River, Michigan. *North American Journal of Fisheries Management*, *28*, 1009–1019. <https://doi.org/10.1577/M07-051.1>
- Cochran-Biederman, J. L., Wyman, K. E., French, W. E., & Loppnow, G. L. (2015). Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology*, *29*, 175–186. <https://doi.org/10.1111/cobi.12374>
- Croghan, C., & Egeghy, P. P. (2003). Methods of dealing with values below the limit of detection using SAS. Southeastern SAS User Group, St. Petersburg, FL.

- DeLonay, A. J., Chojnacki, K. A., Jacobson, R. B., Albers, J. L., Braaten, P. J., Bulliner, E. A., and Wildhaber, M. L. (2016a). Ecological requirements for pallid sturgeon reproduction and recruitment in the Missouri River: A synthesis of science, 2005–2012. U.S. Geological Survey Scientific Investigations Report 2015–5145. Reston, VA.
- DeLonay, A. J., Jacobson, R. B., Chojnacki, K. A., Braaten, P. J., Buhl, K. J., Eder, B. L., Elliott, C. M., Erwin, S. O., Fuller, D. B., Haddix, T. M., Ladd, H. L. A., Mestl, G. E., Papoulias, D. M., Rhoten, J. C., Wesolek, C. J., & Wildhaber, M. L. (2016b). Ecological requirements for pallid sturgeon reproduction and recruitment in the Missouri River—annual report 2013. U.S. Geological Survey, Open-File Report 2015–1197. Reston, VA
- DNRC (Montana Department of Natural Resources and Conservation). (2014). Toston Dam (Broadwater-Missouri). Montana Department of Natural Resources and Conservation, Helena, MT.
- Elliott, C. M., DeLonay, A. J., Chojnacki, K. A., & Jacobson, R. B. (2020). Characterization of pallid sturgeon (*Scaphirhynchus albus*) spawning habitat in the Lower Missouri River. *Journal of Applied Ichthyology*, 36, 25–38. <https://doi.org/10.1111/jai.13994>
- Feist, G., Schreck, C. B., Fitzpatrick, M. S., & Redding, J. M. (1990). Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. *General and Comparative Endocrinology*, 80, 299–313.
- Fitzpatrick, M. S., Redding, J. M., Ratti, F. D., & Schreck, C. B. (1987). Plasma testosterone concentration predicts the ovulatory response of coho salmon (*Oncorhynchus kisutch*) to gonadotropin-releasing hormone analog. *Canadian Journal of fisheries and aquatic sciences*, 44, 1351–1357.
- Fitzpatrick, M. S., Van Der Kraak, G., & Schreck, C. B. (1986). Profiles of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch*, during final maturation. *General and Comparative Endocrinology*, 62, 437–451.
- Fuller, D. A., Jaeger, M. E., and Webb M. A. H. (2008). *Spawning and associated movement patterns of pallid sturgeon in the lower Yellowstone River. Report prepared for the Upper Missouri River Basin pallid sturgeon Workgroup, Western Area Power Administration, and U.S. Army Corps of Engineers.* Helena, MT: Montana Fish, Wildlife and Parks.
- Goodman, B. J., Guy, C. S., Camp, S. L., Gardner, W. M., Kappenman, K. M., & Webb, M. A. H. (2013). Shovelnose sturgeon spawning in relation to varying discharge treatments in a Missouri River tributary. *River Research and Applications*, 29, 1004–1015. <https://doi.org/10.1002/rra.2587>

- Gosch, N. J. C., Civiello, A. P., Gemeinhardt, T. R., Bonneau, J. L., & Long, J. M. (2018). Are shovelnose sturgeon a valid diet surrogate for endangered pallid sturgeon during the first year of life?. *Journal of Applied Ichthyology*, *34*, 39–41.
- Griffiths, R. A., & Pavajeau, L. (2008). Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, *22*, 852–861. <https://doi.org/10.1111/j.1523-1739.2008.00967.x>
- Guy, C. S., Treanor, H. B., Kappenman, K. M., Scholl, E. A., Ilgen, J. E., & Webb, M. A. (2015). Broadening the regulated-river management paradigm: a case study of the forgotten dead zone hindering pallid sturgeon recovery. *Fisheries*, *40*, 6–14. <https://doi.org/10.1080/03632415.2014.987236>
- Hagelin, A., Calles, O., Greenberg, L., Piccolo, J., & Bergman, E. (2016). Spawning migration of wild and supplementary stocked landlocked Atlantic salmon (*Salmo salar*). *River Research and Applications*, *32*, 383–389. <https://doi.org/10.1002/rra.2870>
- Holmquist, L. M., Guy, C. S., Tews, A., Trimpe, D. J., & Webb, M. A. (2019). Reproductive ecology and movement of pallid sturgeon in the upper Missouri River, Montana. *Journal of Applied Ichthyology*, *35*, 1069–1083. <https://doi.org/10.1111/jai.13962>
- Jachowski, D. S., & Lockhart, J. M. (2009). Reintroducing the black-footed ferret *Mustela nigripes* to the Great Plains of North America. *Small Carnivore Conservation*, *41*, 58–64.
- Jackson, C. D., & Brown, G. E. (2011). Differences in antipredator behaviour between wild and hatchery-reared juvenile Atlantic salmon (*Salmo salar*) under seminatural conditions. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*, 2157–2166. <https://doi.org/10.1139/f2011-129>
- Kaesler, A. J., & Litts, T. L. (2010). A novel technique for mapping habitat in navigable streams using low-cost side scan sonar. *Fisheries*, *35*, 163–174. <https://doi.org/10.1577/1548-8446-35.4.163>
- Kappenman, K. M., Webb, M. A. H., & Greenwood, M. (2013). The effect of temperature on embryo survival and development in pallid sturgeon *Scaphirhynchus albus* (Forbes & Richardson 1905) and shovelnose sturgeon *S. platyrhynchus* (Rafinesque, 1820). *Journal of Applied Ichthyology*, *29*, 1193–1203. <https://doi.org/10.1111/jai.12301>
- Leathe, S. (2018). Effects of Great Falls reservoirs on Missouri River water temperature. North Western Energy, Sioux Falls, SD.
- Lumsden, H. G., & Drever, M. C. (2002). Overview of the trumpeter swan reintroduction program in Ontario, 1982-2000. *Waterbirds*, *25*, 301–312.

- Marsh, P. C., Dowling, T. E., Kesner, B. R., Turner, T. F., & Minckley, W. L. (2015). Conservation to stem imminent extinction: the fight to save razorback sucker *Xyrauchen texanus* in Lake Mohave and its implications for species recovery. *Copeia*, 2015, 141–156. <https://doi.org/10.1643/OT-14-030>
- Munakata, A., Amano, M., Ikuta, K., Kitamura, S., & Aida, K. (2012). Involvement of sex steroids, luteinizing hormone and thyroid hormones in upstream and downstream swimming behavior of land-locked sockeye salmon *Oncorhynchus nerka*. *Fisheries science*, 78, 81–90. <https://doi.org/10.1007/s12562-011-0436-6>
- NWE (Northwestern Energy). (2016). Hydro operational requirements from FERC license articles and SOP agreements with agencies for NWE's 11 hydropower dams (3 FERC licenses). North Western Energy, Sioux Falls, SD.
- Paragamian, V. L., Kruse, G., & Wakkinen, V. (2001). Spawning habitat of Kootenai River white sturgeon, post-Libby Dam. *North American Journal of Fisheries Management*, 21, 22–33. [https://doi.org/10.1577/1548-8675\(2001\)021<0022:SHOKRW>2.0.CO;2](https://doi.org/10.1577/1548-8675(2001)021<0022:SHOKRW>2.0.CO;2)
- Porreca, A. P., Hintz, W. D., & Garvey, J. E. (2017). Do alluvial sand dunes create energetic refugia for benthic fishes? An experimental test with the endangered pallid sturgeon. *River Research and Applications*, 33, 690–696. <https://doi.org/10.1002/rra.3132>
- Rapp, T., Shuman, D. A., Graeb, B. D. S., Chipps, S. R., & Peters, E. J. (2011). Diet composition and feeding patterns of adult shovelnose sturgeon (*Scaphirhynchus platorynchus*) in the lower Platte River, Nebraska, USA. *Journal of Applied Ichthyology*, 27, 351–355. <https://doi.org/10.1111/j.1439-0426.2011.01743.x>
- Richards, R. R. (2011). Movement of *Scaphirhynchus* species in the Missouri River above Fort Peck Reservoir, Montana. Master's thesis, Montana State University, Bozeman.
- Schooley, J. D., & Marsh, P. C. (2007). Stocking of endangered razorback suckers in the lower Colorado River basin over three decades: 1974–2004. *North American Journal of Fisheries Management*, 27, 43–51.
- Scott, G. L., McCaughey, W. W., & Izlar, K. (2011). Guidelines for whitebark pine planting prescriptions. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. 63, 362–364.
- Scott, M. L., Auble, G. T., & Friedman, J. M. (1997). Flood dependency of cottonwood establishment along the Missouri River, Montana, USA. *Ecological Applications*, 7, 677–690. [https://doi.org/10.1890/1051-0761\(1997\)007\[0677:FDOCEA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1997)007[0677:FDOCEA]2.0.CO;2)

- Serrano, I., Larsson, S., & Eriksson, L. O. (2009). Migration performance of wild and hatchery sea trout (*Salmo trutta* L.) smolts—Implications for compensatory hatchery programs. *Fisheries Research*, *99*, 210–215. <https://doi.org/10.1016/j.fishres.2009.06.004>
- Stober, Q. J. (1962). Some limnological effects of Tiber Reservoir on the Marias River. Master's thesis, Montana State University, Bozeman.
- Talbott, M. J., Van Eenennaam, J. P., Linares-Casenave, J., Doroshov, S. I., Guy, C. S., Struffenegger, P., & Webb, M. A. (2011). Investigating the use of plasma testosterone and estradiol-17 β to detect ovarian follicular atresia in farmed white sturgeon, *Acipenser transmontanus*. *Aquaculture*, *315*, 283–289. <https://doi.org/10.1016/j.aquaculture.2010.10.041>
- USFWS (United State Fish and Wildlife Service). (2012). *Biological procedure and protocols for researchers and managers handling pallid sturgeon*. (40 pp.). Denver, CO: Fish and Wildlife Service.
- USFWS (United States Fish and Wildlife Service). (2014). *Revised recovery plan for the pallid sturgeon (Scaphirhynchus albus)*. (115 pp.). Denver, CO: Fish and Wildlife Service.
- Walters, J. R., Derrickson, S. R., Michael Fry, D., Haig, S. M., Marzluff, J. M., & Wunderle Jr, J. M. (2010). Status of the California Condor (*Gymnogyps californianus*) and efforts to achieve its recovery. *The Auk*, *127*, 969–1001. <https://doi.org/10.1525/auk.2010.127.4.969>
- Webb, M. A. H., Van Eenennaam, J. P., Crossman, J. A., & Chapman, F. A. (2019). A practical guide for assigning sex and stage of maturity in sturgeons and paddlefish. *Journal of Applied Ichthyology*, *35*, 169–186. <https://doi.org/10.1111/jai.13582>

Tables

Table 2.1. Tenth, 25th, Median, 75th, 90th quantiles of monthly median discharge (m³/s) in the Missouri River from 1956 through 2019 during April through July, and median discharge from April through July for 2015, 2016, 2018, and 2019. Data from U.S. Geological Survey stream gage station near Landusky, Montana (stream gage 06115200).

Month	1956–2019					2015	2016	2018	2019
	10 th	25 th	Median	75 th	90 th	Median			
April	152.3	196.4	246.2	293.1	408.3	210.7	205.3	511.1	406.3
May	187.3	253.2	351.1	441.0	610.2	209.5	280.9	869.3	487.0
June	194.3	299.5	407.1	653.1	868.8	301.6	238.7	831.1	375.2
July	139.8	173.5	248.2	332.0	512.0	184.3	171.3	322.8	303.0

Table 2.2. Median water temperature (°C) in the Missouri River for April through July of 2015, 2016, 2018, and 2019 excluding April of 2015 and 2018 when water temperature data were not collected and June of 2018 when the stream gage was inoperable due to flooding. Data from U.S. Geological Survey stream gage station near Landusky, Montana (stream gage 06115200).

Median water temperature (°C)				
Month	2015	2016	2018	2019
April		11.4		10.4
May	14.0	14.8	13.8	12.0
June	19.9	20.7		19.0
July	21.4	23.8	23.5	22.7

Table 2.3. Tenth, 25th, Median, 75th, 90th quantiles of monthly median discharge (m³/s) in the Marias River from 1960 through 2019 excluding 1973 through 2001, when discharge data was not recorded, summarized by month, and median discharge from April through July for 2015, 2016, 2018, and 2019. Data from U.S. Geological Survey stream gage station near Loma, Montana (stream gage 06102050).

Month	1960–1972 and 2001–2019					2015	2016	2018	2019
	10 th	25 th	Median	75 th	90 th	Median			
April	11.5	14.0	15.2	27.1	37.1	15.7	14.0	81.8	18.9
May	13.3	14.9	30.9	40.2	56.6	15.1	13.9	90.3	20.4
June	13.7	18.0	34.5	54.4	79.2	18.3	13.6	50.7	20.4
July	10.0	15.2	19.4	39.7	57.6	18.2	14.9	21.5	75.3

Table 2.4. Median water temperature (°C) in the Marias River for April through July of 2015, 2016, 2018, and 2019. Data from U.S. Geological Survey stream gage station near Loma, Montana (stream gage 06102050).

Month	Median water temperature (°C)			
	2015	2016	2018	2019
April	10.7	11.0	6.0	9.6
May	14.7	15.6	11.9	12.3
June	20.3	20.4	16.5	18.9
July	20.8	22.8	20.0	19.9

Table 2.5. Proportion of substrate types for three mapped reaches in the Missouri River upstream of Fort Peck Reservoir and median of proportion of substrate type at locations with interquartile range (IQR) in parentheses. River kilometer (rkm) of the upper and lower boundary of mapped reaches is denoted. Mapped reaches were within putative spawning reaches (see Figures 3.9 and 3.10) and were selected for mapping when reproductively-active female pallid sturgeon were observed interacting with mature male pallid sturgeon. Mapped reaches included ~0.5 km distance above and below where interactions were observed.

Mapped Reach (rkm)	Proportion substrate type		
	Sand	Gravel	Cobble
3,081.5–3,084.0	0.43	0.56	0.01
3,088.0–3,090.0	0.41	0.53	0.06
3,117.5–3,119.0 ^a	0.38	0.49	0.13
Median (IQR)	0.41 (0.02)	0.53 (0.04)	0.06 (0.06)

^a Substrate imagery collected one year after spawning occurred.

Figures

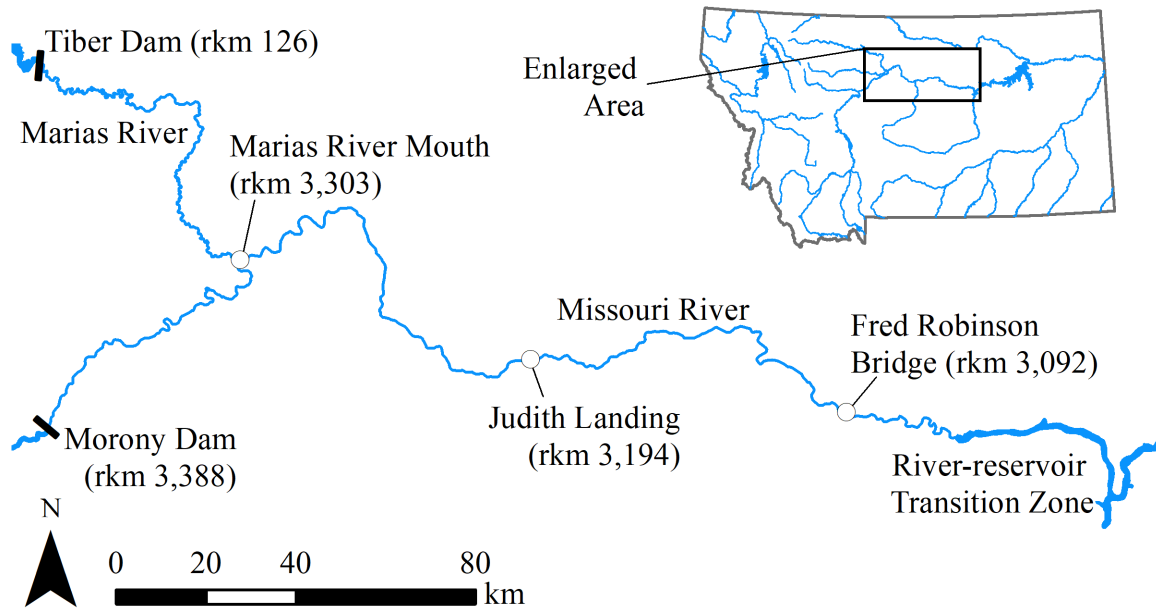


Figure 2.1. Map of the Missouri River from the river-reservoir transition zone at Fort Peck Reservoir, Montana to Morony Dam, Montana (river kilometer [rkm] 3,010–rkm 3,388) and the Marias River from the confluence with the Missouri River to Tiber Dam, Montana (rkm 0–rkm 126). Dams denoted by —, and points of reference denoted by ○.

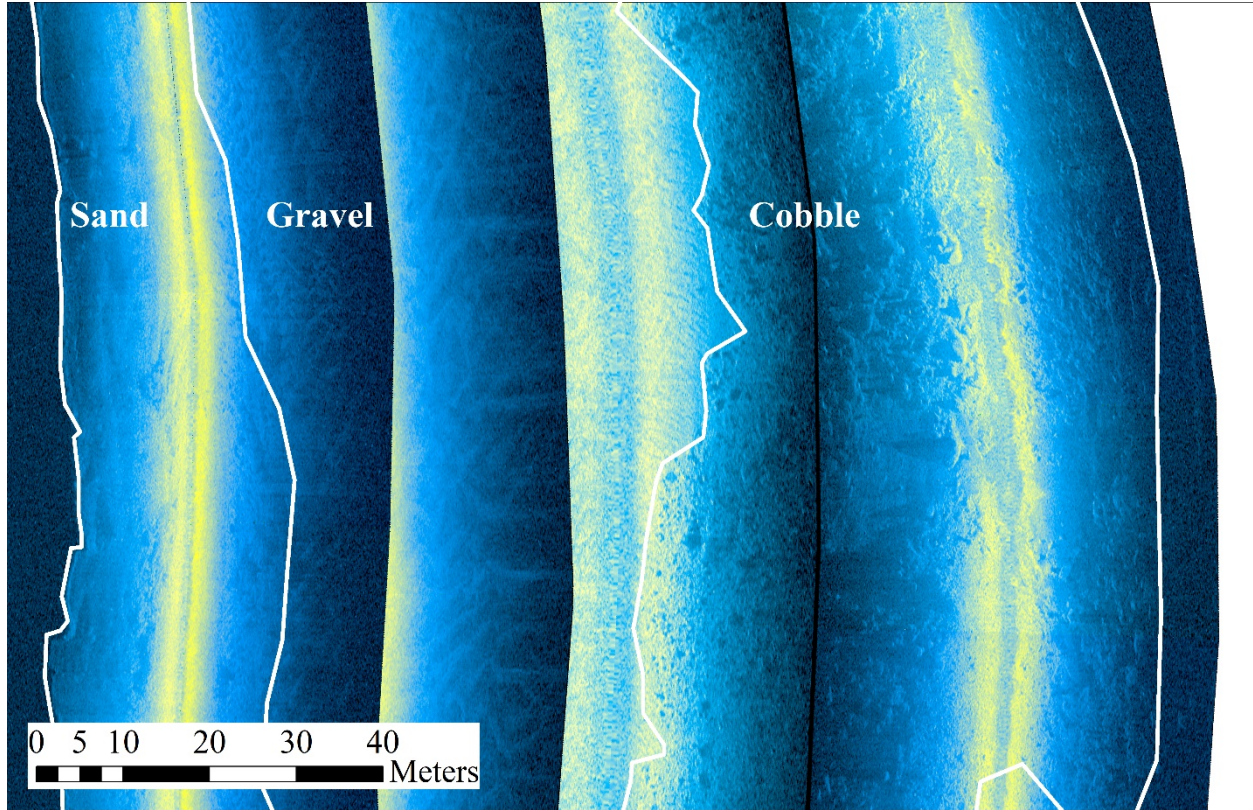


Figure 2.2. Example of substrate imagery and delineation of substrate types (sand, gravel, and cobble). Substrate imagery was collected in the Missouri River near river kilometer 3,117. Substrate imagery was collected and polygons were delineated using methods adapted from Kaeser & Litts (2010).

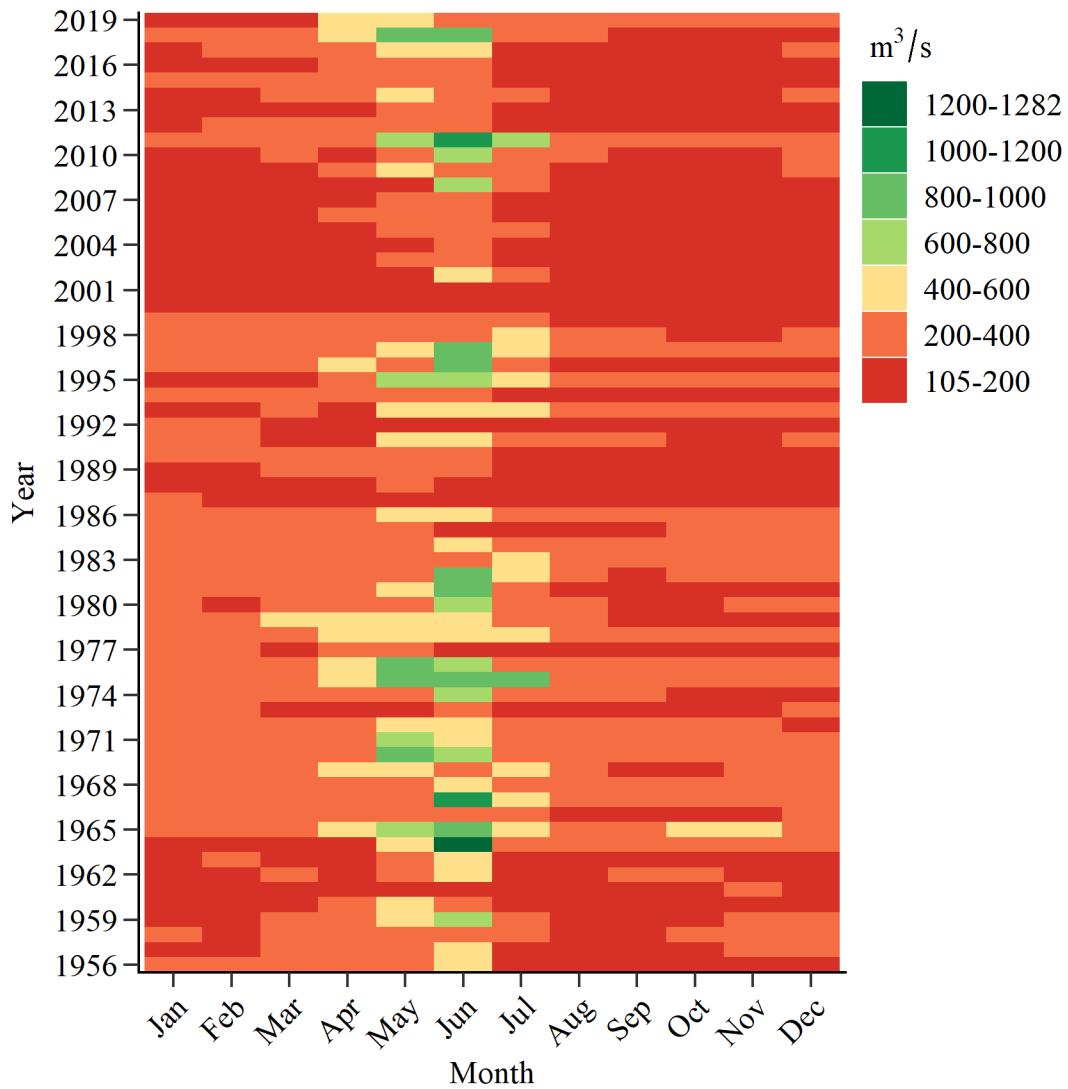


Figure 2.3. Median discharge (m³/s) in the Missouri River by month from 1956 through 2019. Data from U.S. Geological Survey stream gage station near Landusky, Montana (stream gage 06115200).

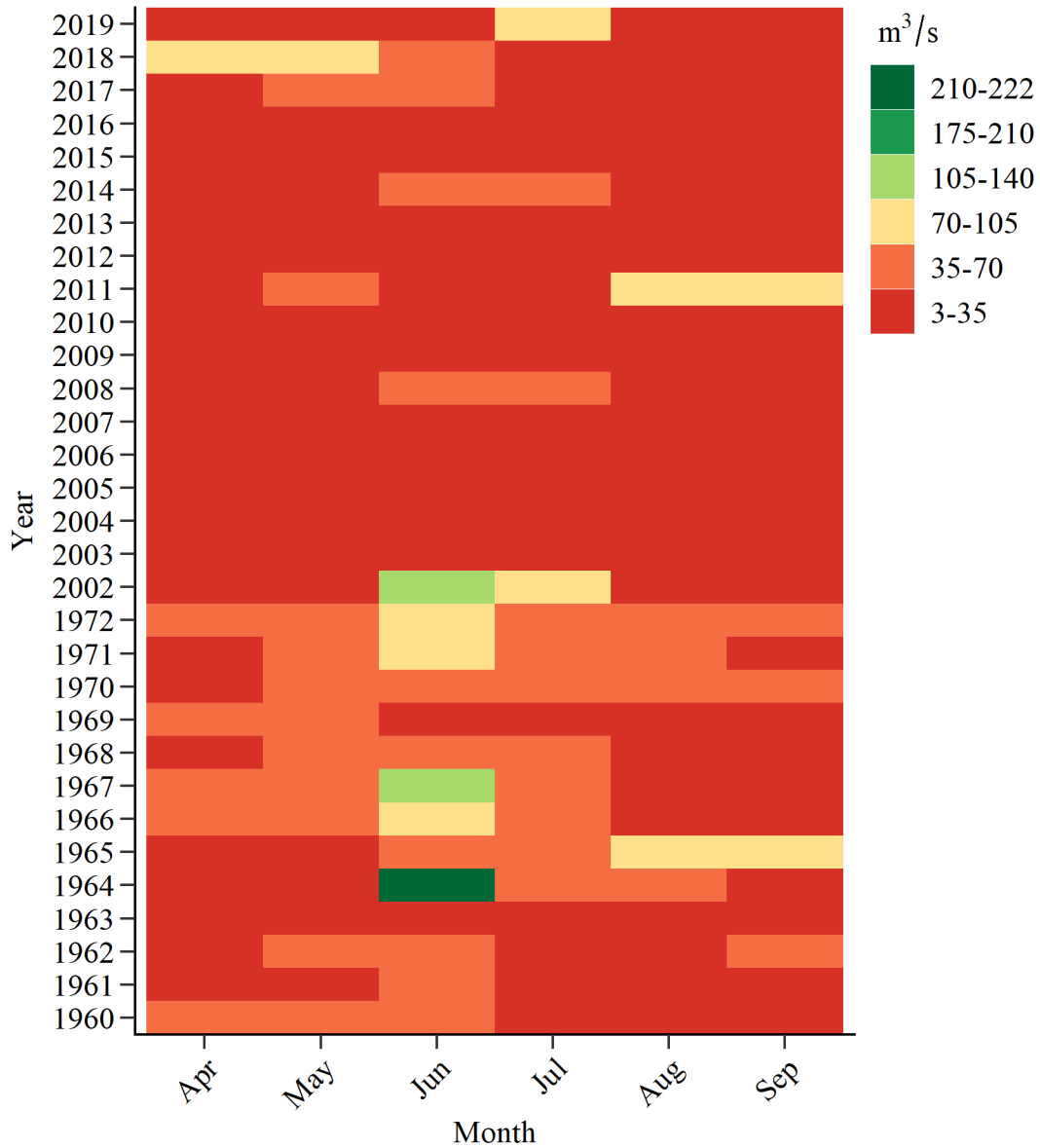


Figure 2.4. Median discharge (m³/s) in the Marias River by month from 1960 through 2019, excluding 1973 through 2001 when discharge data were not recorded. Data from U.S. Geological Survey stream gage station near Loma, Montana (stream gage 06102050).

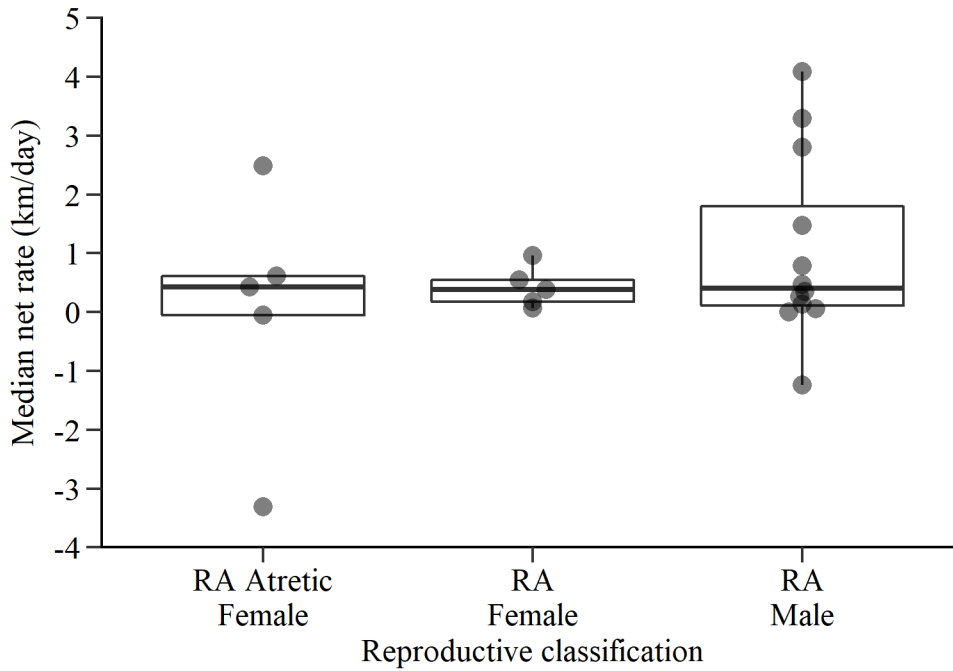


Figure 2.5. Median net movement rate (km/day, circles represent individuals) for pallid sturgeon by reproductive classification during the putative spawning seasons of 2015, 2016, 2018, and 2019 in the Missouri River upstream of Fort Peck Reservoir. Individuals were classified as reproductively-active (RA) atretic female, RA female, and RA male. Box ends represent the 25th and 75th quantiles, horizontal lines are the median, the upper whisker extends to the largest observation no further than 1.5 * interquartile range (IQR) from the 75th quantile, and the lower whisker extends to the smallest observation no further than 1.5 * IQR from the 25th quantile.

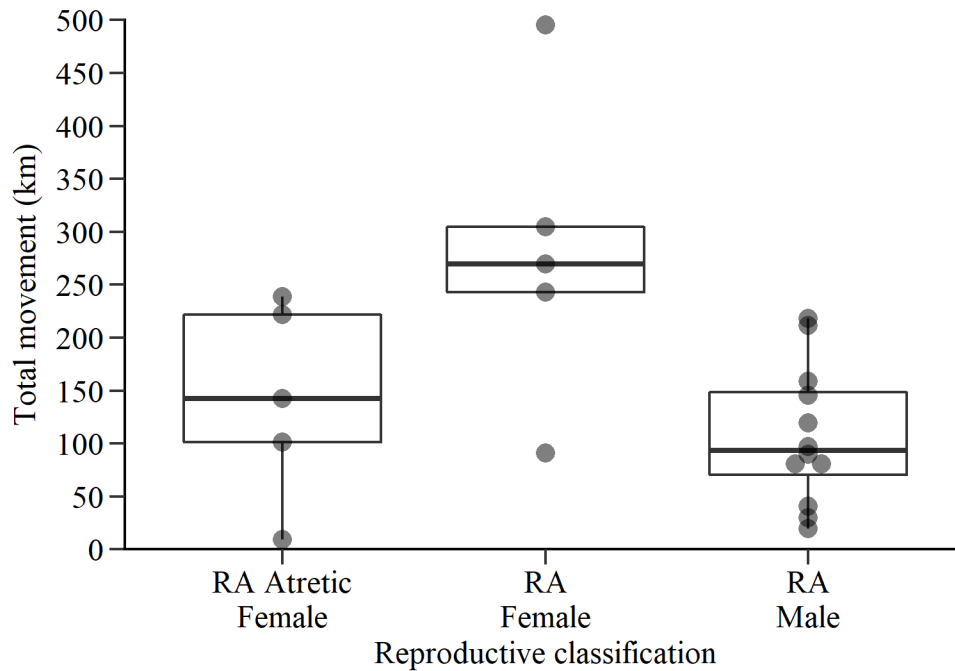


Figure 2.6. Total movement (km, circles represent individuals) for pallid sturgeon by reproductive classification during the putative spawning seasons of 2015, 2016, 2018, and 2019 in the Missouri River upstream of Fort Peck Reservoir. Individuals were classified as reproductively-active (RA) atretic female, RA female, and RA male. Box ends represent the 25th and 75th quantiles, horizontal lines are the median, the upper whisker extends to the largest observation no further than 1.5 * interquartile range (IQR) from the 75th quantile, and the lower whisker extends to the smallest observation no further than 1.5 * IQR from the 25th quantile.

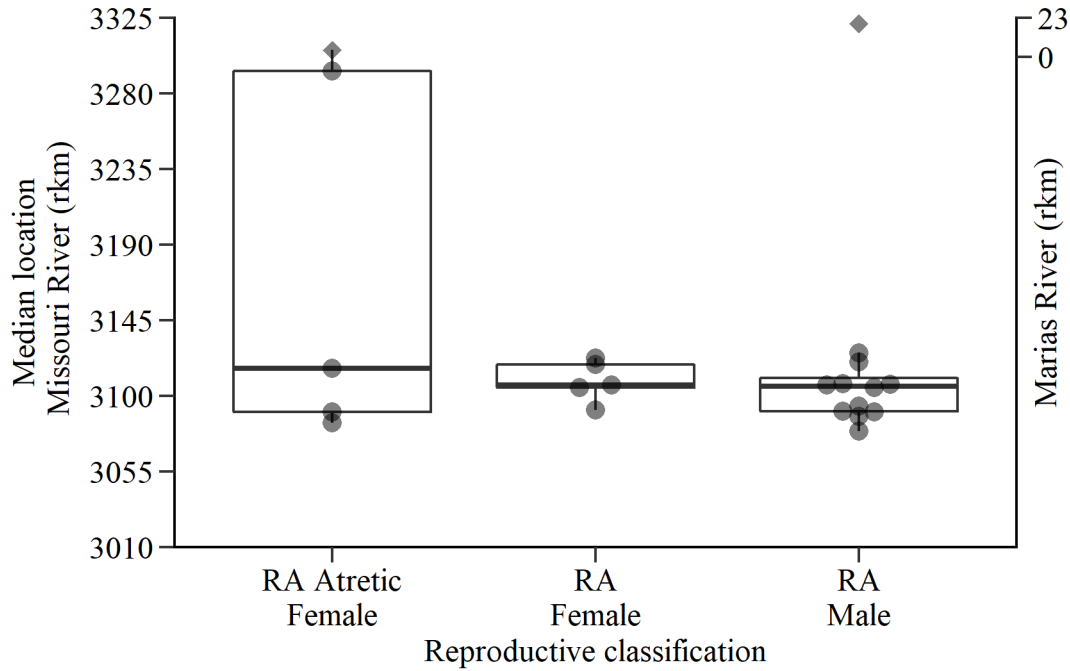


Figure 2.7. Median location (rkm, circles represent locations in the Missouri River and diamonds represent locations in the Marias River) for pallid sturgeon by reproductive classification during the putative spawning seasons of 2015, 2016, 2018, and 2019 upstream of Fort Peck Reservoir. Individuals were classified as reproductively-active (RA) atretic female, RA female, and RA male. Box ends represent the 25th and 75th quantiles, horizontal lines are the median, the upper whisker extends to the largest observation no further than 1.5 * interquartile range (IQR) from the 75th quantile, and the lower whisker extends to the smallest observation no further than 1.5 * IQR from the 25th quantile.

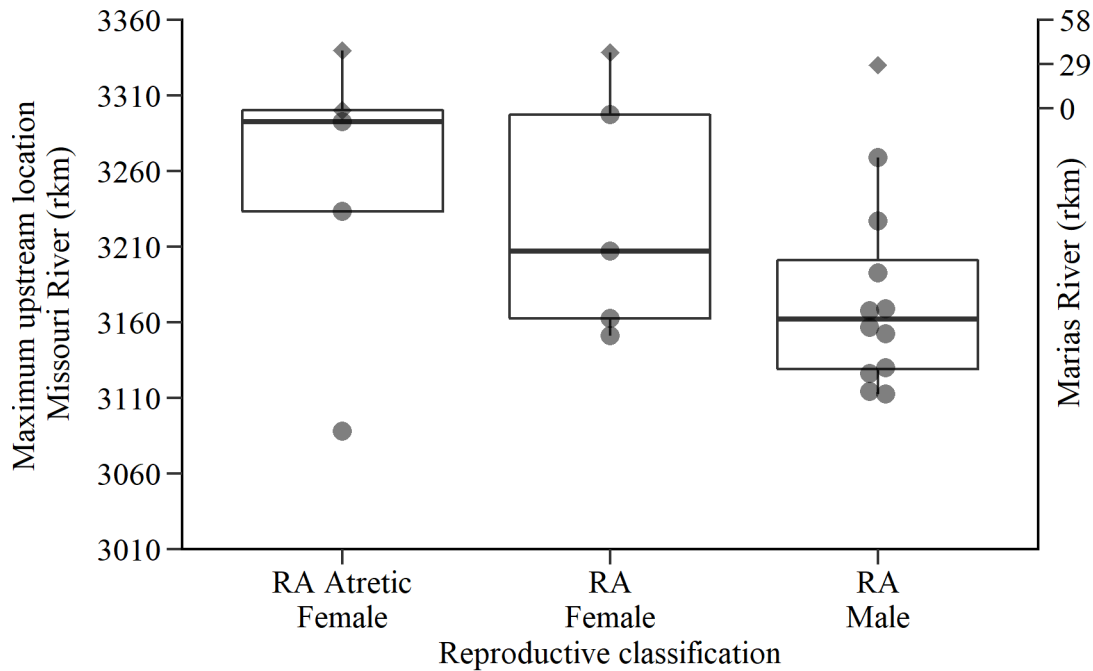


Figure 2.8. Maximum upstream location (rkm, circles represent locations in the Missouri River and diamonds represent locations in the Marias River) for pallid sturgeon by reproductive classification during the putative spawning seasons of 2015, 2016, 2018, and 2019 in the Missouri River upstream of Fort Peck Reservoir. Individuals were classified as reproductively-active (RA) atretic female, RA female, and RA male. Box ends represent the 25th and 75th quantiles, horizontal lines are the median, the upper whisker extends to the largest observation no further than $1.5 \times$ interquartile range (IQR) from the 75th quantile, and the lower whisker extends to the smallest observation no further than $1.5 \times$ IQR from the 25th quantile.

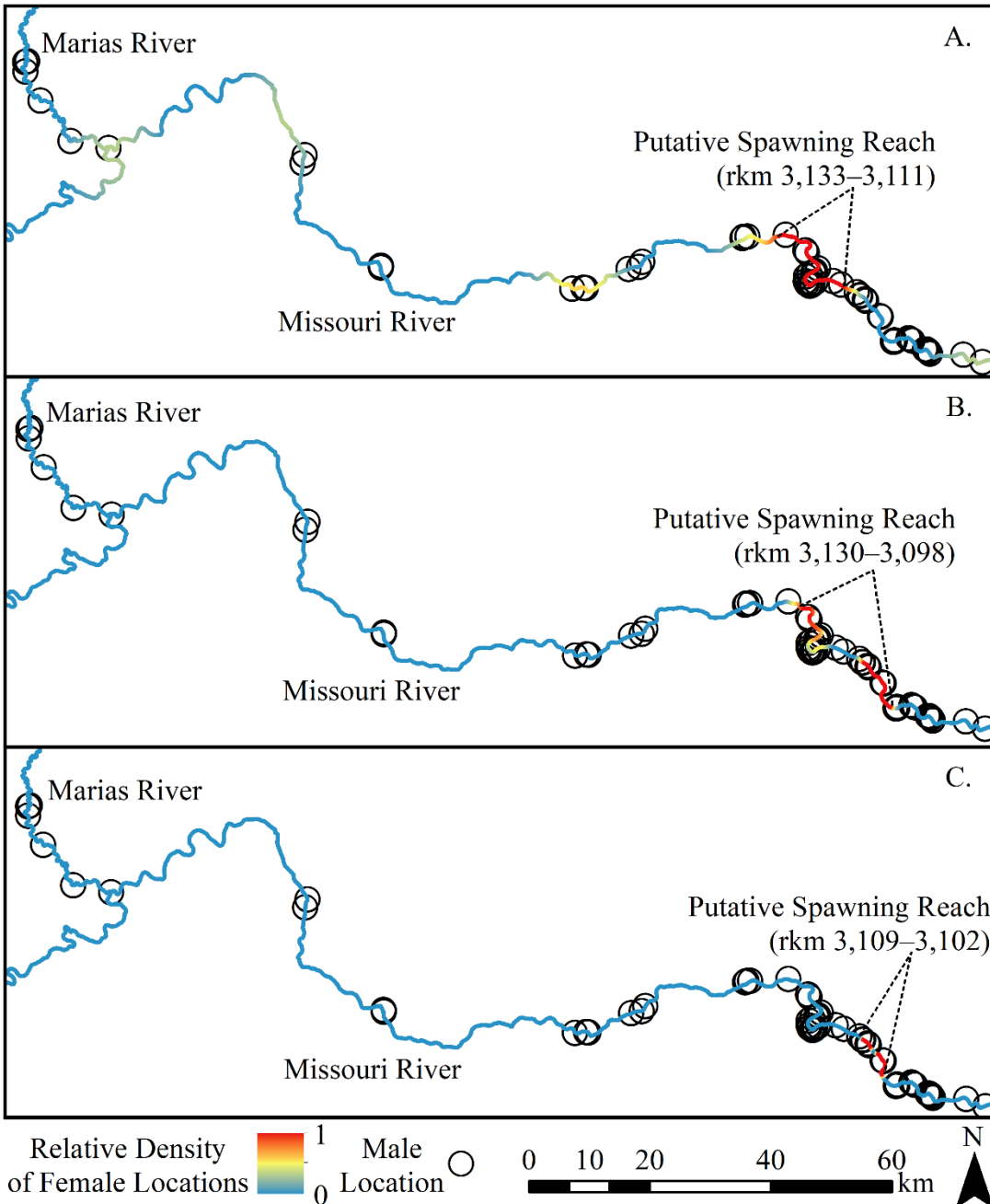


Figure 2.9. Kernel-density maps show relative densities of locations for individual females that successfully ovulated during the spawning season 2018 (A. ID 9_161, B. ID 9_163, and C. ID 9_171). Relative densities closer to one indicate areas where females were most frequently located. Lower and upper bound of putative spawning reach are indicated by the dashed lines and river kilometer (rkm) in parentheses. Mature male locations during the spawning season are indicated by open circles.

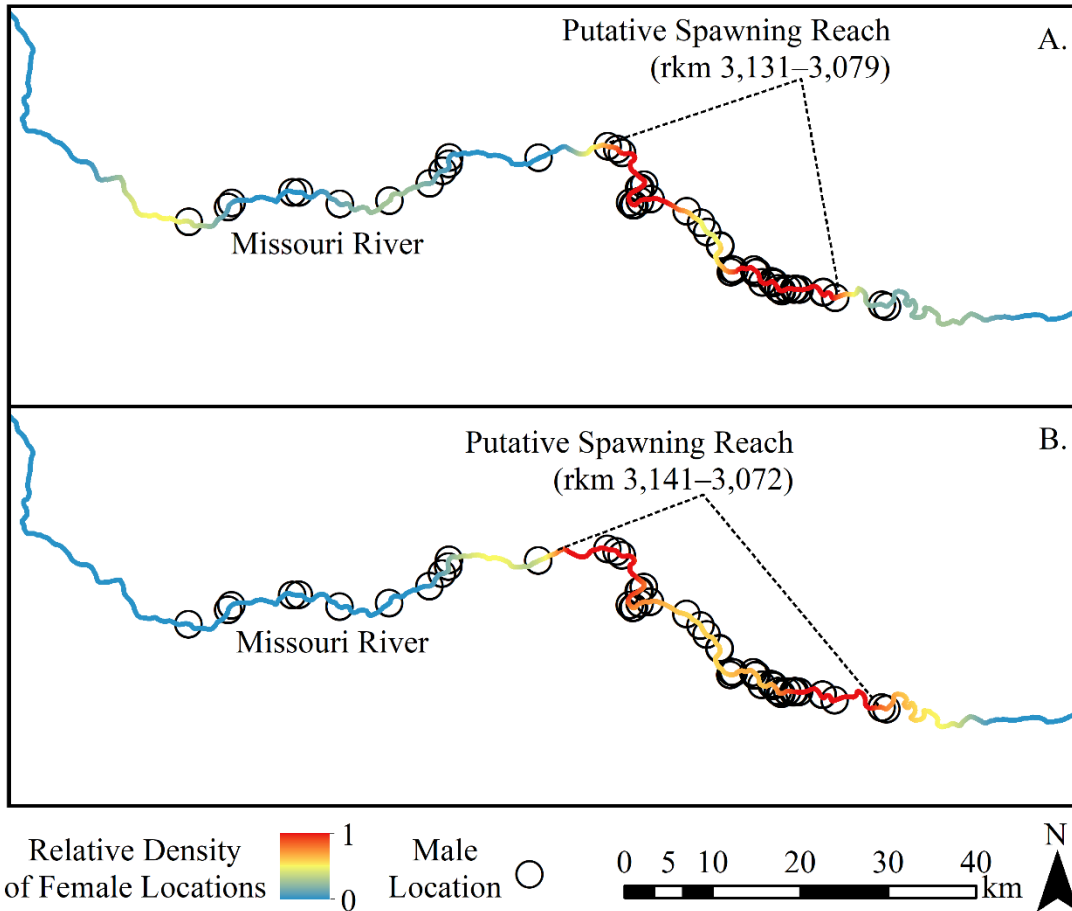


Figure 2.10. Kernel-density maps show relative densities of locations for individual females that successfully ovulated during the spawning season 2019 (A. ID 8_86 and B. ID 8_92). Relative densities closer to one indicate areas where females were most frequently located. Lower and upper bound of putative spawning reach are indicated by the dashed lines and river kilometer (rkm) in parentheses. Mature male locations during the spawning season are indicated by open circles.

REFERENCES CITED

- Al-Chokhachy, R., Heki, L., Loux, T., & Peka, R. (2020). Return of a giant: Coordinated conservation leads to the first wild reproduction of Lahontan cutthroat trout in the Truckee River in nearly a century. *Fisheries*, *45*, 63–73. <https://doi.org/10.1002/fsh.10350>
- Alvarez, D., & Nieceza, A. G. (2003). Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. *Journal of Fish Biology*, *63*, 1565–1577. <https://doi.org/10.1111/j.1095-8649.2003.00267.x>
- Amaral, M., Kozol, A., & French, T. (1997). Conservation status and reintroduction of the endangered American burying beetle. *Northeastern Naturalist*, *4*, 121–132. DOI 10.2307/3858707
- Auer, N. A. (1999). Population characteristics and movements of lake sturgeon in the Sturgeon River and Lake Superior. *Journal of Great Lakes Research*, *25*, 282–293. [https://doi.org/10.1016/S0380-1330\(99\)70737-9](https://doi.org/10.1016/S0380-1330(99)70737-9)
- Bezold, J., & PETERSON, D. L. (2008). Assessment of lake sturgeon reintroduction in the Coosa River system, Georgia–Alabama. In *American Fisheries Society Symposium*, *62*.
- Bovee, K. D., & Scott, M. L. (2002). Implications of flood pulse restoration for *Populus* regeneration on the upper Missouri River. *River Research and Applications*, *18*, 287–298. <https://doi.org/10.1002/rra.672>
- Braaten, P. J., Fuller, D. B., Holte, L. D., Lott, R. D., Viste, W., Brandt, T. F., & Legare, R. G. (2008). Drift dynamics of larval pallid sturgeon and shovelnose sturgeon in a natural side channel of the upper Missouri River, Montana. *North American Journal of Fisheries Management*, *28*, 808–826. <https://doi.org/10.1577/M06-285.1>
- Brown, C., & Laland, K. (2002). Social enhancement and social inhibition of foraging behaviour in hatchery-reared Atlantic salmon. *Journal of Fish Biology*, *61*, 987–998. <https://doi.org/10.1111/j.1095-8649.2002.tb01857.x>
- Burton, M. P., & Idler, D. R. (1987). An experimental investigation of the non-reproductive, post-mature state in winter flounder. *Journal of Fish Biology*, *30*, 643–650. <https://doi.org/10.1111/j.1095-8649.1987.tb05793.x>
- Cherr, G. N., & Clark Jr, W. H. (1984). Jelly release in the eggs of the white sturgeon, *Acipenser transmontanus*: an enzymatically mediated event. *Journal of Experimental Zoology*, *230*, 145–149. <https://doi.org/10.1002/jez.1402300120>

- Chiotti, J. A., Holtgren, J. M., Auer, N. A., & Ogren, S. A. (2008). Lake sturgeon spawning habitat in the Big Manistee River, Michigan. *North American Journal of Fisheries Management*, 28, 1009–1019. <https://doi.org/10.1577/M07-051.1>
- Cochran-Biederman, J. L., Wyman, K. E., French, W. E., & Loppnow, G. L. (2015). Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology*, 29, 175–186. <https://doi.org/10.1111/cobi.12374>
- Croghan, C., & Egeghy, P. P. (2003). Methods of dealing with values below the limit of detection using SAS. Southeastern SAS User Group, St. Petersburg, FL.
- DeLonay, A. J., Chojnacki, K. A., Jacobson, R. B., Albers, J. L., Braaten, P. J., Bulliner, E. A., and Wildhaber, M. L. (2016a). Ecological requirements for pallid sturgeon reproduction and recruitment in the Missouri River: A synthesis of science, 2005–2012. U.S. Geological Survey Scientific Investigations Report 2015–5145. Reston, VA.
- DeLonay, A. J., Jacobson, R. B., Chojnacki, K. A., Braaten, P. J., Buhl, K. J., Eder, B. L., Elliott, C. M., Erwin, S. O., Fuller, D. B., Haddix, T. M., Ladd, H. L. A., Mestl, G. E., Papoulias, D. M., Rhoten, J. C., Wesolek, C. J., & Wildhaber, M. L. (2016b). Ecological requirements for pallid sturgeon reproduction and recruitment in the Missouri River—annual report 2013. U.S. Geological Survey, Open-File Report 2015–1197. Reston, VA.
- DeLonay, A. J., Jacobson, R. B., Papoulias, D. M., Simpkins, D.G., Wildhaber, M. L., Reuter, J. M., Bonnot, T. W., Chojnacki, K. A., Korschgen, C. E., Mestl, G. E., & Mac, M. J. (2009) Ecological requirements for pallid sturgeon reproduction and recruitment in the Lower Missouri River – A research synthesis 2005-08. U.S. Geological Survey Scientific Investigations Report 2009-5201. Reston, VA.
- DNRC (Montana Department of Natural Resources and Conservation). (2014). Toston Dam (Broadwater-Missouri). Montana Department of Natural Resources and Conservation, Helena, MT.
- Doroshov, S. I., Moberg, G. P., & Van Eenennaam, J. P. (1997). Observations on the reproductive cycle of cultures white sturgeon, *Acipenser transmontanus*. *Environmental Biology of Fishes*, 48, 265–278.
- Eichelberger, J. S., Braaten, P. J., Fuller, D. B., Krampe, M. S., & Heist, E. J. (2014). Novel single-nucleotide polymorphism markers confirm successful spawning of endangered pallid sturgeon in the upper Missouri River basin. *Transactions of the American Fisheries Society*, 143, 1373–1385. <https://doi.org/10.1080/00028487.2014.935479>
- Elliott, C. M., DeLonay, A. J., Chojnacki, K. A., & Jacobson, R. B. (2020). Characterization of pallid sturgeon (*Scaphirhynchus albus*) spawning habitat in the Lower Missouri River. *Journal of Applied Ichthyology*, 36, 25–38. <https://doi.org/10.1111/jai.13994>

- Erickson, D. L., & Webb, M. A. (2007). Spawning periodicity, spawning migration, and size at maturity of green sturgeon, *Acipenser medirostris*, in the Rogue River, Oregon. *Environmental Biology of Fishes*, *79*, 255–268.
- Feist, G., Schreck, C. B., Fitzpatrick, M. S., & Redding, J. M. (1990). Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. *General and Comparative Endocrinology*, *80*, 299–313.
- Fitzpatrick, M. S., Redding, J. M., Ratti, F. D., & Schreck, C. B. (1987). Plasma testosterone concentration predicts the ovulatory response of coho salmon (*Oncorhynchus kisutch*) to gonadotropin-releasing hormone analog. *Canadian Journal of fisheries and aquatic sciences*, *44*, 1351–1357.
- Fitzpatrick, M. S., Van Der Kraak, G., & Schreck, C. B. (1986). Profiles of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch*, during final maturation. *General and Comparative Endocrinology*, *62*, 437–451.
- Forsythe, P. S., Crossman, J. A., Bello, N. M., Baker, E. A., & Scribner, K. T. (2012). Individual-based analyses reveal high repeatability in timing and location of reproduction in lake sturgeon (*Acipenser fulvescens*). *Canadian Journal of Fisheries and Aquatic Sciences*, *69*, 60–72. <https://doi.org/10.1139/f2011-132>
- Fuller, D. A., Jaeger, M. E., and Webb M. A. H. (2008). *Spawning and associated movement patterns of pallid sturgeon in the lower Yellowstone River. Report prepared for the Upper Missouri River Basin pallid sturgeon Workgroup, Western Area Power Administration, and U.S. Army Corps of Engineers*. Helena, MT: Montana Fish, Wildlife and Parks.
- Goodman, B. J., Guy, C. S., Camp, S. L., Gardner, W. M., Kappenman, K. M., & Webb, M. A. H. (2013). Shovelnose sturgeon spawning in relation to varying discharge treatments in a Missouri River tributary. *River Research and Applications*, *29*, 1004–1015. <https://doi.org/10.1002/rra.2587>
- Goren, M. (2009). Saving critically endangered fish species—utopia or a practical idea? The story of the Yarqon bleak—*Acanthobrama telavivensis* (Cyprinidae) as a test case. *Aqua*, *15*, 1–12.
- Gosch, N. J. C., Civiello, A. P., Gemeinhardt, T. R., Bonneau, J. L., & Long, J. M. (2018). Are shovelnose sturgeon a valid diet surrogate for endangered pallid sturgeon during the first year of life?. *Journal of Applied Ichthyology*, *34*, 39–41.
- Griffiths, R. A., & Pavajeau, L. (2008). Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, *22*, 852–861. <https://doi.org/10.1111/j.1523-1739.2008.00967.x>

- Guy, C. S., Treanor, H. B., Kappenman, K. M., Scholl, E. A., Ilgen, J. E., & Webb, M. A. (2015). Broadening the regulated-river management paradigm: a case study of the forgotten dead zone hindering pallid sturgeon recovery. *Fisheries*, *40*, 6–14. <https://doi.org/10.1080/03632415.2014.987236>
- Hagelin, A., Calles, O., Greenberg, L., Piccolo, J., & Bergman, E. (2016). Spawning migration of wild and supplementary stocked landlocked Atlantic salmon (*Salmo salar*). *River Research and Applications*, *32*, 383–389. <https://doi.org/10.1002/rra.2870>
- Hamel, M. J., Spurgeon, J. J., Steffensen, K. D., & Pegg, M. A. (2020). Uncovering unique plasticity in life history of an endangered centenarian fish. *Scientific Reports*, *10*, 12866. <https://doi.org/10.1038/s41598-020-69911-1>
- Hesse, L. W. (1987). Taming the wild Missouri River: what has it cost?. *Fisheries*, *12*(2), 2–9. [https://doi.org/10.1577/1548-8446\(1987\)012<0002:TTWMRW>2.0.CO;2](https://doi.org/10.1577/1548-8446(1987)012<0002:TTWMRW>2.0.CO;2)
- Holmquist, L. M., (2017). Reproductive ecology of hatchery-origin and wild pallid sturgeon in the Missouri River above Fort Peck Reservoir, Montana. Master's thesis, Montana State University, Bozeman.
- Holmquist, L. M., Guy, C. S., Tews, A., & Webb, M. A. (2019a). First maturity and spawning periodicity of hatchery-origin pallid sturgeon in the upper Missouri River above Fort Peck Reservoir, Montana. *Journal of Applied Ichthyology*, *35*, 138–148. <https://doi.org/10.1111/jai.13751>
- Holmquist, L. M., Guy, C. S., Tews, A., Trimpe, D. J., & Webb, M. A. (2019b). Reproductive ecology and movement of pallid sturgeon in the upper Missouri River, Montana. *Journal of Applied Ichthyology*, *35*, 1069–1083. <https://doi.org/10.1111/jai.13962>
- Ireland, S. C., Beamesderfer, R. C. P., Paragamian, V. L., Wakkinen, V. D., & Siple, J. T. (2002). Success of hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) following release in the Kootenai River, Idaho, USA. *Journal of Applied Ichthyology*, *18*, 642–650. DOI: 10.1046/j.1439-0426.2002.00364.x
- IUCN (International Union for Conservation of Nature and Natural Resources). (2020). *The IUCN redlist of threatened species. IUCN Global Species Programme Red List Unit, Cambridge, United Kingdom*. Retrieved October 10, 2020, from www.iucnredlist.org
- Jachowski, D. S., & Lockhart, J. M. (2009). Reintroducing the black-footed ferret *Mustela nigripes* to the Great Plains of North America. *Small Carnivore Conservation*, *41*, 58–64.
- Jackson, C. D., & Brown, G. E. (2011). Differences in antipredator behaviour between wild and hatchery-reared juvenile Atlantic salmon (*Salmo salar*) under seminatural

- conditions. *Canadian Journal of Fisheries and Aquatic Sciences*, 68, 2157–2166. <https://doi.org/10.1139/f2011-129>
- Jonsson, B., Jonsson, N., & Finstad, A. G. (2013). Effects of temperature and food quality on age and size at maturity in ectotherms: an experimental test with Atlantic salmon. *Journal of Animal Ecology*, 82, 201–210. <https://doi.org/10.1111/j.1365-2656.2012.02022.x>
- Jordan, G. R., Heist, E. J., Braaten, P. J., DeLonay, A. J., Hartfield, P., Herzog, D. P., Kappenman, K. M., & Webb, M. A. H. (2016). Status of knowledge of the pallid sturgeon (*Scaphirhynchus albus* Forbes and Richardson, 1905). *Journal of Applied Ichthyology*, 32, 191–207. <https://doi.org/10.1111/jai.13239>
- Kaesler, A. J., & Litts, T. L. (2010). A novel technique for mapping habitat in navigable streams using low-cost side scan sonar. *Fisheries*, 35, 163–174. <https://doi.org/10.1577/1548-8446-35.4.163>
- Kappenman, K. M., Webb, M. A. H., & Greenwood, M. (2013). The effect of temperature on embryo survival and development in pallid sturgeon *Scaphirhynchus albus* (Forbes & Richardson 1905) and shovelnose sturgeon *S. platyrhynchus* (Rafinesque, 1820). *Journal of Applied Ichthyology*, 29, 1193–1203. <https://doi.org/10.1111/jai.12301>
- Leathe, S. (2018). Effects of Great Falls reservoirs on Missouri River water temperature. North Western Energy, Sioux Falls, SD.
- Lumsden, H. G., & Drever, M. C. (2002). Overview of the trumpeter swan reintroduction program in Ontario, 1982-2000. *Waterbirds*, 25, 301–312.
- Marsh, P. C., Dowling, T. E., Kesner, B. R., Turner, T. F., & Minckley, W. L. (2015). Conservation to stem imminent extinction: the fight to save razorback sucker *Xyrauchen texanus* in Lake Mohave and its implications for species recovery. *Copeia*, 2015, 141–156. <https://doi.org/10.1643/OT-14-030>
- Meiri, I., Gothilf, Y., Zohar, Y., & Elizur, A. (2002). Physiological changes in the spawning gilthead seabream, *Sparus aurata*, succeeding the removal of males. *Journal of Experimental Zoology*, 292, 555–564. <https://doi.org/10.1002/jez.10072>
- Munakata, A., Amano, M., Ikuta, K., Kitamura, S., & Aida, K. (2012). Involvement of sex steroids, luteinizing hormone and thyroid hormones in upstream and downstream swimming behavior of land-locked sockeye salmon *Oncorhynchus nerka*. *Fisheries science*, 78, 81–90. <https://doi.org/10.1007/s12562-011-0436-6>
- Newman, D. M., Jones, P. L., & Ingram, B. A. (2008). Age-related changes in ovarian characteristics, plasma sex steroids and fertility during pubertal development in captive female Murray cod *Maccullochella peelii peelii*. *Comparative Biochemistry and*

Physiology Part A: Molecular & Integrative Physiology, 150, 444–451.
<https://doi.org/10.1016/j.cbpa.2008.05.005>

- NWE (Northwestern Energy). (2016). Hydro operational requirements from FERC license articles and SOP agreements with agencies for NWE's 11 hydropower dams (3 FERC licenses). North Western Energy, Sioux Falls, SD.
- Okuzawa, K. (2002). Puberty in teleosts. *Fish Physiology and Biochemistry*, 26, 31–41.
<https://doi.org/10.1023/A:1023395025374>
- Paragamian, V. L., Kruse, G., & Wakkinen, V. (2001). Spawning habitat of Kootenai River white sturgeon, post-Libby Dam. *North American Journal of Fisheries Management*, 21, 22–33. [https://doi.org/10.1577/1548-8675\(2001\)021<0022:SHOKRW>2.0.CO;2](https://doi.org/10.1577/1548-8675(2001)021<0022:SHOKRW>2.0.CO;2)
- Pfleger, M. O., Rider, S. J., Johnston, C. E., & Janosik, A. M. (2016). Saving the doomed: Using eDNA to aid in detection of rare sturgeon for conservation (Acipenseridae). *Global ecology and conservation*, 8, 99–107. <https://doi.org/10.1016/j.gecco.2016.08.008>
- Porreca, A. P., Hintz, W. D., & Garvey, J. E. (2017). Do alluvial sand dunes create energetic refugia for benthic fishes? An experimental test with the endangered pallid sturgeon. *River Research and Applications*, 33, 690–696. <https://doi.org/10.1002/rra.3132>
- Rapp, T., Shuman, D. A., Graeb, B. D. S., Chipps, S. R., & Peters, E. J. (2011). Diet composition and feeding patterns of adult shovelnose sturgeon (*Scaphirhynchus platorynchus*) in the lower Platte River, Nebraska, USA. *Journal of Applied Ichthyology*, 27, 351–355.
<https://doi.org/10.1111/j.1439-0426.2011.01743.x>
- Richards, R. R. (2011). Movement of *Scaphirhynchus* species in the Missouri River above Fort Peck Reservoir, Montana. Master's thesis, Montana State University, Bozeman.
- Rideout, R. M., & Tomkiewicz, J. (2011). Skipped spawning in fishes: more common than you might think. *Marine and Coastal Fisheries*, 3, 176–189.
<https://doi.org/10.1080/19425120.2011.556943>
- Schooley, J. D., & Marsh, P. C. (2007). Stocking of endangered razorback suckers in the lower Colorado River basin over three decades: 1974–2004. *North American Journal of Fisheries Management*, 27, 43–51.
- Scott, G. L., McCaughey, W. W., & Izlar, K. (2011). Guidelines for whitebark pine planting prescriptions. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. 63, 362–364.

- Scott, M. L., Auble, G. T., & Friedman, J. M. (1997). Flood dependency of cottonwood establishment along the Missouri River, Montana, USA. *Ecological Applications*, 7, 677–690. [https://doi.org/10.1890/1051-0761\(1997\)007\[0677:FDOCEA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1997)007[0677:FDOCEA]2.0.CO;2)
- Serrano, I., Larsson, S., & Eriksson, L. O. (2009). Migration performance of wild and hatchery sea trout (*Salmo trutta* L.) smolts—Implications for compensatory hatchery programs. *Fisheries Research*, 99, 210–215. <https://doi.org/10.1016/j.fishres.2009.06.004>
- Stober, Q. J. (1962). Some limnological effects of Tiber Reservoir on the Marias River. Master's thesis, Montana State University, Bozeman.
- Szczepkowski, M., Kolman, R., & Szczepkowska, B. (2015). Impact of feed ration on growth and the results of sterlet, *Acipenser ruthenus* L., artificial reproduction. *Aquaculture Research*, 46, 2147–2152. <https://doi.org/10.1111/are.12370>
- Talbott, M. J., Van Eenennaam, J. P., Linares-Casenave, J., Doroshov, S. I., Guy, C. S., Struffenegger, P., & Webb, M. A. (2011). Investigating the use of plasma testosterone and estradiol-17 β to detect ovarian follicular atresia in farmed white sturgeon, *Acipenser transmontanus*. *Aquaculture*, 315, 283–289. <https://doi.org/10.1016/j.aquaculture.2010.10.041>
- Thomas, M. L., & Simmons, L. W. (2011). Crickets detect the genetic similarity of mating partners via cuticular hydrocarbons. *Journal of evolutionary biology*, 24, 1793–1800. <https://doi.org/10.1111/j.1420-9101.2011.02319.x>
- Thorsen, A., Witthames, P. R., Marteinsdóttir, G., Nash, R. D., & Kjesbu, O. S. (2010). Fecundity and growth of Atlantic cod (*Gadus morhua* L.) along a latitudinal gradient. *Fisheries Research*, 104, 45–55. <https://doi.org/10.1016/j.fishres.2010.03.020>
- Tripp, S. J., Phelps, Q. E., Colombo, R. E., Garvey, J. E., Burr, B. M., Herzog, D. P., & Hrabik, R. A. (2009). Maturation and reproduction of shovelnose sturgeon in the middle Mississippi River. *North American Journal of Fisheries Management*, 29, 730–738. <https://doi.org/10.1577/M08-056.1>
- Trippel, E. A., & Harvey, H. H. (1990). Ovarian atresia and sex ratio imbalance in white sucker, *Catostomus commersoni*. *Journal of Fish Biology*, 36, 231–239. <https://doi.org/10.1111/j.1095-8649.1990.tb05598.x>
- U.S. Office of the Federal Register (1990). Endangered and threatened wildlife and plants; determination of endangered status for the pallid sturgeon. *Federal Register* 55:173(September 6, 1990):36641–36647.

- USFWS (United State Fish and Wildlife Service). (2012). *Biological procedure and protocols for researchers and managers handling pallid sturgeon*. (40 pp.). Denver, CO: Fish and Wildlife Service.
- USFWS (United States Fish and Wildlife Service). (2014). *Revised recovery plan for the pallid sturgeon (Scaphirhynchus albus)*. (115 pp.). Denver, CO: Fish and Wildlife Service.
- Walters, J. R., Derrickson, S. R., Michael Fry, D., Haig, S. M., Marzluff, J. M., & Wunderle Jr, J. M. (2010). Status of the California Condor (*Gymnogyps californianus*) and efforts to achieve its recovery. *The Auk*, *127*, 969–1001. <https://doi.org/10.1525/auk.2010.127.4.969>
- Webb, M. A. H. (2010). Determine spawning interval of white sturgeon in the Columbia River. White sturgeon mitigation and restoration in the Colombia and Snake rivers upstream from Bonneville Dam. Annual Progress Report, April 2010-March 2011.
- Webb, M. A. H. (2011). Determine spawning interval of white sturgeon in the Columbia River. White sturgeon mitigation and restoration in the Colombia and Snake rivers upstream from Bonneville Dam. Annual Progress Report, April 2011-March 2012.
- Webb, M. A. H., Van Eenennaam, J. P., Crossman, J. A., & Chapman, F. A. (2019). A practical guide for assigning sex and stage of maturity in sturgeons and paddlefish. *Journal of Applied Ichthyology*, *35*, 169–186. <https://doi.org/10.1111/jai.13582>
- Webb, M. A., & Doroshov, S. I. (2011). Importance of environmental endocrinology in fisheries management and aquaculture of sturgeons. *General and Comparative Endocrinology*, *170*, 313–321. <https://doi.org/10.1016/j.ygcen.2010.11.024>
- Webb, M. A., Van Eenennaam, J. P., Doroshov, S. I., & Moberg, G. P. (1999). Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. *Aquaculture*, *176*, 315–329. [https://doi.org/10.1016/S0044-8486\(99\)00108-8](https://doi.org/10.1016/S0044-8486(99)00108-8)
- Zhang, H., Jarić, I., Roberts, D. L., He, Y., Du, H., Wu, J., Wang, C., & Wei, Q. (2020). Extinction of one of the world's largest freshwater fishes: Lessons for conserving the endangered Yangtze fauna. *Science of the Total Environment*, *710*, 136242. <https://doi.org/10.1016/j.scitotenv.2019.136242>
- Zhang, Y., Qu, Q., Sun, D., Liu, X., Suo, L., & Zhang, Y. (2011). Vitellogenin in Amur sturgeon (*Acipenser schrenckii*): induction, purification and changes during the reproductive cycle. *Journal of applied ichthyology*, *27*, 660–665. <https://doi.org/10.1111/j.1439-0426.2011.01698.x>
- Zohar, Y., & Billard, R. (1984). Annual and daily changes in plasma gonadotropin and sex steroids in relation to teleost gonad cycles. *Transactions of the American Fisheries*

Society, 113, 444–451. [https://doi.org/10.1577/1548-8659\(1984\)113<444:AADCIP>2.0.CO;2](https://doi.org/10.1577/1548-8659(1984)113<444:AADCIP>2.0.CO;2)