

ORIGINAL ARTICLE

Reproductive indices and observations of mass ovarian follicular atresia in hatchery-origin pallid sturgeon

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Abstract

The Pallid Sturgeon (*Scaphirhynchus albus*) Conservation Propagation and Stocking Program began stocking in the Missouri River above Fort Peck Reservoir in 1998 with 1997-year-class pallid sturgeon. Within the 1997-year class, all hatchery-origin pallid sturgeon females that reached reproductive maturation by 2016 underwent mass ovarian follicular atresia. Using combined historical and contemporary data, we described the spawning periodicity for female and male pallid sturgeon, characterized age- and size-at-first spawning, and evaluated what proportion of females experience mass ovarian follicular atresia during the first and subsequent reproductive cycles. Pallid sturgeon reached their first reproductive cycle at older ages and larger sizes than described for other populations. Females were functionally and physiologically capable of spawning at 21 years and males at 15 years. Immature pallid sturgeon as old as 20 years were documented. We found that more female pallid sturgeon underwent mass ovarian follicular atresia during the presumed-first reproductive cycle or known-first reproductive cycle than females during subsequent reproductive cycles (62.5% compared to 33.3%) indicating that effects related to reproductive maturation may be occurring. Nonetheless, mass ovarian follicular atresia appears to also occur for reasons not related to reproductive maturation. Females had biennial reproductive cycles, and males had annual and biennial reproductive cycles. Population models should account for females undergoing mass ovarian follicular atresia in their first reproductive cycle and subsequent cycles thereby increasing the age at first-successful spawning and reducing the estimated size of the spawning stock.

KEYWORDS

fisheries ecology, reproductive physiology, sturgeons

1 | 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 released from a conservation propagation program may have reduced fitness and not make meaningful contributions to population recovery. Success of the conservation propagation program for white sturgeon (*Acipenser transmontanus*) in the Kootenai River relies in part on successful adaptation of hatchery fish once released into the wild, survival of hatchery fish beyond sexual maturity, and retention of wild sturgeon life history characteristics and genetics in the

hatchery-reared population (Ireland et al., 2002). Successfully supplementing a population with captive-origin individuals 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, 2009). Therefore, it is imperative to evaluate reproductive success and reproductive indices of individuals 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., Lahontan cutthroat trout [*Oncorhynchus clarkii henshawi*; 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 many 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. Office of the Federal Register, 1990). The Pallid Sturgeon Conservation Propagation and Stocking Program was initiated to prevent extirpation of the species and preserve genetic diversity (USFWS (United State Fish and Wildlife Service), 2014). Stocking pallid sturgeon above Fort Peck Reservoir began in 1998 with the 1997-year-class (hereafter, referred to as hatchery-origin) 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 no detectable recruitment since the species has been monitored (USFWS (United State Fish and Wildlife Service), 2014).

Previous research successfully documented reproductively mature hatchery-origin pallid sturgeon in the Missouri River above Fort Peck Reservoir (i.e., post-vitellogenic females and spermatogenic males; herein referred to as spawning capable) and characterized spawning periodicity in males (Holmquist, Guy, Tews, & Webb, 2019) using plasma sex steroids, gonadal biopsies, or both (see Webb et al., 2019). There were 13 hatchery-origin pallid sturgeon that had been spawning capable by the conclusion of the 2016 spawning season—10 spawning capable males and three spawning capable females (Holmquist, Guy, Tews, & Webb, 2019). The minimum biological reproductive cycle for female sturgeon is 2 years (Doroshov et al., 1997; Zhang et al., 2011), and hatchery-origin pallid sturgeon experiencing different environmental conditions entered the spawning capable phase at different ages and sizes. Hatchery-origin pallid sturgeon above Fort Peck Reservoir reach these reproductive phases when they are older and larger than those kept in captivity (Holmquist, Guy, Tews, & Webb, 2019; Jordan et al., 2016). Contemporary age and size estimates of hatchery-origin pallid sturgeon during their presumed-first

reproductive cycle (i.e., during the first documented reproductive cycle of an individual for which no evidence suggested maturation had occurred prior) above Fort Peck Reservoir vary from 15–19 years, 1011–1198 mm fork length, and 4848–8700 g for males and 18–19 years, 1070–1140 mm fork length, and 6400–6940 g for females (Holmquist, Guy, Tews, & Webb, 2019). However, ten hatchery-origin pallid sturgeon (1997-year class) that were annually sampled from 2014–2016 had not experienced a spawning capable phase (Holmquist, Guy, Tews, & Webb, 2019) indicating they remained immature and that the variation of age and size at the first reproductive cycle may be greater than currently described. Younger and smaller reproductively mature hatchery-origin pallid sturgeon 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 1775 g and was presumed to be spawning capable at 11 years of age based on plasma steroid concentrations, and the youngest and smallest female (also captured in the fall) was 14 years of age, 1078 mm, and 5900 g and had vitellogenic ovarian follicles (M. A. H. Webb, U.S. Fish and Wildlife Service, unpubl. data). No direct observations of successful spawning of these fish were made. With individuals becoming spawning capable, conservation-propagation efforts have been successful to this point, however, no successful spawning of captive-origin individuals released in the wild has been documented to date. Therefore, investigating reproductive indices (e.g., age and size at first maturity, spawning periodicity, 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 (United State Fish and Wildlife Service), 2014).

Mass ovarian follicular atresia has been documented in hatchery-origin pallid sturgeon ($n = 3$) above Fort Peck Reservoir (Holmquist, 2017). It is unknown if the hatchery-origin pallid sturgeon that underwent mass ovarian follicular atresia during their presumed-first reproductive cycle will successfully ovulate during their subsequent reproductive cycles. Furthermore, it is unknown what proportion of females fail to complete oocyte maturation and ovulation during their first cycle. Not accounting for mass ovarian follicular atresia could result in overestimation of the spawning population size.

We designed this study to answer key questions about reproductive indices of hatchery-origin pallid sturgeon. Specifically, (1) at what age and size do pallid sturgeon spawn for the first time, (2) what proportion of pallid sturgeon experience mass ovarian follicular atresia, and (3) what is the spawning periodicity for female and male pallid sturgeon? Answering these questions will provide a more accurate assessment of the reproductive potential for the population of hatchery-origin pallid sturgeon above Fort Peck Reservoir.

2 | STUDY AREA

The study area is within the Great Plains Management Unit described in USFWS (United State Fish and Wildlife Service) (2014)

and includes the Missouri River from the upstream end of Fort Peck Reservoir to Morony Dam (river kilometer [rkm] 3010 to rkm 3388) and the Marias River from the confluence with the Missouri River to Tiber Dam (rkm 0 to rkm 126; Figure 1). The study area is on the northern and western peripheries of the distribution of pallid sturgeon (Longitude = 47°24'–48°18'; Latitude = –111°6' to –107°54'; USFWS (United State Fish and Wildlife Service), 2014).

3 | METHODS

3.1 | Fish sampling

Pallid sturgeon were sampled between early May and late July of 2018 and 2019. Additionally, pallid sturgeon were sampled by Montana Fish, Wildlife & Parks and collaborators from 2011 through 2017 using similar methods. Pallid sturgeon included in this study were 1997-year-class hatchery-origin fish that had been previously radio telemetered as part of a concurrent study conducted by Montana Fish, Wildlife & Parks and past research conducted by Holmquist, Guy, Tews, and Webb (2019). Pallid sturgeon were located using telemetry and captured using drifted 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. 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. During 2018 and 2019, known spawning capable females (see Section 3.2) were resampled as water temperature neared 24°C to determine ovulatory outcome. Similar to Holmquist, Guy, Tews, Trimpe, 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 (DeLonay et al., 2016; Fuller et al., 2008), and water temperatures during spawning are estimated to be from 12–24°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, while 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), implanted with a transmitter (model MCFT2-3 L; Lotek, Newmarket, ON) and a PIT tag if the PIT tag was missing, assessed for sex and stage of maturity, and were subsequently added to the study. Radio transmitters were inserted using methods described by Holmquist (2017).

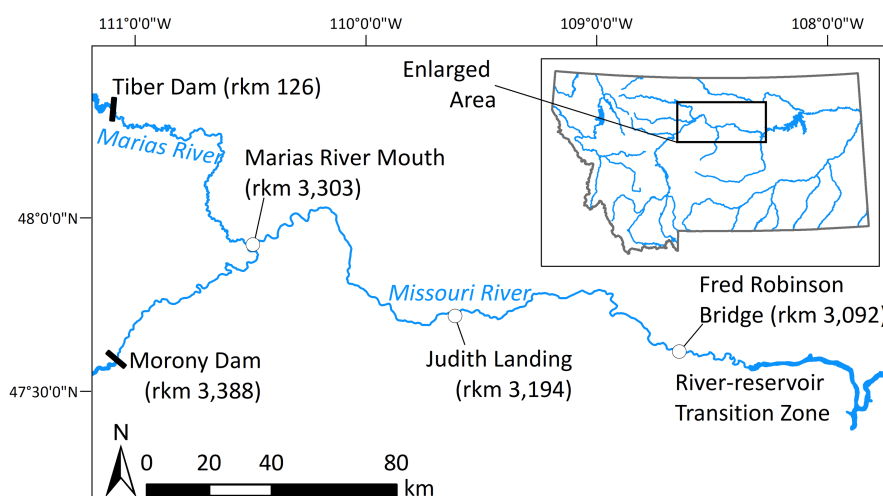
Biological samples and metrics were collected from all captured pallid sturgeon. Handling and sampling procedures conformed to protocols developed for pallid sturgeon (USFWS (United State Fish and Wildlife Service), 2012). Passive integrated transponder (PIT) tag codes were recorded and used to identify individual pallid sturgeon. Pallid sturgeon were measured (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 centrifuged ($1228\times g$ for 5 min). Plasma was stored at –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 vitellogenic or spawning capable. Tissue was collected through a small abdominal incision (1–2 cm) made just off the midline as described by Webb et al. (2019), and preserved in 10% phosphate-buffered formalin for histological analysis following the methods described in McGarvey et al. (2020). Incisions were closed with evenly spaced 1–3 single interrupted sutures, using size 0 absorbable suture material attached to a CP-1 suture needle (Ethicon PDS®II).

3.2 | 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) and quantified by radioimmunoassay as described in Fitzpatrick et al. (1986)

FIGURE 1 Map of the Missouri River from the river-reservoir transition zone at fort peck reservoir, Montana to Morony dam, Montana (river kilometer [rkm] 3010–rkm 3388) and the Marias River from the confluence with the Missouri River to Tiber dam, Montana (rkm 0–rkm 126). Dams denoted by \blacksquare , and points of reference denoted by \circ .



modified by Feist et al. (1990). A slightly more concentrated charcoal solution (6.25 g charcoal and 4.0 g dextran/L phosphate buffered saline with gelatin) was used for all assays. Testosterone and E2 concentrations were validated by verifying that serial dilutions were parallel to standard curves. 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. Fish with plasma concentrations of T greater than 38 ng/ml and E2 less than 0.30 ng/ml were assigned as spawning-capable males. Fish with T greater than 10 ng/ml and E2 greater than 0.30 ng/ml were assigned as vitellogenic or spawning-capable females. Fish with T concentrations less than 38 ng/ml and E2 less than 0.30 ng/ml were assigned as a non-reproductively active male (pre-meiotic) or non-reproductively active female (previtellogenic) if the respective sex of the fish was determined by histology or known from prior capture. These fish were immature or not reproductively active in that year. Pallid sturgeon were assigned as non-reproductively active and unknown sex if sex was not determinable or known. Fish that were consistently non-reproductively active (i.e., ≥ 3 years) were considered immature.

A spawning-capable male pallid sturgeon based on steroid concentrations would have testicular cysts with germ cells that were meiotic (spermatocytes, spermatids, and/or spermatozoa). Vitellogenic and spawning-capable females were differentiated by collection of ovarian follicles that were examined under a microscope, processed histologically, or both. A vitellogenic female would not be able to spawn during the year she was sampled, while a spawning-capable female would be capable of spawning during the year she was sampled. A non-reproductively active male would have germ cells that had not initiated meiosis in testicular cysts, and a non-reproductively active female would not have initiated vitellogenesis: these fish were not physiologically capable of spawning during the year in which they were sampled. For hatchery-origin male pallid sturgeon classified as reproductively active using T concentrations but without corresponding gonadal tissue, movement during spawning season was used to validate reproductive state, and for one male that remained sedentary throughout the spawning season, reproductive state was reassigned as non-reproductive (see Holmquist, Guy, Tews, & Webb, 2019). Additionally, histological analysis was used to inform ovulatory outcome of all spawning-capable females. Post-ovulatory ovarian follicles indicated ovulatory success, and ovulatory failure was indicated by $>50\%$ of the ovarian follicles undergoing atresia (Hunter et al., 1992).

3.3 | Reproductive status

Reproductive histories were used to characterize age and size at expected-first spawning (i.e., age and size when an individual is

functionally and physiologically capable of a successful spawning event). Females were not considered to have reached expected-first-spawn if they underwent mass ovarian follicular atresia during their first observed reproductive cycle. Collected age, length, and weight data for all pallid sturgeon, including those characterized by Holmquist, Guy, Tews, and Webb (2019), were summarized for males and females at expected-first spawning. Additionally, age, length, and weight of hatchery-origin pallid sturgeon that were immature were compared to those of male and female hatchery-origin pallid sturgeon at expected-first spawning. A linear model was created to illustrate the length-weight relationship of pallid sturgeon in all categories (male and female at expected-first spawn and immature). 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 (Korner-Nievergelt et al., 2015). 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 (Korner-Nievergelt et al., 2015).

3.4 | Mass ovarian follicular atresia

The proportion of pallid sturgeon that experienced mass ovarian follicular atresia was evaluated. Furthermore, historical data on reproductive status of individual female pallid sturgeon were used to determine if spawning-capable females were having their first reproductive cycle or a subsequent cycle, and proportion of females experiencing mass ovarian follicular atresia was reported for each group.

3.5 | Spawning periodicity

Reproductive histories of individual hatchery-origin pallid sturgeon that were captured in separate years were used to describe spawning periodicity (i.e., number of years between spawning events). Pallid sturgeon that were spawning capable in consecutive years were defined as having annual reproductive cycles. Pallid sturgeon that were spawning capable every other year were defined as having a biennial reproductive cycle. Annual 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).

4 | RESULTS

4.1 | Reproductive status

Female hatchery-origin pallid sturgeon initiated their presumed-first reproductive cycle between 18 and 22 years old. However, expected-first spawning for females occurred at 21 or 22 years old.

Male hatchery-origin pallid sturgeon experienced expected-first spawning between 15 and 22 years old (Table 1). Length of female pallid sturgeon at expected-first spawning varied from 1138 to 1233 mm and weight varied from 6440 to 8980 g (Table 1). Length and weight for male pallid sturgeon was less and varied from 917 mm to 1215 mm and 4760 to 8765 g (Table 1). Length and weight values for immature pallid sturgeon overlapped values for female and male pallid sturgeon at expected-first spawning. The overlap in length and weight among categories were at the ends of the distributions, that is, smaller female and male pallid sturgeon at expected-first spawning overlapped with larger immature pallid sturgeon (Figure 2).

4.2 | Mass ovarian follicular atresia

Hatchery-origin pallid sturgeon experienced mass ovarian follicular atresia during six of the eleven observed reproductive cycles (54.5%), and mass ovarian follicular atresia was less prevalent in subsequent reproductive cycles than in first or presumed-first reproductive cycles (Table 2). One of three (33.3%) hatchery-origin female pallid sturgeon underwent mass ovarian follicular atresia during a subsequent reproductive cycle (Figure 3; Group A). Two of two (100%) hatchery-origin female pallid sturgeon underwent mass ovarian follicular atresia during their first reproductive cycle (Figure 3; Group A or Group B) and three of six (50.0%) hatchery-origin female pallid sturgeon underwent mass ovarian follicular atresia during their presumed-first reproductive cycle (Figure 3; Group A or Group B). All females underwent mass ovarian follicular atresia

during the late phase of vitellogenesis or post-vitellogenesis as indicated by the presence of yolk and melanin in the atretic bodies and, in some cases, the size of the ovarian follicles. If some fish in their presumed-first reproductive cycle were not having their first reproductive cycle, several different proportions could occur. The most extreme proportions could range from a minimum of two out of five (40%) to a maximum of five out of five (100%) individuals experiencing mass ovarian follicular atresia during the first reproductive event. Similarly, the number of fish experiencing atresia at subsequent-spawning events could range from a minimum of one out of six (17%) to a maximum of four out of six (67%).

4.3 | Spawning periodicity

Spawning periodicity was estimated for three hatchery-origin female pallid sturgeon that were observed completing more than one reproductive cycle—all had biennial reproductive cycles (Table 3). Female hatchery-origin pallid sturgeon ID OE09 was spawning capable in 2015 and underwent mass ovarian follicular atresia. This female was vitellogenic in 2016 and spawning capable in 2019. This reproductive pattern suggests a biennial reproductive cycle. Female hatchery-origin pallid sturgeon ID 3B15 and 4033 were spawning capable in 2016 and 2018 indicating biennial reproductive cycles (Table 3). In addition to the three female hatchery-origin pallid sturgeon described above, pallid sturgeon ID 6862 was spawning capable in 2018 and not spawning capable in 2019 indicating a biennial or longer reproductive cycle. The highest number of spawning-capable hatchery-origin female pallid sturgeon documented in a spawning season occurred in 2018 with six spawning-capable females—the prior highest was two spawning-capable 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) experienced annual reproductive cycles and two (IDs 4E58 and 2A09) experienced biennial reproductive cycles (Table 4). One male pallid sturgeon that experienced an annual reproductive cycle (spawning capable in 2015 and 2016, ID 2518) did not maintain an annual cycle because the fish was not spawning capable in 2019 indicating that periodicity of individuals has plasticity and is not a rigid trait. One additional male (ID 503F) was spawning capable in 2018 (determined by elevated plasma sex steroids in the fall of 2017) and not spawning capable in 2019 indicating a biennial or longer reproductive cycle.

TABLE 1 Summary statistics by reproductive status for age (years), fork length (mm), and weight (g) of 1997 year-class hatchery-origin pallid sturgeon at expected-first spawning and 1997 year-class hatchery-origin pallid sturgeon known to be immature

Variable	Summary statistic	Status		
		Immature	Male	Female
Age	N	11	15	6
	Mean (SE)	19 (0.13)	18 (0.59)	21 (0.21)
	Median	19	19	21
	Minimum	18	15	21
	Maximum	20	22	22
Length	N	11	15	6
	Mean (SE)	1033 (26)	1099 (20)	1174 (14)
	Median	1046	1097	1170
	Minimum	873	917	1138
	Maximum	1144	1215	1233
Weight	N	11	15	6
	Mean (SE)	5031 (462)	6355 (370)	7841 (402)
	Median	5060	5750	7903
	Minimum	2190	4760	6440
	Maximum	7060	8765	8980

5 | DISCUSSION

Age- and size-at-presumed-first reproductive cycle and expected-first spawning was further evaluated than previously possible as additional hatchery-origin pallid sturgeon had achieved these reproductive phases. Female pallid sturgeon in this study exhibited biennial spawning periodicity and experienced their first reproductive cycles between ages 18 and 22; however, expected-first spawning

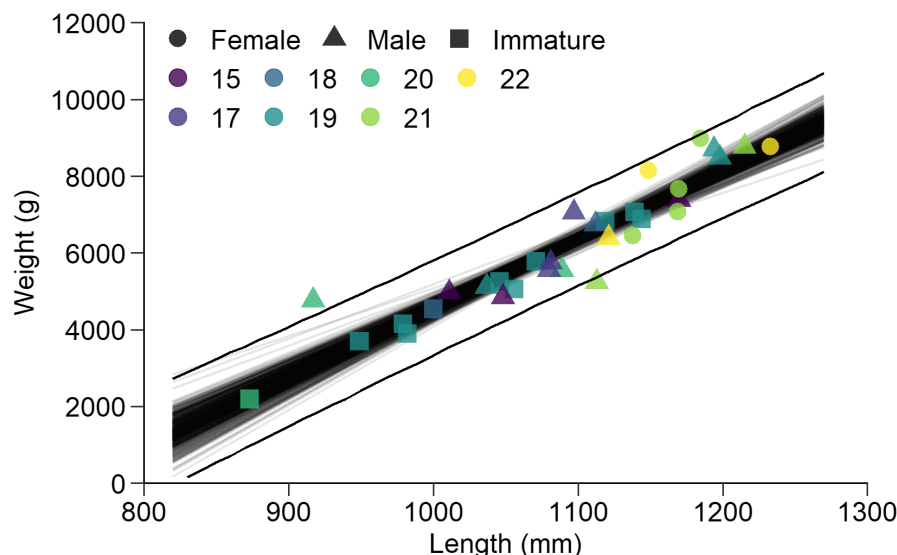


FIGURE 2 Plot of length (mm) and weight (g) of immature, male at expected-first spawn, and female at expected-first spawn hatchery-origin 1997-year-class pallid sturgeon. Color of point represents age of fish varying from the youngest (15 years) to the oldest (22 years). Translucent lines represent 500 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).

TABLE 2 Percent of hatchery-origin pallid sturgeon that underwent mass ovarian follicular atresia by spawning event type (first reproductive cycle, presumed-first reproductive cycle, and subsequent reproductive cycle)

First reproductive cycle	Presumed-first reproductive cycle	Subsequent reproductive cycle
100% (n = 2)	50% (n = 6)	33% (n = 3)

did not occur until ages 21 and 22 given the occurrence of mass follicular atresia. Male pallid sturgeon had annual and biennial spawning periodicities and reached expected-first spawning between ages 15 and 22. The spawning periodicities of pallid sturgeon in this study were similar to pallid sturgeon in other portions of the upper Missouri River (Jordan et al., 2016) and to shovelnose sturgeon (*Scaphirhynchus platyrhynchus*; Tripp et al., 2009). Additionally, male pallid sturgeon becoming spawning capable 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 reproductive cycle above Fort Peck Reservoir continued to exceed those of hatchery-origin pallid sturgeon in other downstream locations (DeLonay et al., 2009; Holmquist, Guy, Tews, & Webb, 2019; Jordan et al., 2016). Therefore, location-specific characterizations of age- and size-at-first reproductive cycle and at expected-first spawning are imperative to providing accurate information to stock-assessments.

Overlap between the age and size of immature pallid sturgeon and pallid sturgeon during their presumed-first reproductive cycle above Fort Peck Reservoir suggested that age and size alone did not indicate when a hatchery-origin pallid sturgeon would become reproductively mature. Two other metrics that may be related to achieving reproductive maturity and first spawning included lipid stores and feed ration (Szczechowski et al., 2015; Webb & Doroshov, 2011). Providing feed with high lipid content to Atlantic salmon (*Salmo salar*) decreases age and size at first maturity (Jonsson et al., 2013; note that initiation of reproductive maturation and spawning occur within the same year for salmonids). Therefore, feed ration and feed quality

can both contribute to reproductive development. Therefore, even a pallid sturgeon that was successful at foraging may have delayed maturity if forage quality was poor. Future studies may consider if reproductive development may be associated with metrics other than age and size (e.g., feed ration or feed quality); although, hatchery origin pallid sturgeon from the 1997-year-class in the Missouri River above Fort Peck Reservoir are considered to be in good condition and have grown quickly (MTFWP, 2022) suggesting forage has not been a limiting factor.

At the population level, pallid sturgeon above Fort Peck Reservoir appeared to achieve reproductive maturity at older ages and larger sizes than pallid sturgeon in other locations. For example, reproductive 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 spawning-capable male and female pallid sturgeon varying from 15 to 22 years old in the Missouri River above Fort Peck Reservoir. Note that reproductive failure during the first reproductive cycle has not been examined in the lower Missouri River and could be a priority for future research. Pallid sturgeon above Fort Peck Reservoir becoming reproductively mature 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, the pallid sturgeon population above Fort Peck Reservoir is at the northern periphery of the range of pallid sturgeon and becoming reproductively mature 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 morhua*; Thorsen et al., 2010).

Mass ovarian follicular atresia was experienced by all hatchery-origin female pallid sturgeon observed during their first reproductive cycles, half of those observed during presumed-first reproductive cycles, and one third of those observed during subsequent reproductive cycles. The higher proportion of mass ovarian follicular atresia during first reproductive cycles and presumed-first reproductive

cycles compared to subsequent reproductive cycles could result from an association between reproductive maturation and mass ovarian follicular atresia. A spawning “dummy run” has been described in teleosts and entails immature fish exhibiting activation of the brain-pituitary neuroendocrine axis (i.e., endocrine signaling related to reproduction is occurring between the brain and pituitary

gland), but the developing ovarian follicles do not yet have the capacity to respond to the chemical signals; therefore, vitellogenesis (i.e., tertiary oocyte growth) does not occur (Newman et al., 2008; Okuzawa, 2002). In contrast, female hatchery-origin pallid sturgeon that underwent mass ovarian follicular atresia in their first or presumed-first reproductive cycles completed vitellogenesis but

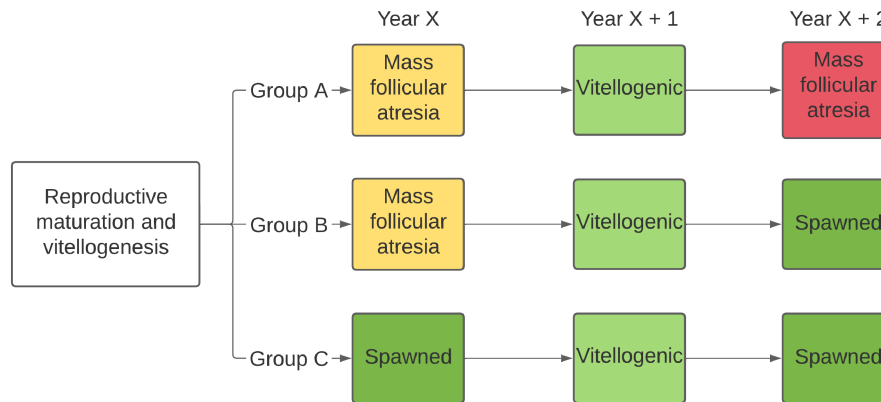


FIGURE 3 A graphical model of possible reproductive patterns female pallid sturgeon may experience beginning at reproductive maturation and ending at the second reproductive cycle. Group a represents fish that experience ovarian follicular atresia in their first reproductive cycle during year x and atresia during the next spawning cycle due to environmental conditions or interactions among conspecifics. Group B represents fish that experience ovarian follicular atresia in their first reproductive cycle during year x and successfully spawn during the next spawning cycle. Group C represents fish that successfully spawn in their first reproductive cycle during year x and during the next spawning cycle.

TABLE 3 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 to be spawning capable between 2015 and 2019

ID	Steroid	Reproductive year				
		2015	2016	2017	2018	2019
0E09 ^a	T	62.18^b	29.73 ^c			33.71
	E2	0.67^b	ND ^c			4.32
3B15 ^a	T		52.42^b	24.71	0.19^{b,d}	
	E2		1.83^b	0.47	ND^{b,d}	
4033 ^a	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^d	
	E2		ND		ND^d	
5A67	T					65.05
	E2					0.43

Note: Bold font denotes spawning-capable status of an individual.

ND denotes steroid concentrations below detectable levels.

^aPallid sturgeon data from 2015 to 2016 included in Holmquist, Guy, Tews, and Webb (2019).

^bReproductive cycle concluded by mass follicular atresia.

^cOriginally sample 3/16/2016 (data shown) and then resampled 6/27/2016 after vitellogenesis was initiated (T = 25.45, E2 = 1.51).

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

TABLE 4 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 to be spawning capable between 2011 and 2019

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

Note: Bold font denotes spawning-capable status of an individual.

ND denotes steroid concentrations below detectable levels.

^aPallid sturgeon data from 2011 to 2016 included in Holmquist, Guy, Tews, and Webb (2019).

^bSample collected in the fall and assumed spawning capable the following spring.

failed to undergo oocyte maturation and ovulation. Pallid sturgeon undergoing mass ovarian follicular atresia during their first reproductive cycle may be experiencing a break in the brain-pituitary-gonadal axis occurring at a later stage of development than in teleosts (i.e., after vitellogenesis but before ovulation). Despite the apparent increased prevalence of mass ovarian follicular atresia in fish experiencing a first reproductive cycle, prevalence of atresia in fish that are not experiencing a first reproductive cycle suggests that other factors are also contributing to mass ovarian follicular atresia in this population. Additionally, two wild pallid sturgeon were previously documented undergoing mass follicular atresia for reasons not

associated with reproductive maturation—one in 2014 and one in 2015 (Holmquist, 2017).

Incidences of mass ovarian follicular atresia not related to reproductive maturation may have been caused by various environmental factors or interactions among conspecifics. Environmental conditions such as water temperature have been shown to result in mass ovarian follicular atresia in other sturgeon species (Webb et al., 1999). However, there is little support for this cause as upstream dams on the Missouri River have little effect on the water temperature in the study area (Leathe, 2018), and water-temperature regime in the Missouri River above Fort Peck Reservoir probably

resemble historical conditions. Diet has been linked to incidences of atresia in Labrador cod (e.g., cod with more pelagic diets had decreased prevalence of mass ovarian follicular atresia compared to those with more benthic diets, Sherwood et al., 2007). Again, there is little support for this cause as the population of pallid sturgeon included in this study were in good condition and have grown quickly (MTFWP, 2022). Current rarity of spawning-capable individuals above 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 mass ovarian follicular atresia in white suckers (*Catostomus commersonii*; Trippel & Harvey, 1990) and could contribute to mass 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 mass ovarian follicular atresia if male fish are removed from the spawning population (Meiri et al., 2002). Spawning-capable female pallid sturgeon unable to find a suitable mate would likely not spawn and would undergo mass ovarian follicular atresia. Future research may verify the prevalence of mass ovarian follicular atresia during the first reproductive cycle and, also determine how prevalent mass ovarian follicular atresia is following the first reproductive cycle. Furthermore, how environmental conditions and interactions among conspecifics might contribute to incidences of mass ovarian follicular atresia may be considered.

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 find any indications of female pallid sturgeon having a reproductive cycle longer than 2 years, although our small sample size could be the reason, as 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 spawning-capable female pallid sturgeon, continued sampling that complements the current historical data would be useful to further evaluate the proportion of females with a 2- or 3-year spawning periodicity. Interestingly, one hatchery-origin male pallid sturgeon had varying duration of the reproductive cycle by having an annual periodicity (i.e., being spawning capable two consecutive years), but the following year was not spawning capable. 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, 2010; Webb, 2011 and lake sturgeon 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, 2011), 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, as well as other stressors.

Sex-steroid concentrations are dynamic with short-term variation despite predictable trends associated with stages of 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) with ripe ovarian follicles had an E2 concentration below the level used to indicate a spawning-capable female. This was the first study to document a spawning-capable female with an E2 concentration below 0.30 ng/ml, and we suggest lowering the discriminating E2 concentration to 0.28 ng/ml based on the quantification of E2 in the plasma of a spawning-capable female. Additionally, one hatchery-origin male pallid sturgeon (ID 2A09) with a biennial reproductive cycle had a slightly elevated testosterone concentration of 39.10 ng/ml but was determined to be non-reproductively active by Holmquist, Guy, Tews, and Webb (2019) based on sedentary behavior and a lack of interaction with other spawning-capable 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 could be continually adapted to represent the best available knowledge, and short-term variation in sex-steroid concentrations may 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 above Fort Peck Reservoir do successfully spawn, and mass ovarian follicular atresia appears to be more prevalent during first reproductive cycles than in subsequent cycles. Population models may account for females undergoing mass ovarian follicular atresia in their first reproductive cycle (e.g., of the individuals experiencing their first reproductive cycles, 40%–100% may undergo ovarian follicular atresia). This degenerative process will delay when pallid sturgeon are physiologically and functionally able to successfully spawn for the first time resulting in an older age and larger size at first spawning. Rates of atresia during subsequent reproductive cycles may be similarly accounted for (e.g., during subsequent reproductive cycles 17%–67% of individuals expected to spawn may undergo ovarian follicular atresia). This will also ensure that the spawning stock biomass is not biased by the lack of inclusion of the rate of mass ovarian follicular atresia (see Corriero et al., 2021). If the rates of mass ovarian follicular atresia are high in subsequent reproductive cycles and are not incorporated into

population models seeking to evaluate recovery or population viability (e.g., Nelson et al., 2021), the spawning stock biomass could be overestimated. Therefore, it is beneficial for population models to account for females undergoing mass follicular atresia in their first reproductive cycle and subsequent cycles thereby increasing the age at first successful spawning and reducing the estimated size of the spawning stock.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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