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Articles

Evidence of Limited Recruitment of Pallid Sturgeon in the Lower Missouri River

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Abstract

Pallid Sturgeon *Scaphirhynchus albus* are endemic to the Missouri and Mississippi river basins and are rare throughout their range. The species was listed as federally endangered with little to no evidence of natural recruitment. Since population augmentation was initiated as a recovery objective in the early 1990s, thousands of hatchery-origin Pallid Sturgeon have been stocked in the lower Missouri River (Gavins Point Dam [river kilometer 1,305.1] to the confluence of the Mississippi River [river kilometer 0.0]). Efforts to discriminate natural reproduction and recruitment of wild-origin Pallid Sturgeon from hatchery-origin fish has been hampered by tag loss in hatchery-origin sturgeon, inconsistent documentation of hatchery parental crosses, and the failure to collect tissue samples for genotyping all broodstock. However, the recent reconstruction of missing parental genotypes from known hatchery-origin progeny and from cryopreserved milt made it possible to examine Pallid Sturgeon recruitment. Therefore, our objectives were to 1) determine the likelihood that unmarked Pallid Sturgeon captured from the lower Missouri River were the result of natural recruitment and 2) examine the length distribution of wild- and hatchery-origin fish to determine if a difference exists by origin and examine the life-stage distribution. Genetic analysis showed that from 2003 to 2015, 358 “presumptive wild-origin” Pallid Sturgeon were captured in the lower Missouri River and the comparison between the length distributions of wild- and hatchery-origin fish did not provide any additional clarification into potential wild-origin fish. Low recruitment may be due to a small breeding population, high mortality of early life stages, hybridization with Shovelnose Sturgeon *Scaphirhynchus platyrhynchus*, or transport of drifting free embryos or larvae



into inhospitable habitats. Determining what factors are limiting recruitment is the important next step for the recovery of Pallid Sturgeon in the lower Missouri River.

Keywords: endangered species; recovery; *Scaphirhynchus*

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Introduction

The Pallid Sturgeon *Scaphirhynchus albus* is a benthic, obligate rheophile endemic to turbid, main-stem rivers and larger tributaries of the Mississippi and Missouri river basins (Forbes and Richardson 1905; Bailey and Cross 1954; Kallemeyn 1983). Early accounts of the species suggest that Pallid Sturgeon were not common in research collections or commercial catches (Forbes and Richardson 1905; Bailey and Cross 1954), especially compared to the sympatric Shovelnose Sturgeon *Scaphirhynchus platyrhynchus*. Populations of both *Scaphirhynchus* species declined throughout the 20th century resulting in the larger, less common Pallid Sturgeon being federally listed as endangered (USFWS 1990) pursuant to the U.S. Endangered Species Act (ESA 1973, as amended). Similar to other sturgeon species, researchers have attributed Pallid Sturgeon population declines to river management practices, habitat modifications, and past overharvest (USFWS 1993, 2014; Jacobson et al. 2015a, 2016b).

The U.S. Fish and Wildlife Service (USFWS) developed the Pallid Sturgeon Recovery Plan with input from other federal and state agencies to prevent imminent localized extirpation and maintain the genetic diversity of the species until fisheries managers could implement effective actions to improve natural recruitment and reestablish a self-sustaining population (USFWS 1993, 2014). The Pallid Sturgeon Recovery Plan listed conservation augmentation as a priority action to augment the existing Pallid Sturgeon population. The Pallid Sturgeon Conservation Augmentation Program (PSCAP) was initiated to supplement the diminished Pallid Sturgeon population in 1992 when morphologically identified Pallid Sturgeon collected from the Mississippi River were artificially propagated at the Missouri Department of Conservation's Blind Pony State Fish Hatchery (Sweet Spring, Missouri). In 1997 and 1999, propagation efforts continued in the lower Missouri River, and multiple hatchery facilities have

sustained these efforts as an annual activity since 2001. Artificial propagation and population augmentation efforts were conducted under the Pallid Sturgeon Range-Wide Stocking and Augmentation Plan (USFWS 2008), which artificially propagated wild-origin broodstock and reared their progeny to larger sized fish (i.e., > 100 mm) prior to release into the wild. Stocking of these larger sized (i.e., fall age-0 and age-1) fish reduces the likelihood of mortality and minimizes bottlenecks associated with early life stages.

Hatchery personnel physically marked hatchery-origin fish from propagation efforts using a variety of methods, including T-bar tags, coded-wire tags (CWT), passive integrated transponders (PITs), scute removal, and elastomer marks (Steffensen et al. 2008; USFWS 2008). Tag retention, especially PIT tags in hatchery-origin Pallid Sturgeon, has been variable, which impedes the ability of researchers to discern hatchery-origin fish when recaptured. McQuown et al. (2000) developed microsatellite markers to identify hatchery-origin fish using genetic parentage analysis, provided that genotypes of the parental broodstock were retained (DeHaan et al. 2008; USFWS 2008). However, efforts to collect and archive genetic tissue samples of broodstock used by PSCAP were incomplete prior to 2008. Lapses in the documentation of broodstock crosses combined with an incomplete genetic sample archive of broodstock used in the augmentation program resulted in genetically undocumented hatchery-origin progeny stocked in the lower Missouri River, which confounded our ability to detect hatchery-origin fish. This caused a systemic inability to determine whether captured unmarked Pallid Sturgeon were of wild or hatchery origin.

Genetic analyses to confirm the identity of unmarked Pallid Sturgeon captured from the lower Missouri River (river kilometer [rkm] 591.4–1,211.8) from 2003 to 2015 determined that 39.7% (538 of 1,355) of the Pallid Sturgeon PIT-tagged and released from the 2001 and 2002 hatchery year classes had lost their tags (K.D. Steffensen, Nebraska Game and Parks Commission, personal observation). Sturgeon more readily retained

other tagging regimes (i.e., CWT, elastomers, and scute removal) with 98.2% of captured hatchery-origin sturgeon readily identifiable through physical marks. Additionally, after the parental genetics database became more robust, DeHaan et al. (2008) demonstrated that 24 putatively wild-origin Pallid Sturgeon collected in the lower Missouri River were hatchery-origin fish that had shed their external tags.

Researchers have rarely detected successful natural reproduction in the lower Missouri River. However, sampling efforts throughout the lower Missouri River collected seven (i.e., did not match any known parental genotypes) age-0 Pallid Sturgeon (DeLonay et al. 2016b; E.J. Heist, Southern Illinois University, personal observation; R.L. Ruskamp, Nebraska Game and Parks Commission, personal communication) indicating some successful reproduction has occurred by Pallid Sturgeon in the lower Missouri River. The rarity of these observations confounds efforts to determine the timing, frequency, and geographic distribution of successful reproductive events, or the suite of environmental variables associated with successful reproduction needed to guide further recovery actions (Jacobson et al. 2016b).

Recruitment of Pallid Sturgeon, defined as either survival through the first year of life (recruitment to age 1) or survival to adulthood (recruitment to the reproductive adult population) is an important metric that researchers use to measure the success of numerous population-level and habitat restoration efforts and the overall recovery of the species. Prior to 2014, loss of hatchery tags coupled with incomplete documentation and genetic information for hatchery-origin Pallid Sturgeon prevented a detailed assessment of recruitment. Data recovery efforts, reconstruction of missing parental genetics from known hatchery-origin progeny (DeLonay et al. 2016b; E.J. Heist, Southern Illinois University, personal observation), and the use of cryopreserved milt to obtain genotypes from known parents (DeLonay et al. 2016b; M.L. Bartron, USFWS, unpublished data) have resolved much of this uncertainty. It is now possible to reexamine Pallid Sturgeon monitoring data from the lower Missouri River with greater clarity to assess the evidence supporting natural recruitment in this portion of the species' range and gain additional insights into potential recruitment if the length distributions of wild- and hatchery-origin fish differ. Therefore, the objectives of this study were to 1) use available genetic data to determine the likelihood that unmarked Pallid Sturgeon captured from the lower Missouri River were the result of natural recruitment and 2) examine the length distribution of wild- and hatchery-origin fish to determine if a difference exists by origin and examine the life-stage distribution.

Methods

Sampling methods

Researchers utilized data collected by the Pallid Sturgeon Population Assessment Project (PSPAP) and targeted broodstock collection activities from 2003 to

2015 (Welker et al. 2016; Welker and Drobish 2016; Steffensen et al. 2017; Data S1, *Supplemental Material*). Sampling occurred in the main-stem Missouri River downstream of Gavins Point Dam (rkm 1,305.1) to the confluence of the Missouri and Mississippi rivers (rkm 0.0) and lower reaches of the major tributaries, including the James, Big Sioux, Platte, Kansas, Grand, and Osage rivers (Figure 1). Annual efforts expended varied among reaches of the lower Missouri River; therefore, researchers did not compare annual capture rates and the spatial distribution of sturgeon captured among reaches.

Researchers used common handling protocols throughout the Missouri River basin; these included a suite of gears that effectively captured all size classes of Pallid Sturgeon (Welker et al. 2016; Welker and Drobish 2016; Steffensen et al. 2017). All collection efforts conformed to the USFWS Pallid Sturgeon Handling Protocols and were permitted under USFWS handling permits. Researchers weighed (g) and measured (fork length, mm) captured Pallid Sturgeon, and collected morphometric and meristic data to verify species identification. Researchers thoroughly examined each Pallid Sturgeon collected for physical marks or tags. The presence of a physical tag (i.e., PIT tag, CWTs, elastomer, or scute mark) indicated hatchery origin and the tagging scheme provided some hatchery information such as year class, parents, stocking location, stocking length–weight, and age at stocking. Researchers collected a tissue sample (1-cm² caudal or pectoral fin clip) for genetic analyses from unmarked individuals morphologically identified as Pallid Sturgeon and submitted tissue samples to the USFWS Northeast Fishery Center Conservation Genetics Lab (Lamar, Pennsylvania) or the Southern Illinois University Center for Fisheries, Aquaculture, & Aquatic Sciences (Carbondale, Illinois) for genetic analysis. A PIT tag number provided a unique identifier for each Pallid Sturgeon and data was restricted to first capture to prevent pseudoreplication. We excluded any unmarked sturgeon from which we did not collect a genetic sample from this analysis to avoid overestimation of recruitment.

Objective 1: Recruitment via genetic analysis and discrimination

We extracted genomic DNA using the Purgene method (Qiagen, Valencia, CA). The DNA concentrations were standardized for polymerase chain reaction (PCR). DeHaan et al. (2008) identified 17 microsatellite loci that are used for parentage analysis in Pallid Sturgeon: *Spl15*, *Spl18*, *Spl19*, *Spl26*, *Spl30*, *Spl34*, *Spl35*, *Spl36*, *Spl40*, *Spl56*, *Spl60*, *Spl101*, *Spl105*, *Spl106*, *Spl119*, *Spl158*, and *Spl173* (McQuown et al. 2000). Our analysis included these 17 loci; we added an additional 2 loci (*Spl12* and *Spl53*; McQuown et al. 2000) to improve species identification in the lower Missouri River baselines.

We created multiplex reactions to streamline the amplification process: we created five pre-PCR multiplex

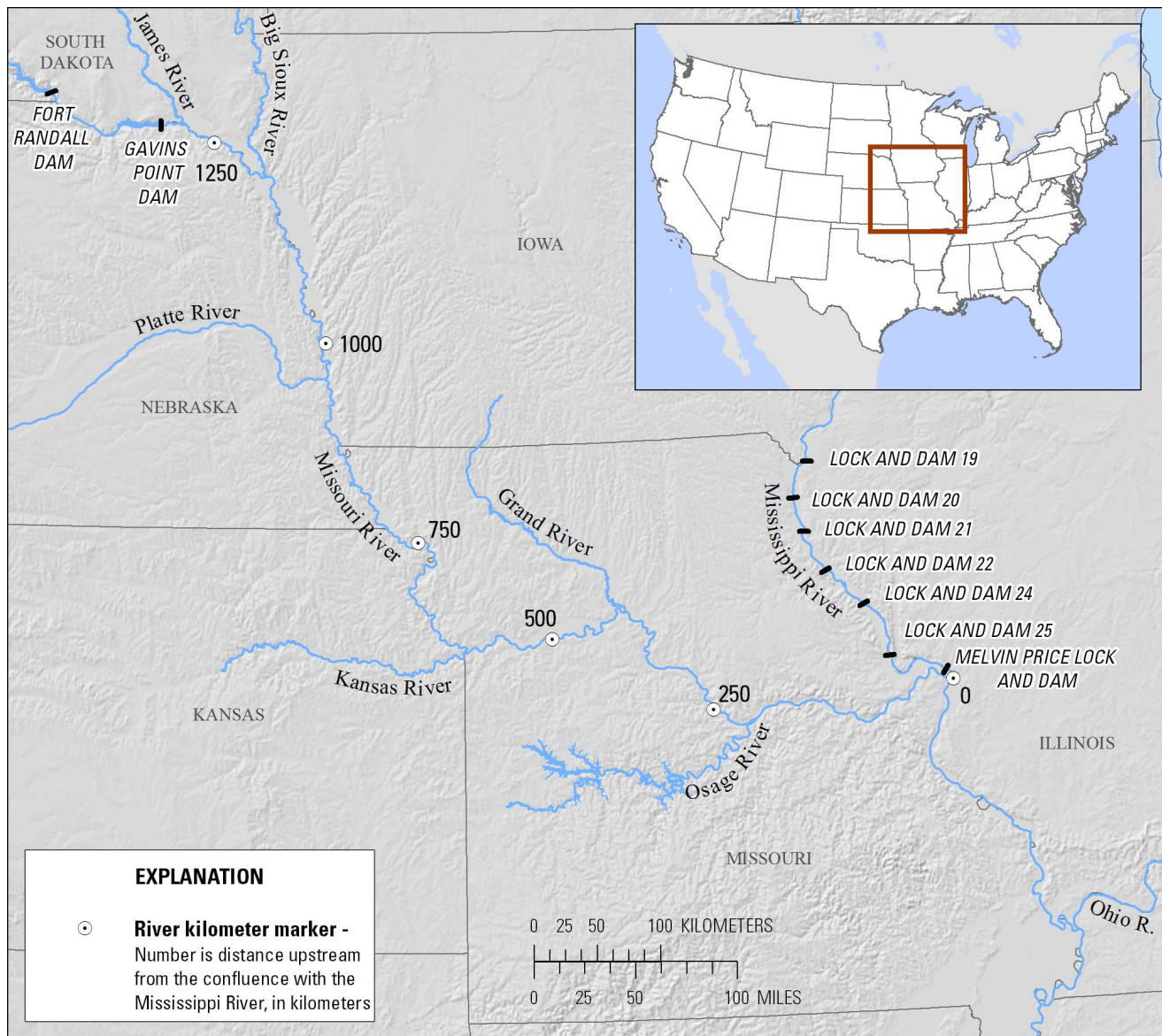


Figure 1. Map of the lower Missouri River from Gavins Point Dam (river kilometer 1,305.1) to the confluence with the Mississippi River (river kilometer 0.0).

reactions, with three to five loci within each reaction. We amplified loci *Spl26*, *Spl40*, *Spl53*, and *Spl105* separately. We added loci *Spl26*, *Spl40*, and *Spl105* to one of the multiplexes post-PCR. We did not add locus *Spl53* to a multiplex reaction; we ran it individually. For the multiplex reactions, reagent concentrations were the same. Each 20- μ L PCR reaction consisted of 1.5 μ L of genomic DNA extract, 1.5 \times PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl), 3.75 mM MgCl₂, 0.3175 mM each dNTP, 0.12–0.80 μ M of each primer (forward primer fluorescently labeled; Applied Biosystems, Foster City, CA), 0.06 units of Taq polymerase (Promega Corporation, Madison, WI), and deionized water added to achieve the final volume. Single PCR reactions, *Spl26*, *Spl40*, *Spl53*, and *Spl105*, were 10- μ L PCR reactions, and each consisted of 1.5 μ L of genomic DNA extract, 1.5 \times PCR

buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl), 3.75 mM MgCl₂, 0.3175 mM each dNTP, 0.06–0.24 μ M of each primer (forward primer fluorescently labeled; Applied Biosystems), 0.06 units of Taq polymerase (Promega Corporation), and deionized water added to achieve the final volume. The amplification cycle consisted of an initial denaturing at 94°C for 2 min; 35 cycles of 94°C denaturing for 45 sec, 56°C annealing for 45 sec, 72°C extension for 2 min; and a 30-min extension at 72°C. Locus *Spl105* had an annealing temperature of 50°C. We visualized genotypes using an ABI 3130XL Genetic Analyzer (Applied Biosystems). We used Genescan and Genmapper software from Applied Biosystems to identify alleles at each of the 19 loci. To estimate genotyping error, we extracted and amplified a random sample of 10% of all project samples, and genotyped them a

second time. We compared the initial and secondary genotypes to determine if genotyping error is an issue but detected genotype error in less than 1% of samples run.

Objective 1: data analysis

We accomplished genetic-based species assignments and detection of hybridization using the computer program NewHybrids (NewHybrids v1.1, University of California–Berkeley, Berkeley, CA; Anderson and Thompson 2002), a Bayesian-based method that can be used when baseline groups differ in allele frequency but do not have fixed differences, which is the case with Pallid and Shovelnose Sturgeon in the upper and middle Missouri River. NewHybrids computes the posterior probability that each individual belongs to one of the two baseline groups, or to one of four classes of hybrids: F_{1S} , F_{2S} , and directional backcrosses. Individuals were classified as a Pallid Sturgeon if the probability of assignment to known Pallid Sturgeon was 95% or greater. However, for individuals sampled in the lower portion of the Missouri River basin (Recovery Priority Management Areas 3 and 4), the probability of assignment was reduced to 90% or greater due to the slightly reduced ability to distinguish Pallid and Shovelnose Sturgeon based on allele-frequency differences between both species. We developed thresholds based on simulations with representative baseline groups to evaluate assignment of each of the classes of hybrid offspring.

We used genetic parentage analysis to determine if an individual Pallid Sturgeon captured originated from the PSCAP on the Missouri River or wild-origin (i.e., naturally produced). Researchers have obtained multilocus genotypes at 17 microsatellite loci for all but one hatchery-spawned adult since 2000. We conducted parentage assignments using Cervus (Cervus v3.0; Kalinowski et al. 2007). Genetic parentage assignments allow for 0 or 1 mismatch in parental–offspring triplet genotype comparisons. DeHaan et al. (2008) determined that by using 17 highly variable loci and allowing a single mismatch to accommodate for errors, the probability of an incorrect match were reduced to virtually zero. We compared parentage assignments to the hatchery-spawning database maintained by USFWS Missouri River Fish and Wildlife Conservation Office. Because genotypes are missing for six to eight broodstock, we identified individuals not assigned to the known parental genotypes as “presumptive wild origin.”

Objective 2: Length data

We calculated annual mean and median fork length (mm) and the associated confidence intervals to determine the degree of overlap between wild- and hatchery-origin Pallid Sturgeon populations. Potential recruitment may be assumed if population length distributions were not overlapping. Additionally, we subdivided presumptive wild-origin Pallid Sturgeon into

three length-group classifications to quantify when recruitment was detected in the lower Missouri River. These length groups included juvenile (< 600 mm, preontogenetic diet shift to piscivory; Grohs et al. 2009), subadult (600 – 800 mm), and adult (> 800 mm, minimum length at maturity; Steffensen et al. 2013a; Wildhaber et al. 2015).

Results

Objective 1: recruitment

The PSPAP collected 4,487 unique morphologically identified Pallid Sturgeon from 2003 to 2015 from the lower Missouri River. Of those, we readily identified 3,370 as hatchery-origin Pallid Sturgeon using physical tags or marks. We did not collect genetic samples for 117 unmarked sturgeon; therefore, we excluded these from further analysis. Genetic testing of the remaining 1,000 (22.3%) unmarked morphologically identified Pallid Sturgeon was required to detect potentially unmarked hatchery-origin fish and avoid overestimation of natural recruitment.

Genetic testing of unmarked morphologically identified Pallid Sturgeon revealed that 82 unmarked fish likely had some degree hybridization with Shovelnose Sturgeon, and we excluded these from further analyses. We only retained those sturgeon that strongly assigned as genetically pure Pallid Sturgeon (NewHybrids $P > 0.95$; Schrey et al. 2011; Jordan et al., in review). Genetic testing also indicated that 560 fish matched parental genotypes of known hatchery crosses made by the PSCAP and we classified these as hatchery-origin fish. The remaining 358 unmarked fish did not match parental genotypes of known hatchery crosses and we classified these fish as “presumptive wild origin” (Table 1). Overall, presumptive wild-origin Pallid Sturgeon only accounted for 8.0% of all Pallid Sturgeon collected by the PSPAP during 13 y of sampling. The PSPAP also frequently recaptured presumptive wild-origin Pallid Sturgeon ($n = 131$) as well as hatchery-origin ($n = 962$) fish, which we did not include in the above capture data. Annual recapture rates varied from 0.0% during the first several years of monitoring to 48.1% in 2014 for wild-origin Pallid Sturgeon. Comparatively, hatchery-origin fish recapture rates generally increased annually to 33% in 2014 and 2015.

Objective 2: length data

Lengths of known hatchery-origin Pallid Sturgeon collected by the PSPAP from 2003 to 2015 varied widely, ranging from 111 to 1,075 mm; however, there was less variation in the lengths of presumptive wild-origin Pallid Sturgeon, which ranged from 532 to 1,197 mm (Figure 2). The confidence intervals consistently overlapped, which did not allow conclusive determination of origin based on fork length. Restricting the hatchery-origin length data to the two missing genotyped year classes (1992 and 2001) slightly improves the wild-origin

Table 1. The total number of presumptive wild-origin and hatchery-origin Pallid Sturgeon *Scaphirhynchus albus* captured and recaptured in the lower Missouri River from 2003 to 2015.

| Year | Presumptive wild origin | | | Hatchery origin | | |
|-------|-------------------------|-----------|-------------|-----------------|-----------|-------------|
| | Capture | Recapture | % Recapture | Capture | Recapture | % Recapture |
| 2003 | 0 | 0 | 0.0 | 6 | 1 | 14.3 |
| 2004 | 7 | 0 | 0.0 | 28 | 0 | 0.0 |
| 2005 | 5 | 0 | 0.0 | 54 | 1 | 1.8 |
| 2006 | 9 | 0 | 0.0 | 73 | 2 | 2.7 |
| 2007 | 17 | 2 | 10.5 | 234 | 7 | 2.9 |
| 2008 | 65 | 4 | 5.8 | 474 | 46 | 8.8 |
| 2009 | 42 | 9 | 17.6 | 486 | 81 | 14.3 |
| 2010 | 27 | 14 | 34.1 | 526 | 107 | 16.9 |
| 2011 | 46 | 9 | 16.4 | 486 | 84 | 14.7 |
| 2012 | 18 | 7 | 28.0 | 490 | 131 | 21.1 |
| 2013 | 38 | 25 | 39.7 | 377 | 160 | 29.8 |
| 2014 | 41 | 38 | 48.1 | 356 | 176 | 33.1 |
| 2015 | 43 | 23 | 34.8 | 340 | 166 | 32.8 |
| Total | 358 | 131 | 26.8 | 3,930 | 962 | 19.7 |

discrimination for the larger adult-sized fish but lacks discrimination for the juvenile and subadults (Figure 3).

Only 19.6% of the presumptive wild-origin Pallid Sturgeon captured were of juvenile (< 600 mm; *n* = 3) or subadult (600–800 mm; *n* = 67) size. The PSPAP captured juvenile-size Pallid Sturgeon only in 2007, 2011, and 2015, whereas subadult-size Pallid Sturgeon were captured annually from 2005 through 2015. Capture frequencies ranged from 2 fish in 2006 to 12 fish in 2008 with an overall mean of 6.3 fish per year. Comparatively, 91.6% of hatchery-origin fish were juvenile or subadult size. Despite the fact that known juvenile hatchery-origin fish ranging from approximately 100 to 600 mm constitute a substantial portion of the fish collected by the PSPAP, no presumptive wild-origin Pallid Sturgeon less than 500 mm were collected by the PSPAP from 2003 to 2015, and only three juveniles (500–600 mm) were collected (Figure 2). The broad variation of lengths of known hatchery-origin Pallid Sturgeon collected annually clearly indicates that juvenile wild Pallid Sturgeon are susceptible to capture and readily identifiable using the protocols of the PSPAP monitoring program.

Discussion

Evidence for natural Pallid Sturgeon recruitment in the lower Missouri River was limited considering the effort expended during 13 y of PSPAP sampling. Only 358 (8.0%) of the 4,487 individual Pallid Sturgeon captured from 2003 to 2015 are considered to be presumptive wild-origin fish and the comparison between the length distributions of wild- and hatchery-origin populations did not provide any additional clarification into potential presumptive wild-origin fish. Only 19.6% of presumptive wild-origin Pallid Sturgeon were of juvenile (< 600 mm; 0.8%) or subadult size (600–800 mm; 18.7%), or approximately 1.6% of all Pallid Sturgeon collected by PSPAP crews. The best evidence of recruitment of Pallid

Sturgeon on the lower Missouri River arises from the capture of distinct presumed-wild subadult fish and unmarked adult fish captured each year. No wild-origin Pallid Sturgeon less than 500 mm were detected, despite annual captures of known hatchery-origin fish ranging from 100 to 500 mm. Monitoring data show that juvenile-sized hatchery-origin Pallid Sturgeon were collected annually, which indicates it is not likely a gear effect limiting the detection of juvenile wild-origin fish. Presumptive wild-origin juveniles in the lower Missouri River are rare, which requires continued species recovery efforts, as the existing level of natural recruitment is insufficient to sustain the species (Steffensen et al. 2013b; Jacobson et al. 2015b, 2016a).

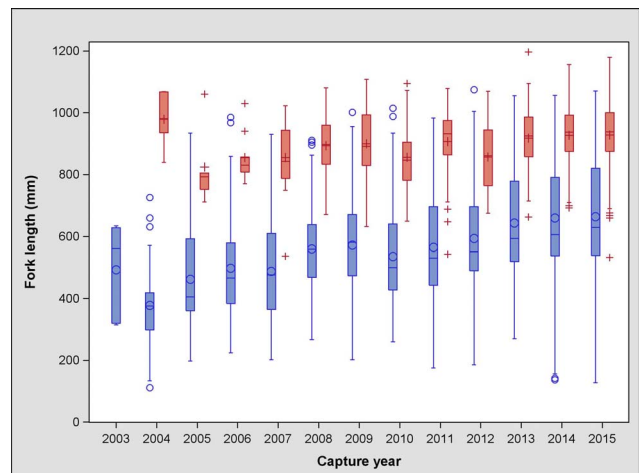


Figure 2. Fork-length distribution for presumptive wild- (red bars) and hatchery-origin (blue bars) Pallid Sturgeon *Scaphirhynchus albus* captured in the lower Missouri River from 2003 to 2015. The line within the box represents the median and the +/- represents the mean fork length (mm). The box represents the upper and lower 25% confidence intervals and the whiskers represent the 95% confidence intervals.

Table 2. Summary of hatchery-origin Pallid Sturgeon *Scaphirhynchus albus* stocked into the lower Missouri River, the middle Mississippi River, and the lower reaches of select tributaries. Year classes impacted by broodstock with missing genotypes, which limits the ability to assign to parentage and the number of progeny produced, are presented by year class.

| Year class | No. of female broodstock ^a | No. of male broodstock ^a | Broodstock missing a genotype | No. of progeny produced by broodstock missing genotype | Total no. of progeny stocked ^a | Proportion unassignable to known broodstock ^b |
|-------------------|---------------------------------------|-------------------------------------|-------------------------------|--|---|--|
| 1992 ^c | 2 or 3 | 3 or 4 | Potentially 2 broodfish | Unknown ^d | 4,182 | Unknown ^d |
| 1997 | 5 | 4 | 2 females and 3 males | 2,816 | 2,851 | 0.988 |
| 1999 | 1 | 3 | — | — | 532 | 0.000 |
| 2001 | 3 | 9 | 1 male | 12 | 7,453 | 0.002 |
| 2002 | 1 | 5 | — | — | 9,241 | 0.000 |
| 2003 | 3 | 11 | — | — | 10,129 | 0.000 |
| 2004 | 8 | 19 | — | — | 39,255 | 0.000 |
| 2005 | 3 | 4 | — | — | 3,654 | 0.000 |
| 2006 | 3 | 4 | — | — | 3,642 | 0.000 |
| 2007 | 4 | 3 | — | — | 4,515 | 0.000 |
| 2008 | 2 | 4 | — | — | 6,663 | 0.000 |
| 2009 | 5 | 10 | — | — | 14,594 | 0.000 |
| 2010 | 4 | 5 | — | — | 6,812 | 0.000 |
| 2011 | 4 | 7 | — | — | 21,736 | 0.000 |
| 2012 | 1 | 2 | — | — | 99 | 0.000 |
| 2013 | 3 | 3 | — | — | 6,035 | 0.000 |
| 2014 | 2 | 6 | — | — | 19,582 | 0.000 |
| 2015 | 2 | 4 | — | — | 12,513 | 0.000 |
| Totals | — | — | — | 2,828–7,010 | 173,488 | 0.016–0.040 |

^a From Huenemann 2018.

^b K.A. Chojnacki, U.S. Geological Survey, personal observation.

^c Depending on the authoritative source, either three females and four males or two females and three males were spawned. The genotypes of five broodstock (two of one gender and three of the other) used to produce the 1992 year class were posthoc reconstructed (DeLonay et al. 2106a; E.J. Heist, Southern Illinois University, personal observation). Genetic samples from known offspring of the other broodstock parent(s) were insufficient to facilitate genotype reconstruction.

^d The proportion of unassignable offspring cannot be determined from existing data.

As of 2015, approximately 96.0–98.4% of hatchery-origin Pallid Sturgeon stocked in the lower Missouri and middle Mississippi rivers were confidently detected using genotypes of documented broodstock. Detecting all hatchery-origin Pallid Sturgeon is still problematic due to the three hatchery-year classes (i.e., 1992, 1997, and 2001) with missing broodstock genotypes; however, this a conservative estimate of the number of undetectable hatchery-origin fish, and the proportion surviving in the wild is likely substantially lower (Table 2). We cannot determine a precise estimate of the proportion of progeny within the 1992 year class produced by these genetically undocumented parental broodstock crosses, but is likely low as genotype reconstruction has not occurred because too few unassigned progeny were recaptured (DeLonay et al. 2016a; E.J. Heist, Southern Illinois University, personal observation). Fisheries managers marked the 1992 and 1997 year classes, which represent upwards of 96% of the hatchery-origin fish without archived parental genotypes, with CWTs prior to being stocked. We detected CWTs in 88% of the fish that we subsequently assigned to the 1992 year class, indicating a low level of tag loss or missed detections (K.D. Steffensen, Nebraska Game and Parks Commission, personal observation). The rate of Pallid Sturgeon with undetected CWT is likely overestimated as not all field crews were able to scan captured sturgeon for CWT in the early years (i.e., 2003 and 2004) of the PSPAP. Only

one fish had a CWT detected that did not assign to the known genotypes of the 1992 known broodstock. This single, unassigned CWT-tagged fish could be the progeny of undocumented 1992 or 1997 parents. The

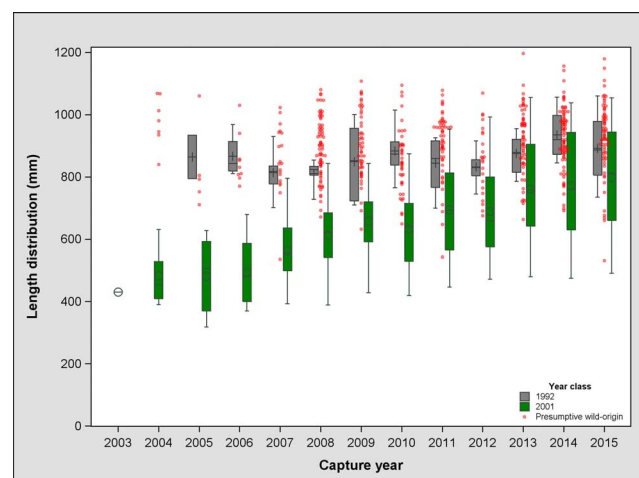


Figure 3. Length distribution for presumptive wild-origin (red dots) compared to the missing genotype hatchery-origin Pallid Sturgeon *Scaphirhynchus albus* (colored bars) captured in the lower Missouri River from 2003 to 2015. No 1997 year class hatchery-origin Pallid Sturgeon were determined due to lack of parental genotypes.

general lack of unassigned CWT-tagged fish following broodstock reconstruction suggests that contributions from any undocumented broodstock to the 1992 and 1997 hatchery year class may be negligible.

While detecting recruitment was confounded by the presence of potentially undetectable hatchery-origin Pallid Sturgeon, it is likely that a substantial portion of the 359 presumptive wild-origin Pallid Sturgeon is the result of natural reproduction. We do not know how long potentially undetectable hatchery-origin fish (e.g., 1992, 1997, and 2001 year classes) will persist in the lower Missouri River as Pallid Sturgeon are a long-lived species and there are inherent difficulties of aging sturgeon to determine the maximum age using wild-origin fish (Whiteman et al. 2004; Koch et al. 2011; Hamel et al. 2014). Reconstruction of the remaining missing broodstock genotypes used to produce hatchery-origin Pallid Sturgeon would improve the detection of natural recruitment but the likelihood of reducing the uncertainty for the three missing year classes (i.e., 1992, 1997, and 2001) remains minimal as the parental genetic samples were lost or not taken and lack of their progeny recaptures prevented genetic reconstruction. Researchers have closely monitored the collection of broodstock genetic samples since those early issues, so missing a parental genotype is unlikely.

Distinguishing natural reproduction from hatchery augmentation is important to identify as natural reproduction and recruitment are foremost recovery criteria in the Pallid Sturgeon Recovery Plan (USFWS 2014). The potential to reclassify Pallid Sturgeon from endangered to threatened can occur when a genetically diverse, self-sustaining population is naturally occurring for two generations. We do not completely understand the causes of the low level of recruitment, but they may be related to a reduced adult breeding population, hybridization with the sympatric Shovelnose Sturgeon, high mortality rates during early life stages related to embryos and larvae drifting into inhospitable habitats, or predation. As Pallid Sturgeon recovery efforts continue, determining what factors are limiting recruitment is vital. If researchers frequently detect natural reproduction, depending upon the level of recruitment, the need for the PSCAP could likely be reduced or eliminated. As parental broodstock genotypes are fully documented, monitoring efforts for Pallid Sturgeon on the lower Missouri River will be able to detect natural recruitment for juveniles and subadults with complete certainty, providing fundamental information for recovery efforts.

Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content or functionality of any supplemental material. Queries should be directed to the corresponding author for the article.

Data S1. Excel spreadsheet containing the Pallid Sturgeon *Scaphirhynchus albus* capture and recapture data from the lower Missouri River from 2003 to 2015. Table includes the following fields: PIT tag number

(TagNumber), Origin (Wild/Hatchery-Origin, Hybrid or Missing), Set Date, Capture Year, Species, Fork Length (Length), Weight (W), and the raw genetic data for the 19 loci.

Found at DOI: <https://doi.org/10.3996/022018-JFWM-013.S1> (403 KB XLSX).

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