Article

Demographic and Evolutionary History of Pallid and Shovelnose Sturgeon in the Upper Missouri River

William R. Ardren,* George R. Jordan, Patrick W. DeHaan, Robin S. Waples

W.R. Ardren
U.S. Fish and Wildlife Service, Lake Champlain Fish and Wildlife Conservation Office, Essex Junction, Vermont 05452

Present address: U.S. Fish and Wildlife Service, Midwest Fisheries Center, 555 Lester Avenue, Onalaska, WI 54650

G.R. Jordan
U.S. Fish and Wildlife Service, Montana Fish and Wildlife Conservation Office, 2900 4th Avenue North, Room 301, Billings, Montana 59101

P.W. DeHaan
U.S. Fish and Wildlife Service, Abernathy Fish Technology Center, 1440 Abernathy Creek Road, Longview, Washington 98632

Present address: U.S. Fish and Wildlife Service, Midwest Fisheries Center, Whitney Genetics Laboratory, 555 Lester Avenue, Onalaska, Wisconsin 54650

R.S. Waples
NOAA Fisheries Service, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, Washington 98112

Abstract

Natural-origin Pallid Sturgeon *Scaphirhynchus albus* in the upper Missouri River are predicted to become extirpated as early as 2024. To aid in recovery efforts for this endangered species, we used genetic data from 17 microsatellite loci to infer demographic and evolutionary history of Pallid Sturgeon and a sympatric Shovelnose Sturgeon *S. platorynchus*. Our data indicated a recent sundering of geneflow between these species by overlapping allele size distributions at all loci and low level of genetic divergence ($F_{ST} = 0.10$). Tests for recent bottlenecks by using heterozygosity excess or allele frequency mode-shift tests indicated demographic stability for both species, while the M ratio identified that historic bottlenecks occurred in both species. Estimates of historical effective population size (N_e), based on coalescent modeling of allele size distribution, suggested that the geographic expansion of these species into the upper Missouri River during the late Pleistocene was associated with 10- to 19-fold reductions in N_e. By contrast contemporary estimates of N_e based on linkage disequilibrium revealed that Shovelnose Sturgeon ($N_e = 2{,}983$) had approximately 10 times greater N_e than Pallid Sturgeon ($N_e = 254$). Our results are consistent with the recent collapse of Pallid Sturgeon being caused by dam construction, which occurred between 1930 and 1965. Fortunately, genetic diversity remaining in this long-lived species provided an opportunity to conserve predam Pallid Sturgeon genetic diversity via a successful captive breeding program. We provide recommendations to address key conservation needs, including how to incorporate our estimate of N_e/adult census size of 0.26 (95% confidence interval of 0.16–0.47) into setting demographic recovery goals for Pallid Sturgeon.

Keywords: sturgeon; Bayesian inference; effective population size; demographic history; *Scaphirhynchus*; endangered species

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* Corresponding author: william_ardren@fws.gov

Introduction

Knowing the demographic history of an imperiled species is critical to setting recovery goals and identifying key restoration actions (Frankham 2005; Akçakaya et al. 2018). For example, demographic history is useful for understanding whether a species' decline was associated with a documented threat, such as habitat modification or destruction (Goossens et al. 2006), overharvest (Foley and Lynch 2020), or climate change (Okello et al. 2008). In addition, the magnitude and time period over which the decline occurred are important factors that influence the type of conservation actions used to recover a species, because it provides a historical template for assessing viability (Schwartz et al. 2006; Waples et al. 2007) and allows managers to overcome the shifting baseline syndrome described by Pauly (1995). Unfortunately, important demographic parameters used in recovery planning, such as adult census size (N) of a population before a demographic decline occurred, is unknown for many imperiled species. This information gap is often caused by difficulty in obtaining demographic data, which is common when the species was not of human concern before it became imperiled.

Genetic data provide a way to retrospectively study the demography of a species (Luikart et al. 2010). Examining trends in genetic change overtime from two or more samples provides high statistical power (Waples 1989), but this method often requires samples from archived DNA, such as museum skins (van der Valk et al. 2019) or fish scales (Ardren and Kapuskinski 2003), which are frequently unavailable for imperiled species. Other methods used to infer demographic history based on a single genetic sample do exist, however. Single-sample methods estimate past population size from a variety of sources, including information from coalescent-based modeling, mismatch distribution of DNA sequence data, and theoretical expectations of levels of linkage disequilibrium or heterozygosity (Luikart et al. 2010).

Genetic methods for inferring the demographic history of a species provide estimates of effective population size (N_e) rather than the N of the population being examined. The N_e is the size of an idealized population that would have the same rates of genetic change and inbreeding as the real population under consideration. Real populations do not conform to idealized assumptions, so N_e is usually much lower than N primarily because of larger-than-random variance in family size, unequal sex ratio, and a fluctuating population size over time (Waples 2005). Frankham (1995) reported a mean value of 0.10–0.11 for N_e-to-N ratio (N_e/N) in a meta-analysis of 192 published estimates for 102 species, while a more recent review by Palstra and Ruzzante (2008) found a similar median value of 0.14 in a meta-analysis of 83 studies. Estimates of N_e can be converted to N using these mean N_e/N values; however, these conversions need to consider variability of the ratio among species, populations within species, and even within populations over time (Ardren and Kapuskinski 2003; Palstra and Fraser 2012). In addition, for the N_e/N ratio to be meaningful, it is important to match estimates of N_e and N for the same time periods (Waples 2005).

Sturgeons (family Acipenseridae) are ancient fishes that have existed for at least 200 million y. Across the northern hemisphere, many species are imperiled; of the 25 extant sturgeon species, 13 are considered in danger of extinction due, in part, to reduction in critical habitat, overexploitation, and accumulation of pollutants in sediments (Birstein et al. 1997). Pallid and Shovelnose Sturgeon, in the genus *Scaphirhynchus,* are endemic to the Mississippi River Drainage of the United States. Pallid Sturgeon (*S. albus*) were listed as endangered pursuant to the U.S. Endangered Species Act in 1990 (ESA 1973, as amended; USFWS 1990), whereas their congener, the Shovelnose Sturgeon *S. platorynchus*, has remained relatively common. Pallid Sturgeon are naturally sympatric with Shovelnose Sturgeon, with Shovelnose Sturgeon occurring in most major river drainages in the Mississippi and Missouri River basins (Keenlyne 1997). Pallid Sturgeon occupy a smaller range than Shovelnose Sturgeon that is restricted to the Missouri River, the Mississippi River from the vicinity of the confluence with the Missouri River downstream to the Gulf of Mexico, and the lower portions of several large tributaries (Keenlyne 1997).

Morphological differences between adult Pallid and Shovelnose Sturgeon (Keenlyne et al. 1994) are much greater than genetic differences observed between these species (Phelps and Allendorf 1983; Tranah et al. 2001). Small genetic differences observed between Pallid and Shovelnose Sturgeon suggest that these species have recently diverged during the late Pleistocene (Campton et al. 2000). The degree of genetic and morphological differentiation between Pallid and Shovelnose Sturgeon is greatest in the upper Missouri River and lowest in the southern portion of the Mississippi River drainage (i.e., downstream from the Ohio River confluence), where hybridization between these species is common (Jordan et al. 2019). Reproductive isolation between Pallid and Shovelnose Sturgeon appears to be maintained by temporal, spatial, or behavioral variables or some combination of these variables in the upper Missouri River, as hybrids between the species are rarely observed in that area (Tranah et al. 2001). In addition, upper Missouri River Pallid Sturgeon are the most genetically distinct component within the range of the species (Schrey and Heist 2007; Schrey et al 2011).

The 2014 revised Pallid Sturgeon recovery plan established four management units for Pallid Sturgeon throughout the species range (USFWS 2014) based on genetic data, morphological differences, biogeography of other fish species and speciation associated with

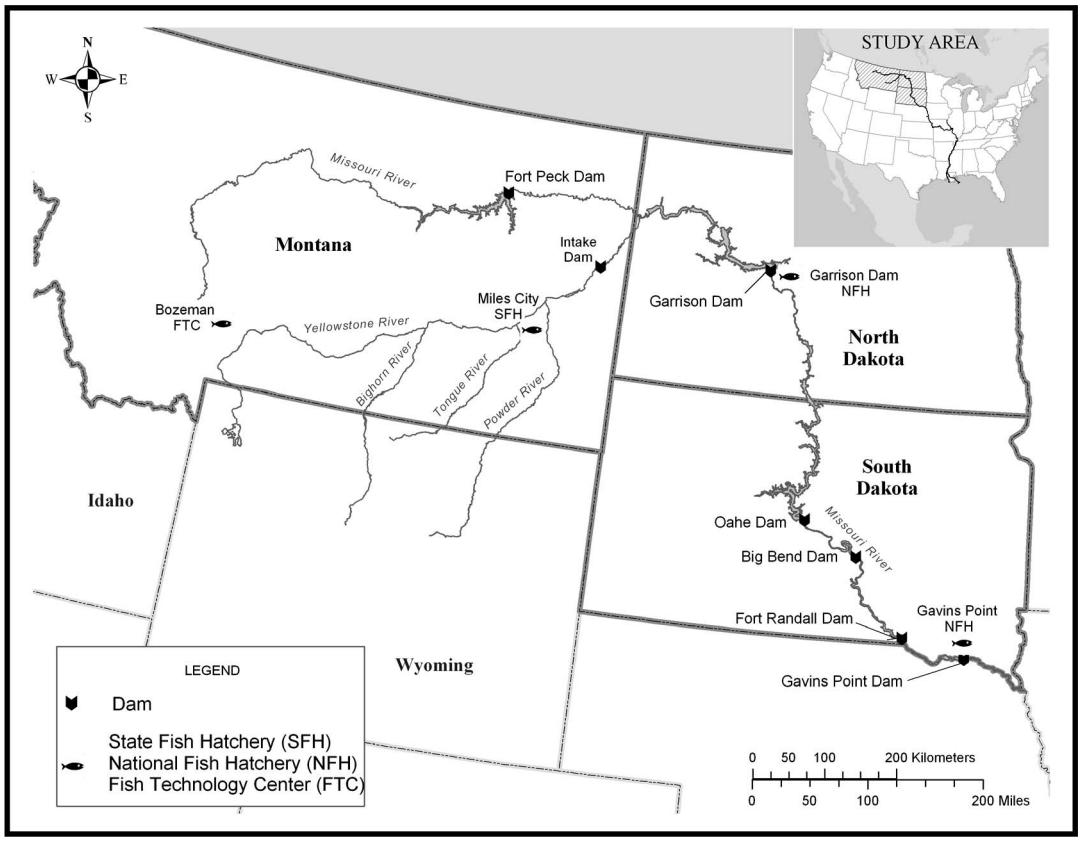

Figure 1. Map of the upper Missouri River Basin depicting main-stem Missouri River dams and locations of facilities associated with Pallid Sturgeon *Scaphirhynchus albus* propagation as of 2022. The inset map provides the location of states in the Great Plains Management Unit relative to the rest of the range of Pallid Sturgeon.

physiographic provinces, and common threats. Pallid Sturgeon from the upper Missouri River are part of the Great Plains Management Unit (GPMU; Figure 1). The GPMU is genetically distinct from the other management units located in the lower Missouri River, middle Mississippi River, and Atchafalaya River (Schrey and Heist 2007). Pallid Sturgeon in the GPMU also occupy the Great Plains Phyisographical Province, which is characterized by a unique fish assemblage and novel habitat characteristics compared with the rest of the species range (Cross et al. 1986; USFWS 2014).

Anthropogenic habitat changes to the upper Missouri River and lower Yellowstone River severely impacted Pallid Sturgeon in the GPMU (Jordan et al. 2016). In the early 1900s, irrigation projects began fragmenting habitats in the lower Yellowstone River, and from the 1930s to the mid-1960s, six main-stem dams were built on the Missouri River, resulting in large reaches of riverine habitat being impounded into reservoirs (Figure 1). Altered water flows and habitat fragmentation caused by these dams impacted Pallid and Shovelnose Sturgeon differently. Natural recruitment for Pallid Sturgeon is rare or nonexistent, while natural Shovelnose Sturgeon recruitment continues to be strong (Braaten et al. 2008). The main hypothesis to explain the differences in recruitment trends for these two closely related species is the lack of unfragmented river habitat for Pallid Sturgeon to complete the larval phase of development (Eichelberger et al. 2014). Pallid Sturgeon may require up to twice the cumulative larval drift distance to complete ontogenetic development as do Shovelnose Sturgeon larvae (Braaten et al. 2008). Specifically, larval Pallid Sturgeon in the upper Missouri River are thought to die after drifting into the lentic environments formed by reservoirs (Guy et al. 2015), while Shovelnose Sturgeon complete their larval drift phase and settle into suitable habitat before drifting into reservoirs (Figure 1).

Based on the lack of documented recruitment and $\hat{N}$ of 158 (95% confidence interval [95% CI] of 129–193) adults, the senescing Pallid Sturgeon in the GPMU may face local extirpation by the year 2024 (Klungle and Baxter 2005). A captive breeding program was established in 1997 to maintain Pallid Sturgeon in the GPMU (USFWS 2008). However, little or no information is available to guide restoration goals for this program because historical records of Pallid Sturgeon abundance are limited in scope and often do not adequately discriminate between Pallid and Shovelnose Sturgeon. Establishing restoration goals for Pallid Sturgeon requires an understanding of historical demographic data, which is lacking (USFWS 2014). Biologists need to know if the Pallid Sturgeon population in the GPMU was historically small or if it recently greatly reduced in size. Manage-

ment of the captive breeding program would also benefit from knowing to what extent Pallid Sturgeon in the GPMU are already suffering from the adverse effects of a genetic bottleneck, such as inbreeding depression and decrease in adaptive potential. Thus, information concerning historic population size, historic levels of genetic variation, and current rates of inbreeding combined with accurate estimates of juvenile survival rates and carrying capacity of the system are key characteristics needed to establish reasonable recovery goals for Pallid Sturgeon (Braaten et al. 2009; USFWS 2014).

Here, we use genetic methods to address critical data gaps needed for conservation of Pallid and Shovelnose Sturgeon in the upper Missouri and lower Yellowstone rivers. In particular, we identify changes in historic N_e for these species and relate these results to known events, such as dam construction and recolonization of the area after the Wisconsin glacial epoch. We also used contemporary estimates of N_e gained from the extant natural population of Pallid Sturgeon in the GPMU to provide an estimate of wild adults in existence before major anthropogenic influences that would have produced the current cohorts of extant wild adults. Then, based on the estimated relationship of N_e to N, we provide recommendations for Pallid Sturgeon recovery criteria in the GPMU.

Methods

Study area and sampling locations

Montana Fish Wildlife and Parks and U.S. Fish and Wildlife Service biologists sampled adult sturgeon from two regions of the upper Missouri River Basin using trammel and trawl nets. Biologists sampled pallid ($n =$ 140) and Shovelnose Sturgeon ($n = 94$) in the upper Missouri River downstream from Fort Peck Dam, Montana, and upstream of Garrison Dam, North Dakota. Additionally, they sampled both species from the lower Yellowstone River downstream from river mile 220 to its confluence with the Missouri River (Figure 1). Biologists collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and collected Shovelnose Sturgeon from May to July of 2005. The biologists used adult fish size and morphometric measurements that distinguish these two species in the upper Missouri River for field identification of Pallid and Shovelnose Sturgeon (Keenlyne et al. 1994; Willis et al. 2002).

Microsatellite genotyping and inheritance of allele variation

We extracted genomic DNA from a 1-mm^2 piece of fin tissue by placing it in 200 μL of 5% chelex containing 0.2 mg/mL proteinase K, incubating it for 2 h at 56°C, boiling it for 8 min at 100°C, and vortexing it for 30 s. Next, we genotyped all individuals at 17 disomic microsatellite loci: *Spl15*, *Spl18*, *Spl19*, *Spl26*, *Spl30*, *Spl34*, *Spl35*, *Spl36*, *Spl40*, *Spl56*, *Spl60*, *Spl101*, *Spl105*, *Spl106*, *Spl119*, *Spl158*, and *Spl173* (McQuown et al. 2000; Table 1). We used 15 μL of total volume for all PCRs; each reaction contained 2 μL of template DNA, 10× PCR buffer, 0.2 mM dNTPs, 0.5 μM forward and reverse primers, 1.5 mM $MgCl_2$, and 0.2 U of *Taq* polymerase. We used the following PCR cycling conditions: an initial DNA denaturation at 94°C for 3 min, followed by 38 cycles of 1) denaturation at 94°C for 30 s, 2) annealing at 56°C for 30 s, and 3) primer extension at 72°C for 30 s, followed by a final annealing and extension at 72°C for 7 min. We used an Applied Biosystems 3130xl genetic analyzer to visualize PCR products, and multilocus genotypes for each individual were determined using Genemapper 5.0 (Applied Biosystems Inc., Foster City, CA) software. To conduct a quality control analysis, we randomly selected 10% of the individuals that a separate researcher reextracted and regenotyped by following the procedures outlined above to ensure a genotyping error rate of less than 5%. We determined mode of inheritance for all 17 microsatellite loci used in this study for Pallid Sturgeon using the methods of Ardren et al. (1999). See Text S1, *Supplemental Material*, for methods of the microsatellite mode of inheritance analysis.

Identification of hybrids and characterizing population genetic structure

We used the Bayesian clustering method of STRUCTURE 2.1 (Pritchard et al. 2000) to identify potential hybrids and characterize the level of population genetic structure within sample collections. STRUCTURE 2.1 groups individuals into a predefined number (K) of genetic clusters based on multilocus genotypes. We applied the admixture model that assumes gene flow among populations and allows for correlated allele frequencies across clusters. This admixture model assigns a proportion of each individual's genome to each of the clusters, pursuing solutions that maximize Hardy–Weinberg equilibrium and linkage equilibrium within clusters. We used data from all Pallid and Shovelnose Sturgeon for the first part of our analysis, and we performed 20 replicated runs for each putative value of K from 1 to 5. All runs had a burn-in of 30,000 iterations followed by 100,000 iterations. We used a symmetric similarity coefficient to determine the similarity of outcomes among the 20 replicate STRUCTURE runs for each K. We used the LargeKGreedy algorithm of CLUMPP (Jakobsson and Rosenberg 2007) with 1,000 random input sequences to determine the number of distinct modes among the 20 runs at each K by grouping pairs of runs that had a symmetric similarity coefficient of $>$0.9. We used the DISTRUCT software (Rosenberg 2004) to generate graphical displays of STRUCTURE results, with the membership of each individual representing the mean membership over the replicate runs. We categorized individuals as Pallid Sturgeon if their probability of membership to the Pallid Sturgeon group defined by structure (q) value was $>$ 0.98 or as a Shovelnose Sturgeon if their q value was $<$ 0.02 (Boyer et al. 2008).

Table 1. Genetic diversity of Pallid Sturgeon *Scaphirhynchus albus* ($n = 139$) and Shovelnose Sturgeon *S. platorynchus* ($n = 94$) sampled in the upper Missouri River basin at 17 microsatellite loci. We used allele frequencies and size distribution to detect evidence of genetic bottlenecks using moment-based estimators of Cornuet and Luikart (1996), Luikart et al. (1998), and Garza and Williamson (2001). We also used these data to estimate the severity and timing of bottlenecks using the hierarchical Bayesian model of Storz and Beaumont (2002). Allele sizes are in base pairs. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

		Frequency	
Locus	**Allele**	**Pallid**	**Shovelnose**
Spl15	185	0.007	0.080
	191	—	0.011
	193	0.230	0.202
	195	0.547	0.154
	197	0.007	0.261
	199	—	0.005
	201	—	0.005
	203	—	0.085
	205	—	0.011
	207	0.119	0.101
	209	0.058	0.037
	213	0.004	—
	221	—	0.011
	225	0.029	0.011
	239	—	0.011
	243	—	0.016
Spl18	237	—	0.005
	239	0.018	0.005
	241	0.730	0.325
	243	0.198	0.638
	245	0.036	0.011
	247	0.018	0.016
Spl19	233	0.018	0.016
	235	0.004	0.043
	237	0.149	0.388
	239	0.301	0.207
	241	0.315	0.101
	243	0.214	0.218
	245	—	0.011
	247	—	0.016
Spl26	282	—	0.018
	286	—	0.012
	290	0.008	—
	294	0.098	0.053
	296	0.203	0.012
	298	0.271	0.012
	300	—	0.041
	302	0.008	0.006
	304	0.008	0.006
	306	0.004	0.018
	308	0.064	0.065
	310	0.105	0.029
	312	0.015	0.035
	314	0.004	0.053
	316	0.023	0.047
	318	—	0.041
	322	—	0.029
	324	0.011	0.100
	326	0.083	0.059
	328	0.049	0.171
	330	0.034	0.088
	332	0.004	0.029
	334	0.008	0.029
	338	0.004	0.029
	340	—	0.012
	344	—	0.006
Spl30	239	0.007	0.011
	243	—	0.005
	249	0.058	0.005
	253	—	0.080
	255	0.669	0.372
	257	0.076	0.037
	259	0.004	0.154
	261	0.011	0.170
	263	0.148	0.122
	265	0.029	0.037
	269	—	0.005
Spl34	312	—	0.048
	316	0.011	0.181
	318	0.004	0.075
	324	—	0.011
	328	—	0.043
	330	—	0.043
	332	0.303	0.027
	334	0.336	0.069
	336	0.007	0.005
	338	0.157	0.005
	340	—	0.335
	342	0.095	0.059
	344	0.004	—
	346	0.084	0.032
	350	—	0.053
	352	—	0.016
Spl35	228	—	0.181
	230	0.087	0.021
	232	0.069	0.075
	234	0.112	0.112
	236	0.377	0.154
	238	0.116	0.059
	240	—	0.027
	242	—	0.021
	244	—	0.176
	248	—	0.053
	250	0.178	0.011
	252	0.044	0.016
	254	0.007	0.032
	256	0.011	0.005
	258	—	0.016
	260	—	0.032
	262	—	0.005
	264	—	0.005
Spl36	345	—	0.016
	347	—	0.037
	349	—	0.085
	351	0.327	0.053
	353	0.302	0.420
	355	0.036	0.101
	357	0.054	0.048
	359	—	0.064
	361	0.011	0.027
	363	—	0.005
	367	0.223	0.048
	369	0.014	0.005

Table 1. Continued.

Locus	Allele	Frequency	
		Pallid	Shovelnose
	371	0.007	0.016
	373	0.025	0.037
	377	—	0.005
	383	—	0.005
	435	—	0.005
	437	—	0.016
	439	—	0.005
Spl40	213	—	0.005
	215	—	0.027
	219	0.040	0.075
	221	0.051	—
	223	—	0.048
	225	—	0.080
	227	0.294	0.218
	229	0.196	0.090
	231	0.123	0.330
	233	0.239	0.075
	235	0.058	0.016
	239	—	0.005
	241	—	0.021
	243	—	0.011
Spl56	177	—	0.059
	201	—	0.005
	203	—	0.122
	207	—	0.005
	209	—	0.154
	211	0.022	0.080
	215	—	0.106
	217	—	0.005
	219	—	0.037
	221	—	0.011
	223	0.083	0.075
	225	0.108	0.075
	227	0.302	0.048
	229	0.425	0.213
	231	0.061	0.005
Spl60	151	0.004	—
	191	0.007	—
	197	0.007	—
	199	0.460	0.356
	201	0.417	0.612
	203	0.104	0.027
	207	—	0.005
Spl101	269	0.040	0.085
	277	0.032	0.197
	281	0.133	0.410
	285	0.245	0.075
	289	0.464	0.207
	293	0.086	0.016
	297	—	0.011
Spl105	121	0.127	0.016
	125	0.033	0.022
	133	0.540	0.532
	137	0.257	0.398
	141	—	0.005
	145	0.036	0.027
	149	0.007	—
Spl106	210	—	0.005
	214	0.091	0.053
	218	0.007	0.085
	222	0.286	0.229
	226	0.018	0.101
	230	0.493	0.229

Table 1. Continued.

Locus	Allele	Frequency	
		Pallid	Shovelnose
	234	0.073	0.223
	238	0.007	0.016
	242	—	0.005
	246	—	0.037
	250	0.015	0.011
	254	0.011	—
	266	—	0.005
Spl119	228	0.304	0.096
	244	—	0.005
	248	0.051	0.016
	252	—	0.080
	256	0.109	0.277
	260	0.402	0.367
	264	—	0.133
	268	0.134	0.021
	272	—	0.005
Spl158	195	—	0.149
	199	—	0.005
	203	0.148	0.117
	207	0.014	0.165
	211	0.040	0.021
	215	0.266	0.255
	219	0.004	0.075
	223	0.281	0.069
	227	0.212	0.064
	231	0.036	0.080
Spl173	202	0.004	0.011
	206	0.321	0.032
	210	0.004	0.156
	214	0.037	0.108
	218	0.627	0.307
	222	0.004	0.108
	226	0.004	0.269
	230	—	0.011

— = No allele of this size was observed.

We categorized fish as hybrids between the two species if they had a q value between 0.02 and 0.98. In part two of our analysis, we used STRUCTURE in the same way as part one but analyzed each species separately for evidence of population structure. In all cases, we identified the most likely K for each analysis using the method of Pritchard et al. (2000) if the posterior probability of $K = 1$ was the highest and using the ΔK method of Evanno et al. (2005) if the highest posterior probability of K was > 1. For each STRUCTURE analysis, we generated summary data for ΔK and mean and variance of posterior probabilities for each K using STRUCTURE HARVESTER (Earl and Von Holdt 2012).

Within and between population diversity

We used the program HP-RARE 1.0 (Kalinowski 2005) to estimate the average population gene diversities (H_E) and allelic richness (A_R) for each microsatellite locus. To evaluate conformance of genotypic frequencies to Hardy–Weinberg equilibrium, we used the methods of Guo and Thompson (1992) via the program GENEPOP 3.4 (Raymond and Rousset 1995). We used GENEPOP 4.1.2 to

test for linkage disequilibrium at all pairs of loci in each sample using 10,000 dememorizations, 10,000 batches, and 10,000 iterations per batch. We used a sequential Bonferroni correction (Rice 1989) to adjust the statistical significance levels used to detect deviations from Hardy–Weinberg equilibrium or genotypic equilibrium to account for multiple tests. We estimated genetic differences between Pallid Sturgeon and Shovelnose Sturgeon in FSTAT 2.9.3 (Goudet 1995) using the F-statistic F_{ST} (Weir and Cockerham 1984) and generated 95% confidence bounds by bootstrapping over loci.

Effective population size

We estimated the historical N_e of Pallid Sturgeon and Shovelnose Sturgeon populations in the entire upper Missouri River basin that existed thousands of years ago using the heterozygosity-based methods of Ohta and Kimura (1973) and Hartl and Clark (1989). The main difference between these two methods is that Ohta and Kimura (1973) assume a stepwise mutation model (SMM), and Hartl and Clark (1989) assume an infinite allele mutation model (IAM). Both methods assume selective neutrality and closed populations and predict that at mutation-drift equilibrium, N_e is a function of H_E. We used the most commonly applied microsatellite muta-tion rate for fishes, $\mu = 5 \times 10^{-4}$ (Estoup and Angers 1998). Theoretically, true long-term N_e should fall between $\hat{N}_{eSMM}$ and $\hat{N}_{eIAM}$ because these two models represent the extremes of the mutation process for microsatellite loci.

We estimated contemporary N_e of each population using the linkage disequilibrium method of Waples (2006) implemented in the program NeEstimator 2 (Do et al. 2014). This method provides an estimate of the effective number of breeding adults that parented the sampled population. For the linkage disequilibrium method, we excluded all alleles with frequencies less than 0.02 (i.e., $P_{crit} = 0.02$, as recommended by Waples and Do [2010]) and used the jackknife procedure to estimate the confidence intervals associated with the point estimates of effective population size (N_{eLD}) for each population.

Bottleneck tests

We screened Pallid Sturgeon and Shovelnose Sturgeon samples for genetic signatures of recent bottlenecks using the heterozygosity excess method of Cornuet and Luikart (1996). This method is sensitive to recent bottlenecks (0.2–4.0 N_e generations), and we expected that Pallid Sturgeon would exhibit signs of a bottleneck using this test if human activities over the past 150 y have caused a dramatic reduction in population size. We used the program BOTTLENECK v 1.2.02 (Piry et al. 1999) to test for heterozygosity excess using a two-phase model of microsatellite mutation with parameter settings of 95% SMM, 5% IAM, and 12% variance in multistep mutations. We determined significance of heterozygosity excess observed in a population using a one-sided Wilcoxon's signed-rank test ($\alpha = 0.05$) that compared levels of the observed deviation from the null hypothesis of 50:50 heterozygosity deficiency excess ratio based on 5,000 simulation iterations. We also examined the allele frequency distribution over all loci to test for signs of a population bottleneck using the methods of Luikart et al. (1998). This allele frequency mode-shift test is based on the premises that populations that have not experienced a recent bottleneck have a distribution of alleles where the majority of alleles are at frequencies less than 0.1, whereas bottlenecked populations have a shifted allele frequency distribution in which many alleles are at a frequency of >0.1. Finally, we used the methods of Garza and Williamson (2001) to test for evidence of bottlenecks based on the relationship between the number of alleles (k) to the allele size range (r), as calculated by the M ratio using the program M_P_VAL. Because k is reduced faster than r in recently bottlenecked populations, we expect the M ratio (k/r) to be small in a recently bottlenecked population. We assumed a model of microsatellite evolution of 88% one-step mutations (pg) and 2.8 average-sized non-one-step mutations (Δ_g; Garza and Williamson 2001). We used prebottlenecked population sizes of $N_e = 500$ and 5,000 and a microsatellite mutation rate of $\mu = 5 \times 10^{-4}$ to estimate θ ($=4N_e\mu$). We used the program Critical_M (Garza and Williamson 2001) to test for statistical significance of the observed M ratio for Pallid and Shovelnose Sturgeon, with the critical value (M_c) identified using 10,000 simulations of an equilibrium population.

Bayesian model for inferring demographic histories of populations

We used the hierarchical Bayesian model of Storz and Beaumont (2002) to test for genetic evidence of population expansion and contraction using the program MSVAR 1.3 to determine if population declines were the result of historic events, such as expansion into new habitat, or recent events, such as habitat loss due to dam construction. This model estimates the demographic history of a closed population over the time interval x_a using observed microsatellite allele frequencies, mutational rate and demographic priors, and coalescence of microsatellite alleles based on number of tandem repeats. The time interval is more specifically defined as $x_a = g \times t_a$, where g is the generation length, and t_a is the number of generations over which the population has been changing is size. Population size (N) at time x is defined as

$$N_x = N_0 \left(\frac{N_1}{N_0}\right)^{x/x_a} \quad (1)\leftarrow$$

with an initial population size of N_1, current population size of N_0, and time increasing into the past. An SMM with a mutation rate μ is assumed by MSVAR 1.3 for microsatellite loci. We used Beaumont's (1999) Bayesian coalescent-based approach to infer the model parameters $\Phi = \{N_0, N_1, x_a, \mu\}$ via a Markov chain Monte Carlo

approach that samples from estimated posterior distributions of these parameters given prior densities and allele frequency data. Each of the four parameters is inferred separately and independently across loci.

We calculated parameters for Φ from a lognormal distribution

$$p(\Phi|M,V) \quad (2)$$

with means $M = \{M_{N0}, M_{N1}, M_{xa}, M_{\mu}\}$ and variances of $V = \{V_{N0}, V_{N1}, V_{xa}, V_{\mu}\}$. We used a set of hyperprior parameters, H, to update M and V at each locus during the Markov chain Monte Carlo run. We assumed all hyperprior parameters to be specified by normal distributions with means α_{N0}, α_{N1}, α_{xa}, α_{μ} and variances σ_{N0}, σ_{N1}, σ_{xa}, σ_{μ}. Distributions for priors of the variances were normal distributions truncated at 0 with means β_{N0}, β_{N1}, β_{xa}, β_{μ} and variances τ_{N0}, τ_{N1}, τ_{xa}, τ_{μ}. We used distributions of H to calculate the probability density $p(M, V \mid H)$. The posterior distributions of M and V were estimated using a Metropolis–Hastings simulation, as described in Storz and Beaumont (2002).

Generation length (g) is defined as the average age of parents of individuals in a cohort of offspring. Because of limited data on age structure for *Scaphirhynchus* species, we estimated the generation length for each species as age at first reproduction + 1/natural mortality rate (IUCN 2019). We assumed a stable age structure with an earliest age of maturity (averaged over both sexes) of 10 for Pallid Sturgeon (Keenlyne and Jenkins 1993) and 5 for Shovelnose Sturgeon (Keenlyne 1997). We assumed the annual mortality rate for both species to be 5% for adults after reaching sexual maturity (Braaten et al. 2009; Keenlyne 1997). Our estimate for g was 30 y for Pallid Sturgeon and 25 y for Shovelnose Sturgeon.

We used broad "uninformative" priors with large variances to affect posterior distributions as little as possible. To better gauge the influence of the priors on the posterior distributions, we conducted a preliminary analysis with independent runs of the model using four different prior parameter configurations (Text S2, *Supplemental Material*). Estimates for M were very different from the priors, indicating that the genetic signal for all four parameters are very strong (Figure S1, *Supplemental Material*).

We used prior parameter set 2 for the final analysis of Pallid and Shovelnose Sturgeon (Table S1, *Supplemental Material*) datasets. Final analysis for each species included data from all 17 loci, and we based it on two independent Markov chain Monte Carlo chains that were each run for 8×10^8 steps for Pallid Sturgeon and 4×10^9 steps for Shovelnose Sturgeon. We recorded parameter values from the prior distribution every 4×10^5 steps for Pallid and 2×10^6 steps for Shovelnose to obtain 20,000 draws from the posterior distribution of each chain. We used the Gelman–Rubin statistic (calculated in R using the CODA module) to assess the convergence of the chains. As with the preliminary analysis, we combined the last half of each chain to produce an overall set of 20,000 points for each species, and we used the Locfit module for density estimation. We used a random subsample of 100 Pallid Sturgeon from the overall dataset for the preliminary and final analysis. We had to reduce the number of fish to 100 because this was the maximum number of individuals that MSVAR can accommodate.

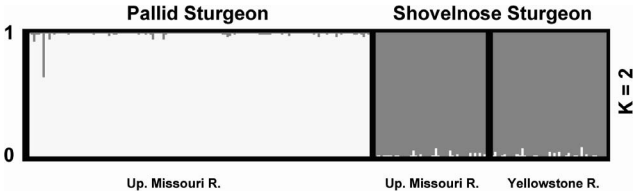

Figure 2. Analysis of genetic population structure for 140 Pallid *Scaphirhynchus albus* and 94 Shovelnose Sturgeon *S. platorynchus* that we sampled from locations in the upper Missouri River Basin. The plot corresponds to the results from an unsupervised Bayesian clustering method assuming the number of genetic clusters $K = 2$. The plot displays mean individual membership of all fish into each of the two clusters. Clusters are represented by different colors, and each fish is represented as a vertical line fractionally allocated into the two genetic clusters. We grouped fish by species identification when sampled in the field and then by location captured. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

Results

Inheritance of microsatellite loci in Pallid Sturgeon

Chi-squared tests showed that progeny genotypic proportions for each of the six full-sib families conformed to Mendelian expectations. None of the parents showed linkage associations, with a logarithm of the odds (LOD) score greater than 3.0 in all pairwise tests. Informative parents for detecting pairwise linkage and mode of segregation are listed by locus in Table S2, *Supplemental Material*.

Genetic population structure

The STRUCTURE analysis provided strong support for reproductive isolation between Pallid and Shovelnose Sturgeon in the upper Missouri River (Figure 2). In part one of the STRUCTURE analysis, where we included both species, we identified $K = 2$ as the most likely number of genetic clusters, with the mean posterior probability of the data peaking at $K = 2$ and a ΔK value for $K = 2$ over 1,000 times higher than other K values (Table S3, *Supplemental Material*). Evaluation of the assignment of individuals to clusters had a clear biological interpretation at a K of 2, with phenotypic identification of species matching the genetic membership to a species. One field-identified Pallid Sturgeon had a q value of 0.70 (Figure 2), indicating that it could be a hybrid between Pallid and Shovelnose Sturgeon; this fish was excluded from the dataset. Conversely, part two of the STRUCTURE

analysis provided no support for multiple genetic clusters when we analyzed Shovelnose and Pallid Sturgeon separately, with $K = 1$ having the highest mean posterior probability for Shovelnose Sturgeon and mean posterior probabilities peaking from $K = 1$ to 3 for Pallid Sturgeon with no peak in ΔK for values from 2 to 5 (Table S3, *Supplemental Material*). Based on these results, we refer to Shovelnose Sturgeon and Pallid Sturgeon sampled in this study as belonging to single random mating populations. The level of genetic differentiation that we observed between Pallid and Shovelnose Sturgeon, as determined by F_{ST}, was 0.10 (95% CI of 0.08–0.13).

Genetic diversity within populations and bottleneck tests

We observed moderate to high levels of genetic diversity in Shovelnose and Pallid Sturgeon across all 17 microsatellite loci (Table 1; Data S1, *Supplemental Material*). Expected heterozygosity (H_E) over all loci was 0.77 for Shovelnose Sturgeon and 0.68 for Pallid Sturgeon. Average number of alleles per locus (N_A) was 11.82 for Shovelnose Sturgeon and 7.82 for Pallid Sturgeon. Shovelnose Sturgeon had an allelic richness (A_R^{170}) of 11.64 (range over loci of 3.90–25.00), and Pallid Sturgeon had an average A_R^{170} of 7.34 (range over loci of 4.98–16.97; Figure 3A). The average number of private alleles that we observed per locus was 4.74 in Shovelnose Sturgeon and 0.47 for Pallid Sturgeon, with the number of private alleles observed in Pallid Sturgeon decreasing as we sampled more Shovelnose and Pallid Sturgeon (Figure 3B). Allele size distributions for Pallid and Shovelnose Sturgeon overlapped extensively, but Pallid Sturgeon had more gaps in observed allele sizes over all loci (Table 1). Both Pallid and Shovelnose Sturgeon conformed to Hardy–Weinberg equilibrium at all loci. We observed significant linkage disequilibrium after Bonferroni correction at 1 of 136 pairs of loci for Pallid Sturgeon, and we observed no significant linkage disequilibrium for Shovelnose Sturgeon. We found no support ($P > 0.05$) for a recent bottleneck in both Pallid and Shovelnose Sturgeon using the heterozygosity excess or allele frequency mode-shift tests (Table 2). The M ratio test indicated evidence of genetic bottlenecks for both Pallid and Shovelnose Sturgeon, with the M ratio less than the critical value at $\theta = 1$ and 10 for both species (Table 2).

Effective population size

Point estimates of historical N_e were higher for Shovelnose Sturgeon based on the IAM and SMM models. However, the range of estimated historical N_e estimates do overlap when considering the IAM and SMM as lower and upper bounds with Pallid Sturgeon ranging from $\hat{N}_{eIAM} = \infty$ to $\hat{N}_{eSMM} = \infty$ and Shovelnose Sturgeon ranging from $\hat{N}_{eIAM} = \infty$ to $\hat{N}_{eSMM} = 4{,}407$ (Table 2). Contemporary $\hat{N}_{eLD}$ for Pallid Sturgeon was 254 (95% CI of 190–369), and Shovelnose Sturgeon had a larger contemporary $\hat{N}_{eLD}$ estimate of 2,983 (95% CI of 692–∞; Table 2).

Storz and Beaumont model of demographic change

We observed good convergence between independent Markov chain Monte Carlo simulations with the 0.975 quantile for Gelman–Rubin statistics below 1.2 for all parameter values (Storz and Beaumont 2002). Pronounced population decline was the most recent demographic event that we identified for Pallid and Shovelnose Sturgeon. The Bayes factor in favor of population decline was 20,000 (all 20,000 point estimates had the historic size greater than the current size) for both species. We report results in $\log_{10}$ scale to facilitate comparison with tables and figures that also report results in $\log_{10}$ scale. Unscaled results are reported in parentheses. For Pallid Sturgeon, the median of the joint distribution was $M_{N0} = 2.74$ (550) and $M_{N1} = 4.02$ (10,471), indicating a 19-fold or 95% contraction in population size (Table 3; Figure 4A). Population decline for Shovelnose Sturgeon was also severe but slightly less than that estimated for Pallid Sturgeon, with a median joint distribution of $M_{N0} = 3.24$ (1,738) and $M_{N1} = 4.23$ (16,982), indicating a 10-fold or 90% contraction in population size (Table 3; Figure 4B). Posterior distributions for time at which Pallid and Shovelnose Sturgeon started to decline (M_{xa}) overlapped to a large extent with a median value of $M_{xa} = 4.48$ (30,200) y before present (YBP) for Pallid Sturgeon and $M_{xa} = 4.36$ (23,120) YBP for Shovelnose Sturgeon (Table 3; Figure 5). The posterior distributions of M_μ were –3.14 (7.24×10^{-4}) and –3.25 (5.62×10^{-4}) for Pallid and Shovelnose Sturgeon, respectively (Table 3); both of these estimates were close to the prior of –3.3 (5.00×10^{-4}) used for M_μ in both species models.

Discussion

Our results provide a good example of how population genetic data can be used to infer critical demographic and evolutionary information for an imperiled species. Here, we discuss how careful interpretation of demographic and evolutionary history inferred from microsatellite DNA data can be integrated into critical conservation issues. This study specifically addresses the following conservation issues for Pallid Sturgeon: 1) evaluating the level of genetic diversity that remains in the GPMU, 2) setting demographic goals for recovery, 3) managing the captive breeding program, and 4) understanding historic and current evolutionary relationships between Pallid and Shovelnose Sturgeon.

Contemporary levels of genetic diversity within and among species

We identified two genetic clusters by Bayesian analysis of population genetic structure that clearly differentiated Pallid and Shovelnose Sturgeon in the upper Missouri River (Figure 2). All but one fish assigned with high probability to a species, indicating that hybridization

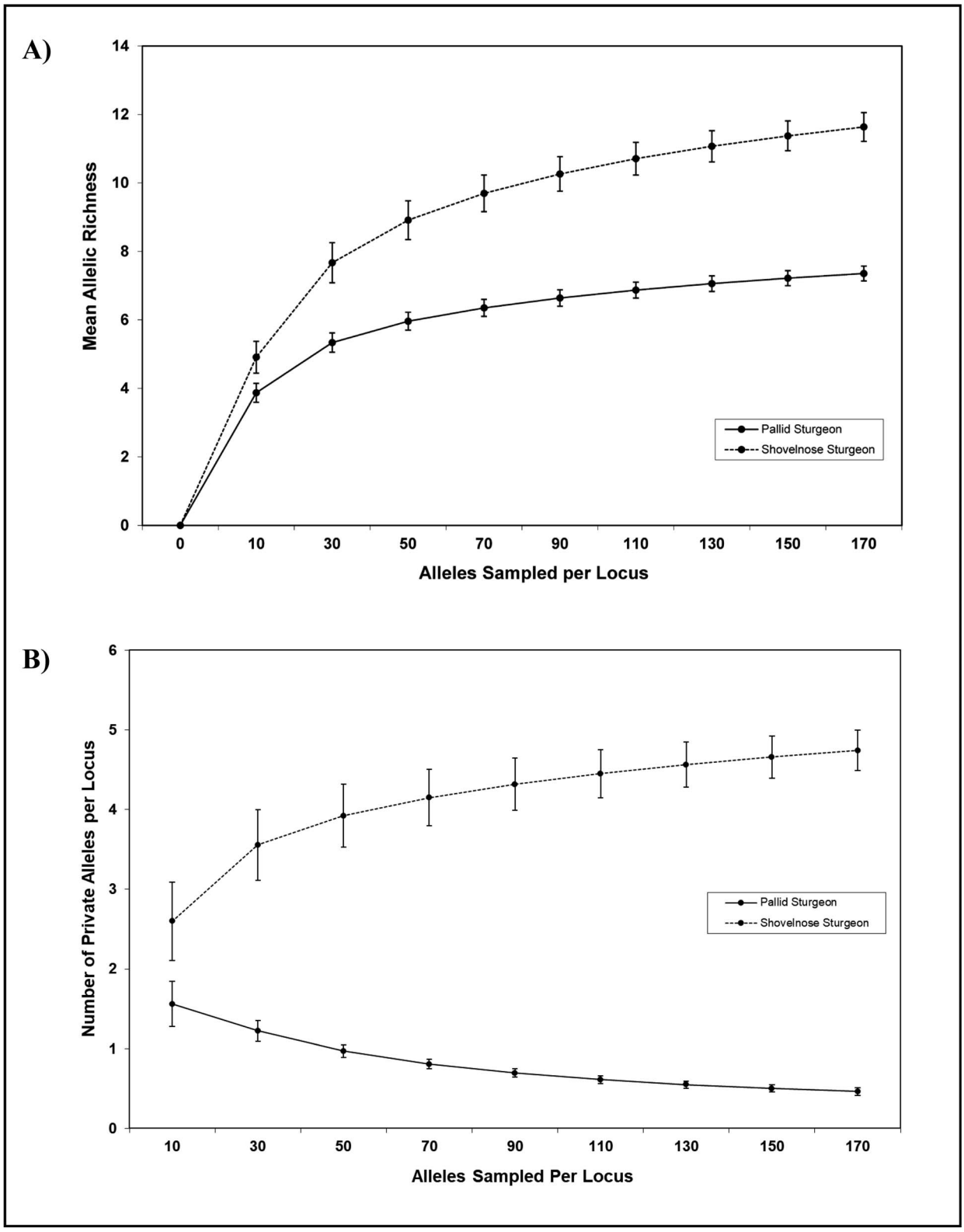

Figure 3. Genetic diversity of Pallid Sturgeon *Scaphirhynchus albus* and Shovelnose Sturgeon *S. platorynchus* from the upper Missouri River Basin. (**A**) Allelic richness observed as a function of the number of alleles sampled. (**B**) Number of private alleles (found only in one species) that we observed as a function of the number of alleles sampled. We report estimates as mean and standard error across 17 microsatellite loci. We based the estimates of the number of private alleles observed on nine pairwise comparisons between these two species in which we sampled 10 to 170 alleles per species. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

Table 2. Estimates of effective population size and results of genetic bottleneck tests based on heterozygosity excess test, allele frequency mode-shift analysis, and M ratio for Pallid *Scaphirhynchus albus* and Shovelnose Sturgeon *S. platorynchus* from the upper Missouri River basin. We estimated long-term effective population sizes with heterozygosity-based methods assuming either a stepwise mutation model (SMM; N_{eSMM}) or infinite allele mutation model (IAM; N_{eIAM}), which we presume to bound the true long-term effective population size (N_e). Contemporary N_e was based on the linkage disequilibrium estimator (N_{eLD}) of Waples (2006). An estimated M ratio $<$ the critical value (M_c) indicates a bottleneck. Theta (θ) values of 1 and 10 correspond to prebottleneck effective population sizes of 500 and 5,000, respectively, assuming a microsatellite mutation rate of 5×10^{-4}. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

	Effective population size			Bottleneck tests				
	Long term		Contemporary	Moment based		M ratio test		
Species	$\hat{N}_{eIAM}$	$\hat{N}_{eSSM}$	$\hat{N}_{eLD}$ (95% CI)	Heterozygote excess	Allele frequency distribution	M ratio	M_c, $\Theta = 1$	M_c, $\Theta = 10$
Pallid Sturgeon	1,070	2,214	254 (190–369)	NS	Norm	0.711	0.840***	0.815***
Shovelnose Sturgeon	1,658	4,407	2,983 (692–∞)	NS	Norm	0.793	0.836**	0.805*

95% CI = 95% confidence interval; NS = Not Significant; Norm = Normal Distribution
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$

between Pallid and Shovelnose Sturgeon is rare in this portion of the species range. We observed no evidence for population genetic structure within either species (Table S3, *Supplemental Material*).

Only the M ratio bottleneck test detected genetic bottlenecks in both species. The heterozygosity excess and allele frequency mode-shift tests did not detect evidence of genetic bottleneck in either species (Table 2). Differences in results among these three tests were likely because of differences in the power of each test to detect declines and time periods over which these methods apply. Models used for heterozygosity excess and allele frequency mode-shift tests are more sensitive to detecting recent bottlenecks than the M ratio, which is more sensitive to detecting historical bottlenecks (Spear et al. 2006). However, these three bottleneck tests do not provide estimates of when the bottleneck occurred. Fortunately, the results from the MSVAR analysis did provide an estimate of the timing of genetic bottleneck for both species occurring approximately 30,000 YBP (Figure 5; Table 3), which is consistent with a historical bottleneck that was only detected by the M ratio test.

Table 3. Modes and quantiles of the posterior distribution of current effective population size $\log_{10}$ (M_{N0}), initial effective population size $\log_{10}$ (M_{N1}), microsatellite mutation rate $\log_{10}$ (M_μ), and time at which the species started a population decline $\log_{10}$ (M_{xa}) for Pallid Sturgeon *Scaphirhynchus albus* and Shovelnose Sturgeon *S. platorynchus* from the upper Missouri River basin based on the hierarchical Bayesian model of Storz and Beaumont (2002). All estimates are reported on a $\log_{10}$ scale to facilitate comparison to Figures 4 and 5. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon from May to July of 2005.

		Quantile		
Parameter	Mode	2.5%	50%	97.5%
Pallid				
M_{N0}	2.86	1.86	2.74	3.63
M_{N1}	3.95	3.11	4.02	4.94
M_μ	− 3.16	− 4.01	− 3.14	− 2.28
M_{xa}	4.49	3.57	4.48	5.42
Shovelnose				
M_{N0}	3.17	2.25	3.24	4.22
M_{N1}	4.20	3.22	4.23	5.25
M_μ	− 3.23	− 4.17	−3.25	− 2.33
M_{xa}	4.38	3.23	4.36	5.92

A lack of evidence for a recent genetic bottleneck for Pallid Sturgeon is consistent with adults sampled in this study coming from a large population not affected by dams and a gradual population decline after dam construction in the upper Missouri River. Also, genetic diversity observed in Pallid Sturgeon (H_E =~~←~~0.68) exceeded the mean value (H_E = 0.46) that DeWoody and Avise (2001) observed in a meta-analysis of 78 freshwater fish species. Absence of a genetic bottleneck signal and the high level of genetic diversity that we observed in this study stand in stark contrast to the current demographic bottleneck Pallid Sturgeon are experiencing due to the absence of natural recruitment in the upper Missouri River over the past 60 y. Retention of genetic diversity in the remnant Pallid Sturgeon population is likely due to the buffering effect of long generation time, presumably larger parental population of the fish we sampled, a gradual demographic decline, and occasional but rare hybridization with Shovelnose Sturgeon. Long generation times can reduce the loss of genetic diversity via a reduction in the pace of genetic drift and inbreeding, both of which act on a generational time scale. These results were consistent with other species of North American sturgeon, which appeared to retain genetic diversity despite significant decreases in population size (King et al. 2001; Israel et al. 2004; DeHaan et al. 2006; Waldman et al. 2019). Studies of other relatively long lifespan fish, such as the Razorback Sucker *Xyrauchen texanus* and Copper Redhorse *Mox-*

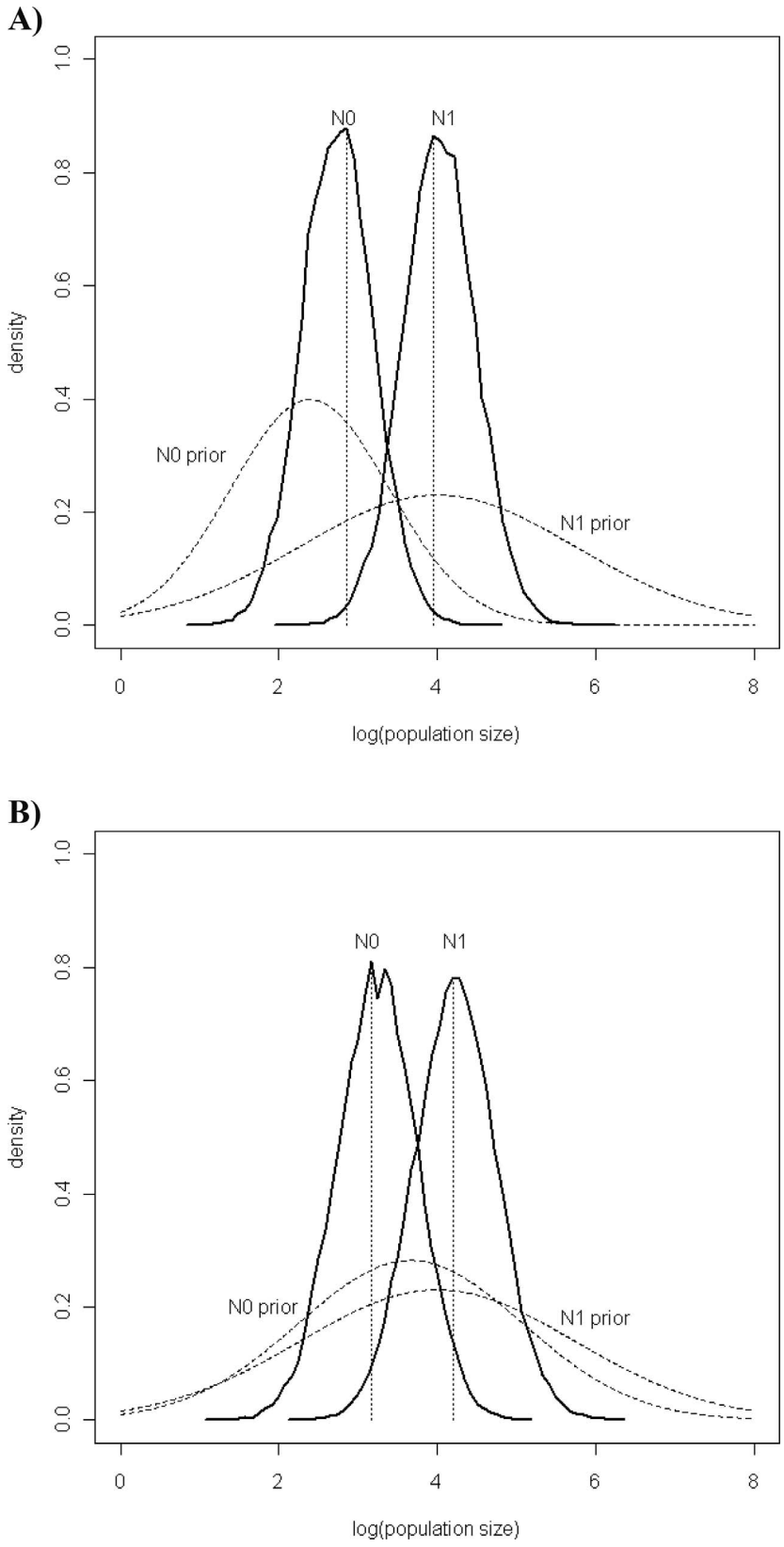

Figure 4. Estimated historic $\log_{10}$ (N_1) and present $\log_{10}$ (N_0) population sizes for Pallid Sturgeon *Scaphirhynchus albus* (**A**) and Shovelnose Sturgeon *S. platorynchus* (**B**) in the upper Missouri River Basin. Thick lines represent the posterior distributions for $\log_{10}$ (N_1) and $\log_{10}$ (N_0) with two independent Markov chain Monte Carlo simulations based on the hierarchical Bayesian model of Storz and Beaumont (2002). Priors for the simulations are shown as dotted lines. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

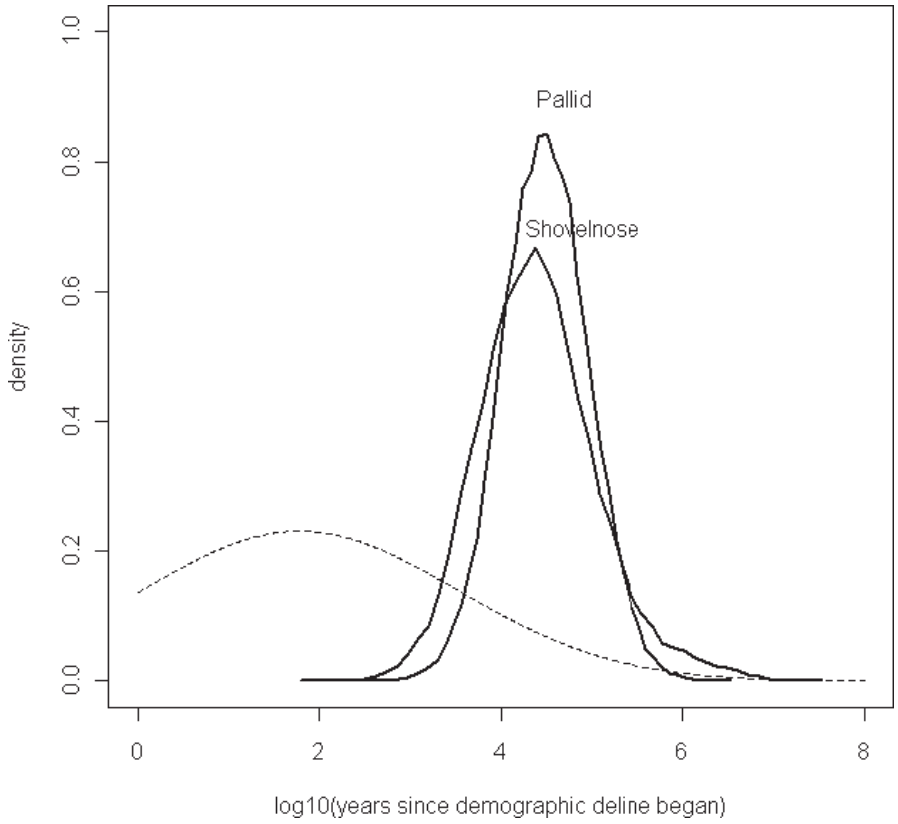

Figure 5. Estimated time when population declines started, $\log_{10}(M_{xa})$, for Pallid *Scaphirhynchus albus* and Shovelnose Sturgeon *S. platorynchus* in the upper Missouri River Basin. Thick lines represent the posterior distributions for $\log_{10}(M_{xa})$ with two independent Markov chain Monte Carlo simulations based on the hierarchical Bayesian model of Storz and Beaumont (2002). Priors for the simulations are shown as dotted lines. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

ostoma hubbsi, also revealed opportunities to conserve genetic diversity that characterized these species before major population declines occurred (Dowling et al. 2005; Lippe et al. 2006).

We also observed high levels of genetic diversity ($H_E = \leftarrow$ 0.77) and no evidence of a genetic bottleneck in Shovelnose Sturgeon, which is consistent with the large adult census size estimated for this population, as indicated by length frequency and catch per unit effort data (Haddix et al. 2009). Shovelnose Sturgeon had an average of four private alleles per locus and twice the number of alleles per locus observed in Pallid Sturgeon (Figure 3). Large current N_e and high levels of genetic diversity in Shovelnose Sturgeon are more likely the result of a large population size that is able to successfully recruit in the upper Missouri River rather than a long generation time buffering the rate of genetic diversity loss.

Historical patterns in genetic diversity

The low number of alleles and small long-term estimates of N_e, based on H_E, suggest that Pallid Sturgeon have been at lower N_e than Shovelnose Sturgeon for long periods of time, likely thousands of years. Both $\hat{N}_{eIAM}$ and $\hat{N}_{eSMM}$ were highest in Shovelnose Sturgeon (Tables 1 and 2). These estimates of long-term N_e are best interpreted as the harmonic mean of N_e for these two species because they recolonized the upper Missouri River approximately 15,000 y ago (Cross et al. 1986). Similar estimates of long-term N_e based on H_E compared with contemporary estimates of N_e based on linkage disequilibrium suggest that Shovelnose Sturgeon have been at a stable abundance in the upper Missouri River over the past 15,000 y (Table 2). By contrast, it appears that Pallid Sturgeon underwent additional reductions in N_e since recolonization of the upper Missouri River, with contemporary estimates of $\hat{N}_{eLD}$ that are 5 to 10 times lower than long-term N_e estimates based on H_E (Table 2). We suspect that this reduction in N_e for Pallid Sturgeon is recent because long-term N_e estimates based on H_E reflect the harmonic mean of N_e over the entire time period, and the harmonic mean is sensitive to low N_e in recent generations.

Setting recovery goals for Pallid Sturgeon

The revised Pallid Sturgeon recovery plan established an interim recovery goal of 5,000 adults for two generations in each of the four management units (USFWS 2014). Because of a lack of genetic, life history, and habitat data for Pallid Sturgeon, this recovery goal

was based on general guidelines established for maintaining genetic variation of an N_e of >500 (Jamieson and Allendorf 2012), an N_e/N of 0.1 (Frankham 1995), and a minimum viability analysis review of 102 vertebrate taxa (Reed et al. 2003). Authors of the revised recovery plan recognized caveats associated with using general guidelines for setting recovery goals (reviewed in Flather et al. 2011) and identified the need to refine the 5,000 adult interim recovery goal using Pallid Sturgeon data when available (USFWS 2014). We acknowledge that using N_e to set recovery thresholds for N needed to maintain genetic diversity is complex (Frankham et al. 2021). However, recent overviews by Laikre et al. (2020, 2021) concluded that the general rule of $N_e > 500$ or $N > 5{,}000$ is robust when N_e/N ratios are not available for a species. Laikre et al. (2021) also highlight that using a population- or species-specific N_e/N and an N_e of 500 will provide the most robust threshold for N needed to maintain long-term genetic diversity.

Braaten et al. (2009) used back estimation age models to estimate Pallid Sturgeon $\hat{N}$ in the upper Missouri River 60 y ago as 968 (95% CI of 790–1,182). We estimated the $\hat{N}_{eLD}$ for this same cohort of Pallid Sturgeon as 254 (95% CI of 190–369), giving us an estimate of $\hat{N}_{eLD}/\hat{N}$ for the upper Missouri River Pallid Sturgeon of 0.26 (95% CI of 0.16–0.47). If we keep the same goal of $N_e > 500$ identified in the revised recovery plan and incorporate the $\hat{N}_{eLD}/\hat{N}$ ratio for Pallid Sturgeon, the requirements of a minimum N capable of maintaining adaptive genetic variability long term is 1,925 (95% CI of 1,065–3,125) adults in the GPMU (Figure 1). In addition, we used the point estimate of 0.26 for $\hat{N}_{eLD}/\hat{N}$ to estimate the upper and lower confidence bounds of historic N (i.e., harmonic mean of N over the past 15,000 y). Our estimate of historic $\hat{N}$ was 6,322, which is the midpoint between upper and lower confidence-bound estimates of 4,120–8,524 based on $\hat{N}_{eIAM} =$ 1,070 and $\hat{N}_{eSMM} =$ 2,214, respectively.

Based on our results, we recommend a recovery goal for N of 1,925–6,322 Pallid Sturgeon in the GPMU. Reaching this recovery goal provides a high probability of maintaining adaptive genetic variation in the population (i.e., $N_e > 500$) and potentially recovering the species to long-term N levels estimated over historic time periods. The range of N for this recommended recovery goal includes the current interim recovery goal of 5,000. Having estimates of historic N provides important context when evaluating the current status and resiliency of a species (Waples et al. 2007). However, as a general rule, using estimates of historic N as a recovery goal is not recommended because current and future conditions have changed, such as amount of available habitat, climate change, or invasive species impacts. In the future, we recommend development of a more refined recovery goal for Pallid Sturgeon in the GPMU based on population viability analysis informed by population growth rates, reproductive success, genetic diversity, and habitat availability for hatchery fish that are becoming sexually mature in the wild (Holmquist et al. 2019). Ultimately, a species status assessment for Pallid Sturgeon will allow for a comprehensive evaluation of the three Rs (resiliency, redundancy, and representation) associated with updating recovery goals for the species over the entire native range (Smith et al. 2018).

Genetic management of Pallid Sturgeon captive breeding program

A captive breeding program is currently the only source of juvenile Pallid Sturgeon in the upper Missouri River. Since 1997, natural-origin adults are captured from the upper Missouri River and transferred to one of four fish hatcheries (Figure 1), where they are artificially mated and returned to the wild. Managers of this captive breeding program have overcome many problems associated with Pallid Sturgeon culture, including disease outbreaks, limited number of broodstock, and lack of information on breeding and culture methods for this endangered species (Webb et al. 2016). In the short term, barring stochastic events in any given production year, these hatcheries can produce enough juvenile fish to reach or exceed the N goal of 1,925 to 6,322 fish that we have recommended based on the results presented in this paper (USFWS 2008).

Efforts to maintain Pallid Sturgeon genetic diversity include development of a genetic management plan (Heist et al. 2013), cryopreservation of sperm from natural-origin males (Wayman et al. 2008), and maintaining a captive population, containing individuals from all captive bred families produced since 2001 at Gavins Point National Fish Hatchery. These proactive efforts have maintained genetic diversity and minimized inbreeding in the captive breeding program (Saltzgiver et al. 2012). However, managers are facing a new challenge as the wild population becomes extirpated, leaving only captive broodstock and hatchery-origin fish captured in the wild to use as broodstock.

Evolutionary relationships between Pallid and Shovelnose Sturgeon

Details concerning the origins of Pallid and Shovelnose Sturgeon have been a topic of active research over the past 20 y (see discussion in Campton et al. [2000]). Pallid and Shovelnose Sturgeon in the upper Missouri River are distinguishable based on a number of traits, including genetics, morphology, biogeographic distribution, and ecological preferences (USFWS 2014). The level of genetic distinction ($F_{ST} =$ 0.10) that we observed between sympatric Pallid and Shovelnose Sturgeon in this study was twice the global F_{ST} of 0.05 observed by Schrey and Heist (2007) among Pallid Sturgeon sampled over the species range using many of the same microsatellite loci that we examined in this study. However, the genetic similarity of these two species suggests that this species pair has recently diverged at these traits.

Our results suggest that Pallid and Shovelnose Sturgeon both experienced extreme population declines approximately 30,000 YBP (Figure 5; Table 3). The timing of this population decline is similar to the estimated date of divergence of 40,000–60,000 YBP between this species pair (Campton et al. 2000). We believe concordant results for time of population decline and similar estimates of large ancestral population size suggests that historical populations of Pallid and Shovelnose Sturgeon characterized by MSVAR analysis likely represent a single protospecies of *Scaphirhynchus* that occurred before these two species diverged. The historic decline documented by MSVAR could therefore simply be the result of processes that caused a decline in the protospecies during the last glacial epoch that also promoted isolation and speciation. Complete overlap in microsatellite allele size at all 17 microsatellite loci (Table 1) is also consistent with the observed decline in these populations being the result of a speciation event that likely occurred during the late Pleistocene.

Glacial refugia in the lower Mississippi River provided an opportunity for allopatric speciation 12,000 to 70,000 YBP, when the upper Missouri River was the only portion of the current Pallid Sturgeon range covered by glaciers (Cross et al. 1986). Subsequent recolonization of the upper Missouri River after glaciers receded provided a unique ecological setting for these newly formed species. Our results are consistent with reinforcement of morphological and ecological preferences that distinguish Pallid and Shovelnose Sturgeon in the upper Missouri River. For example, Pallid Sturgeon in this area are more piscivorous (Gerrity et al. 2006) and occur in swifter water than Shovelnose (Bramblett and White 2001). However, the degree to which the ecological setting reinforces assortative mating between these species in the Mississippi River is less clear (Jordan et al. 2019), and differences in ecological setting are likely to be a major factor in the increased presence of morphological intermediates in areas outside of the upper Missouri River, such as the Atchafalaya River (Allendorf et al. 2001).

Opportunity for recovery

There is hope for restoration of Pallid Sturgeon natural recruitment to the upper Missouri River. Fish passage options have been implemented for an irrigation dam in the lower Yellowstone River that will provide access to 263 km of spawning and rearing habitat. Most importantly, this will provide critically needed larval drift habitat needed for successful natural recruitment (Figure 1). If natural recruitment is restored to the area, we suggest that managers carefully consider the need to continue releasing hatchery-produced fish into the area between Fort Peck Dam and Garrison Dam (e.g., Groombridge et al. [2009]). However, outplanting of hatchery-produced progeny will likely still be the only source of fish for other isolated sections of the upper Missouri River (Erwin et al. 2018).

Supplemental Material

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Text S1. Methods for and results from examining the mode of transmission and segregation of 17 microsatellite loci in six families of Pallid Sturgeon *Scaphirhynchus albus* collected in 2004 from Garrison Dam National Fish Hatchery, North Dakota.

Available: https://doi.org/10.3996/JFWM-21-035.S1 (15 KB DOCX)

Text S2. Influence of priors on posterior distributions for Pallid Sturgeon *Scaphirhynchus albus* from the upper Missouri River that we generated by the hierarchical Bayesian model of Storz and Beaumont (2002) for historic population size (M_{N0}), current population size (M_{N1}), variance in microsatellite mutation rate among loci (V_{μ}), and time at which the species started a demographic decline (M_{xa}). We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007.

Available: https://doi.org/10.3996/JFWM-21-035.S2 (14 KB DOCX)

Data S1. Microsatellite DNA genotypes at 17 loci for 140 Pallid Sturgeon *Scaphirhynchus albus* and 94 Shovelnose Sturgeon *S. platorynchus* from the upper Missouri River that we examined in this study. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

Available: https://doi.org/10.3996/JFWM-21-035.S3 (34 KB TXT)

Table S1. Model parameters and priors for the hierarchical Bayesian model of Storz and Beaumont (2002) that we used to test for genetic evidence of population expansion and contraction in Pallid Sturgeon *Scaphirhynchus albus* and Shovelnose Sturgeon *S. platorynchus* from the upper Missouri River. We ran two chains for each of the four sets of priors for each species. See text for definitions of the parameters historic size before the demographic change occurred, current size since the change occurred, mutation rate at the 17 microsatellite loci examined, and time interval over which the demographic change occurred. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

Available: https://doi.org/10.3996/JFWM-21-035.S4 (18 KB DOCX)

Table S2. Informative families and individuals for examining the mode of transmission and segregation of 17 microsatellite loci in six families of Pallid Sturgeon

Scaphirhynchus albus (A–F) that we collected in 2004 from the upper Missouri River basin.

Available: https://doi.org/10.3996/JFWM-21-035.S5 (15 KB DOCX)

Table S3. Results for a two-part STRUCTURE analysis of genetic structuring of Pallid Sturgeon *Scaphirhynchus albus* and Shovelnose Sturgeon *S. platorynchus* in the upper Missouri River basin. In part one of the analysis, we included both Pallid Sturgeon and Shovelnose Sturgeon. In part two of the STRUCTURE analysis, we analyzed each species separately. In all STRUCTURE analyses, we performed 20 replicated runs for each putative value of *K* from 1 to 5. All runs had a burn-in of 30,000 iterations followed by 100,000 iterations. For each STURCTURE analysis, we generated summary data for ΔK, and mean and variance of posterior probabilities for each *K* were generated using STRUCTURE HARVESTER. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007 and Shovelnose Sturgeon samples from May to July of 2005.

Available: https://doi.org/10.3996/JFWM-21-035.S6 (17 KB DOCX)

Figure S1. Influence of priors on posterior distributions for Pallid Sturgeon *Scaphirhynchus albus* from the upper Missouri River that we generated by the hierarchical Bayesian model of Storz and Beaumont (2002) for (**A**) historic population size $\log_{10}$ (M_{N0}), (**B**) current population size $\log_{10}$ (M_{N1}), (**C**) variance in microsatellite mutation rate among loci $\log_{10}$ (V_{μ}), and (**D**) time at which the species started a demographic decline $\log_{10}$ (M_{xa}). Thick lines represent the posterior distributions based on two independent Markov chain Monte Carlo simulations for each of the four prior sets examined (Table S1, *Supplemental Material*). Priors for the simulations are shown as dotted lines. We collected Pallid Sturgeon samples at either presumed spawning sites or prespawn staging areas from 2000 to 2007.

Available: https://doi.org/10.3996/JFWM-21-035.S7 (195 KB DOCX)

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