Interim Progress Report for PRRIP project "Resolving Pallid Sturgeon Species Identification, Demographics and Hybridization using GT-Seq"

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This report details progress made during the 2023 calendar year with expected progress for 2024-2026.

Student Recruiting and Training – Ph.D. student Junman Huang continues to make progress towards his dissertation proposal involving the use of GTseq (Campbell et al. 2015) for achieving goals related to conservation genetics of pallid sturgeon. Junman presented a poster was titled "GT-seq panel development for *Scaphirhynchus spp*. for high-resolution species identification, broodstock selection, and parentage analysis" at the 2023 meeting of the American Fisheries Society in Grand Rapids, Michigan in August. A timeline for academic benchmarks is presented below. We are waiting to have the plan of study formalized until Junman has a committee meeting where he will present his dissertation proposal. To date Junman has submitted two of the expected five chapters that will make up the proposal/dissertation.

Table 1. Academic benchmarks for Junman Huang's Ph.D. program.

1. Admitted to Graduate School – August 2022.
2. Signed committee form -- December 2022.
3. Dissertation proposal/Plan of Study –December 2024.
4. Preliminary examination –May 2025.
5. Dissertation defense/graduation – May 2027.

GTseq marker development and screening – As described in the previous annual report, we designed GTseq primers based on genomic sequences developed using double digest RADseq (Peterson et al. 2012) as part of Richard Flamio's dissertation research (Flamio et al. 2022). We developed two separate GTseq panels for different purposes. The P-series of loci (where "P" stands for "polymorphic") are more powerful for population genetic analyses including testing for allele frequency differences among the current management units and estimates of effective population size. These markers were chosen based on high levels of heterozygosity in pallid sturgeon in the ddRAD study. The S-series of loci (where "S" stands for "species") had large allele frequency differences between pallid and shovelnose sturgeon in the ddRAD study. The S-series loci are more powerful for discriminating between pallid, shovelnose and hybrid sturgeon. Both panels will be genotyped simultaneously in each GTseq run. We are

currently scoring 144 P-loci and 155 S-loci (299 loci total) and are using both the P-loci and S-loci for project analyses.

Locus validation – In the previous year's annual report we detailed the validation of the markers by demonstrating a greater than 99% agreement in genotypes between the ddRAD study of Flamio et al (2022). This validation demonstrates that two very different methodologies retrieved identical genotypes more than 99% of the time across all loci and individuals, and thus the GTseq genotypes are reliable and the project goal of "Marker development/validation" has been completed.

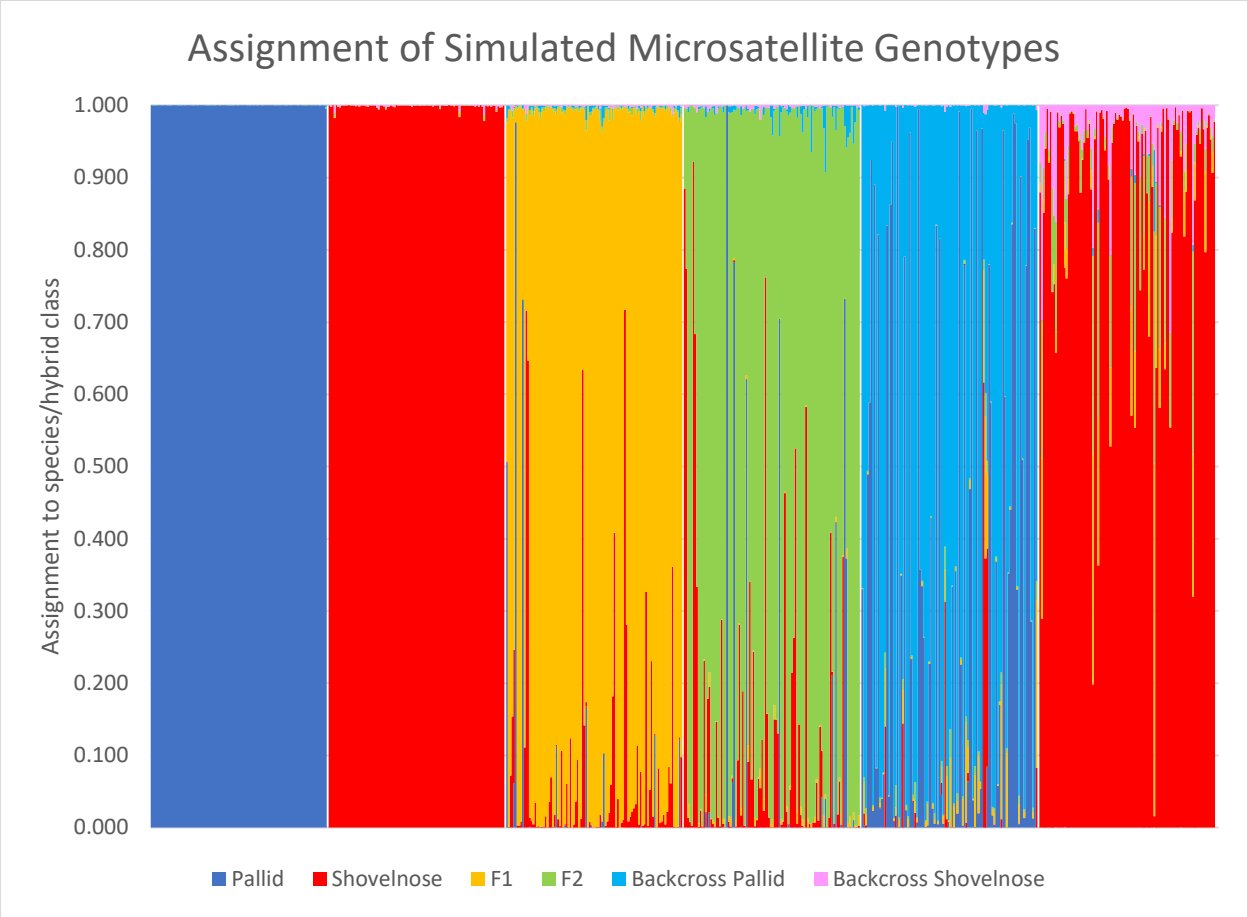
Redefine Species ID -- We used the R-package HYBRIDDETECTIVE (Wringe et al. 2017) to simulate 100 microsatellite and GTseq genotypes for each 6 of classes of individuals: pure pallid sturgeon, pure shovelnose sturgeon, F1 hybrid, F2 hybrid, backcross F1 to pallid sturgeon and backcross F1 to shovelnose sturgeon. We identify a genotype to a particular class when it's NewHybrids score for that class is at least 95%. Allele frequencies were based on our fish identified as pure pallid and shovelnose sturgeon from the Great Plains (GPMU) and Central Lowlands (CLMU) management units, mostly using individuals that make up our NewHybrids (Anderson and Thompson 2002) baseline for species ID. For the microsatellite simulations, 306 pallid and 158 shovelnose sturgeon were used to estimate allele frequencies; for GTseq 200 pallid and 200 shovelnose sturgeon were used. To simulate a genotype of two alleles at each locus, the frequencies of the alleles in each species' baseline at that locus and the proportion of alleles that come from each species for the hybrid class or pure species are considered. For example, to simulate a pure pallid sturgeon genotype the two alleles at each locus are independently simulated based on the frequencies of alleles in the pallid sturgeon baseline. To simulate F_1 genotypes one allele at each locus is simulated from each of the pallid and shovelnose sturgeon baseline. For an F_2 individual, a genotype at each locus has a 50% probability of having one allele from each gene pool (like an F_1) and a 25% probability of getting both alleles from one or the other species. In a backcross individual there is a 50% chance that both alleles come from the pure species involved in the cross and a 50% chance that it receives one allele from each species. This approach follows that of Jordan et al. (2019) which demonstrated that the currently used suite of 19 microsatellite markers are insufficient for reliable identification of pure pallid, shovelnose and hybrid sturgeon.

Microsatellites assigned all pure pallid and shovelnose sturgeon correctly but performed poorly for hybrid classes (Figure 1). For the simulated F_1 and F_2 genotypes, 1% each were assigned as pure pallid sturgeon and 13% of the simulated backcross to pallid category were assigned as pure pallid sturgeon. 58% of simulated backcross to shovelnose sturgeon genotypes were assigned as pure shovelnose sturgeon. However, the GTseq panel unambiguously assigned all 600 simulated genotypes to the appropriate class at greater than 95%.

Based on the results of the validation and simulations, we conclude that the objective titled "Refined species ID and baselines" which was slated for a completion date of December 2023 has been accomplished. We are currently using a NewHybrids baseline file including 200 pallid sturgeon and 200 shovelnose sturgeon from the GPMU and CLMU (both species) and Interior Highlands management unit (IHMU; Pallid sturgeon) for identifying sturgeon to species and hybrid class.

A persistent challenge for the identification of pure pallid sturgeon is that there is no *a priori* knowledge that any individual fish is either a pure pallid or shovelnose sturgeon, and so we do not have the ability to check the assignments against fish of known parentage. However, if groups of full siblings can be identified we can at least be sure that each sibling shares the same species or hybrid class origin. The

1992 year-class of hatchery origin sturgeon presents that possibility, serving as a way to validate GTseq genotypes assigned to siblings that should be the same. In 1992 over 4000 sturgeon were stocked from the first successful attempt at hatchery propagation of pallid sturgeon. It was suspected at the time and verified using microsatellites that at least some of the parents used in that stocking were hybrids . While no tissue samples were retained from the broodstock used in 1992, Ed Heist used known offspring of that cross to reconstruct the genotypes of five parents (two of one sex and three of the other) and identified more than 100 individuals recaptured in the wild from that cross (Ruskamp 2023). Recaptured individuals assign to all the six possible crosses among the five parents, with some families assigning as pure pallid sturgeon and others assigning as hybrids. However, the microsatellite-based assignments lack precision (see Figure 1). As a further means of validating species identification, we will generate GTseq genotypes from the 1992 year-class families and compare the consistency and precision of species/hybrid class assignments within families. We may even be able to reconstruct the GTseq genotypes of the parents to unambiguously determine their species/hybrid class.


Assignment of Simulated Microsatellite Genotypes
Assignment to species/hybrid class
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Pallid
Shovelnose
F1
F2
Backcross Pallid
Backcross Shovelnose

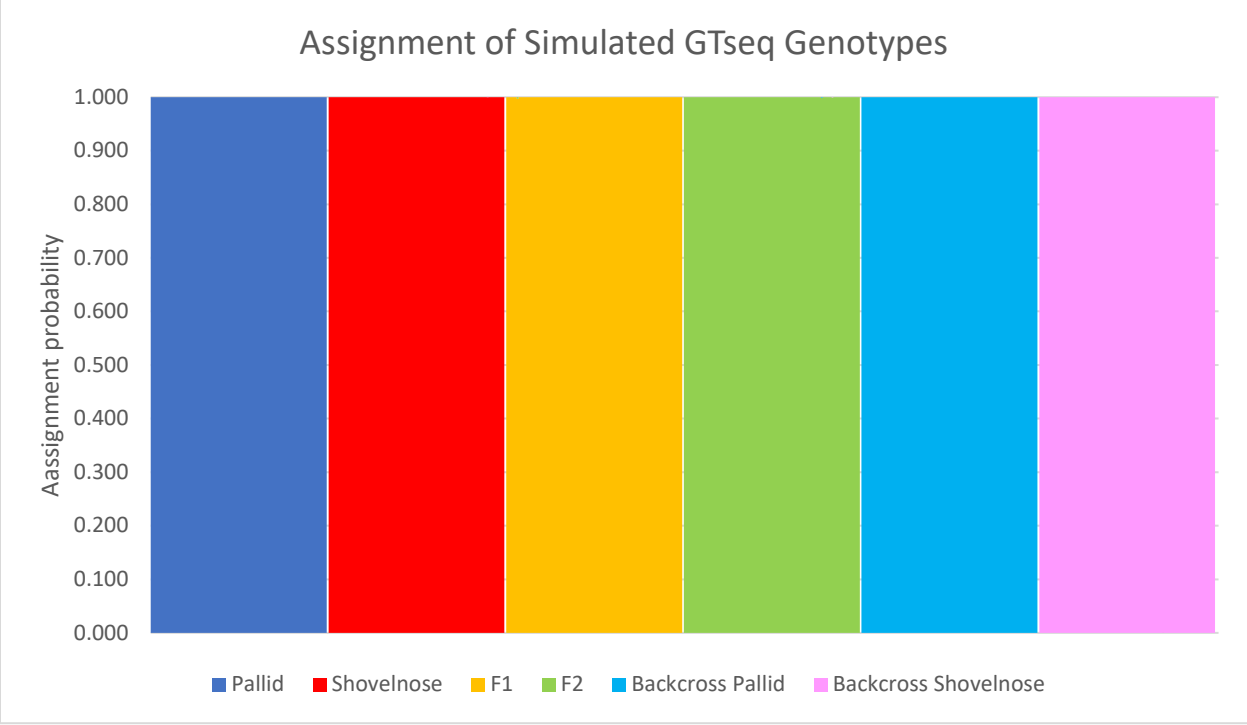

Assignment of Simulated GTseq Genotypes
Aassignment probability
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Pallid
Shovelnose
F1
F2
Backcross Pallid
Backcross Shovelnose

Figure 1. NewHybrids assignment of 100 simulated genotypes of each of six classes using microsatellites (top) and GTseq (bottom). Each figure contains 600 individual bar graphs representing 600 simulated genotypes across the x-axis. Classes (left to right) are pure pallid sturgeon, pure shovelnose sturgeon, F_1 hybrid, F_2 hybrid, backcross to pallid sturgeon and backcross to shovelnose sturgeon. Assignments are shown to pallid sturgeon (blue), shovelnose sturgeon (red), F_1 (yellow), F_2 (green), backcross to pallid (teal) and backcross to shovelnose (pink). Simulated microsatellite genotypes bars are often multicolored because of uncertainty in their assignment, for example most simulated backcross to shovelnose genotypes are mostly red, meaning that they are wrongly assigned as pure shovelnose, with a mixture of orange (F_1) and pink (backcross to shovelnose) assignments. All GTseq simulated genotypes are solid and the correct color because they assign at nearly 100% probability to the correct species or hybrid class.

Population Structure/Management Units –Pallid sturgeon management units (USFWS 2014) were designed based on a combination of genetic data (Campton et al. 2000; Schrey and Heist 2007; Tranah et al. 2001), morphological differences (Kuhajda et al. 2007) and biogeography of other species (Metcalf 1966). Current analyses using microsatellite data (Heist unpublished) indicate small but significant allele frequency differences between the GPMU and either the CLMU or IHMU, but no significant differences between the CLMU and IHMU. Flamio et al. (2022) examined SNP allele frequencies, including the SNPs used for the GTseq markers in this study, among 30 pallid sturgeon each from the GPMU and CLMU, and detected no allele significant frequency differences between these two management units. To test for population structure using our GTseq markers in a larger number of individuals, we selected 43 pallid sturgeon from the GPMU, 180 from the CLMU, and 22 from the IHMU. Each sturgeon was previously checked for hatchery parentage using microsatellites and do not match any known crosses. No pure pallid sturgeon have yet been identified from the Coastal Plains management unit (CPMU), where a previous microsatellite study also detected no pure wild pallid sturgeon (Jordan et al. 2019). We used Genepop 4 (Rousset 2008) to test for population differentiation among the 3 management units for which sufficient samples were available. Consistent with current microsatellite results, we found small but significant differences between the GPMU and either CLMU or IHMU, but no significant differences between the CLMU and IHMU (Table 1). Because the wild pallid sturgeon from the GPMU are very old individuals that likely predate that dams that currently isolate the upper and lower Missouri River basins and because few pallid sturgeon generations have passed in the lower basin since the dams were constructed, these differences in allele frequencies are likely due to past reproductive isolation and not due to recent isolation due to the dams. Thus, the GPMU and CLMU/IHMU likely represent historically isolated populations and we recommend that the current policy of not stocking pallid sturgeon between these management units should continue. The lack of difference between the CLMU and IHMU is not surprising given there are no barriers to movement and numerous fish captured in one MU were recaptured in the other. However, we recognize that there are other reasons beyond genetic distinctiveness for maintaining the current management units (e.g., stocking policy).

Table 1. Population structure among pallid sturgeon management units including Weir and Cockerham's (Weir and Cockerham 1984) unbiased estimator of F_{ST} (above diagonal) and P-value for the test of population differentiation among management units (below diagonal).

	GPMU	CLMU	IHMU
GPMU	----	0.024	0.023
CLMU	<0.001	-----	0.002
IHMU	<0.001	N.S.	-----

Platte River Sample Results

In 2023, 31 fin clips from sturgeon collected in the Platte River were provided by collaborators from the University of Nebraska Lincoln (UNL). We isolated DNA from the samples using standard Qiagen kits. We genotyped the fin clips first with 19 microsatellite loci then with GTseq. Species assignments of both methods were performed using NewHybrids software (Anderson and Thompson 2002) using a microsatellite baseline previously developed based on standardized microsatellite genotyping in collaboration with Lamar Fish Technology Center in Lamar, Pennsylvania. The NewHybrids analyses for the Platte River samples used the new GTseq baseline described above. Parentage assignment was performed with microsatellite using Cervus software (Kalinowski et al. 2007) using genotype from broodstock that produced offspring for stocking in the GPMU and CLMU (Ruskamp 2023).

Thirty of the fin clips were from trotline sampling and one was from a telemetered pallid sturgeon that was discovered dead near Leshara, NE along with dead fish of other species in a fish kill on July 28, 2023. The dead sturgeon was identified as a 2013 year-class hatchery origin fish of GPMU parentage from a family that was known to be stocked in the former RPMA 3 between Fort Randal Dam and the headwaters of Lewis and Clark Lake. Numerous GPMU-origin fish stocked in this region have been recaptured in the CLMU after passing through Gavins Point Dam.

Two of the fin clips, which were identified as hatchery-origin fish in the field, possessed more than two microsatellite alleles at multiple loci. These fish were thus presumed to be triploids, and no further genetic analyses were conducted because the analytical methods we use require a diploid genome. Some degree of spontaneous triploidy is known to occur in sturgeon aquaculture (Schreier et al. 2021).

Of the remaining 28 project fish, three were identified as suspected hybrids in the field. All three of these were identified as pure shovelnose sturgeon using microsatellites, while GTseq identified one of the fish (UNL-832) as more likely to be a F1 backcross to shovelnose sturgeon. Because pallid x shovelnose hybrids are interfertile, we would expect to find such “advanced backcross” fish and it may not be possible to positively identify the exact parentage of such advanced backcrosses.

The remaining 25 fish were identified as hatchery-origin pallid sturgeon of year classes ranging from 2001 through 2018. The NewHybrids scores for the pure pallid sturgeon for microsatellites ranged from 0.870 to 1.000 and one of the fish (UNL-947) would not have been positively identified as a pure pallid sturgeon using microsatellites alone using the standard criterion that a minimum NewHybrids score of 0.95 was required. All 25 fish had NewHybrids scores of 1.000 for the pure pallid sturgeon category using GTseq data. Thus, none of the fin clips collected in the Platte River in 2023 were from wild pallid sturgeon.

We also received two free embryos and one unhatched embryo or egg (referred to as ‘egg”). Both free embryos were confidently identified as shovelnose sturgeon using microsatellites. The identification was confirmed with GTseq for one of the embryos, but the other did not amplify a sufficient number of loci in the first attempt and will be re-run at a later date. The egg failed to amplify sufficient DNA for either microsatellite or GTseq nuclear markers, which is typical of embryos earlier than stage 14 (Kashiwagi et al. 2020).

Table 2. Abbreviated field data including PIT tag number, source of sample, inferred species and origin of sample and parentage for hatchery origin pallid sturgeon.

Number	PIT.Tag.Hexa	Source	Species/ Origin	Mother	Father
UNL-377	3DD.003BA1204E	Trotline	Hatchery pallid 2018	4626711111	4626773563
UNL-389	3DD.003BA12053	Trotline	Hatchery pallid 2018	4626641923	462711443D
UNL-395	4706012165	Trotline	Hatchery pallid 2006	4064021213	7F7D4A7758
UNL-399	4A472F7527	Trotline	Hatchery pallid 2007	47037F460C	454B30016B
UNL-413	4623763D09	Trotline	Hatchery pallid 2004	454910202B	115679374A
UNL-426	3DD.003D4E5F62	Trotline	Hatchery pallid 2018	4626641923	47191F7F39
UNL-475	3DD.003BA12046	Trotline	Hatchery pallid 2018	4715674971	434A582F17
UNL-476	47046F3B54	Trotline	Hatchery pallid 2001	411D262C1F	411D0E2C5F
UNL-523	3DD.003BA12037	Trotline	Hatchery pallid 2009	454B490528	1F477B3A65
UNL-542	3DD.003D4E5B9E	Trotline	Hatchery pallid 2006	4627201358	48683A3B7D
UNL-567	6C00112067	Trotline	Hatchery pallid 2002	116224546A	452A4E1F15
UNL-588	3DD.003BA11FF8	Trotline	Hatchery pallid 2018	4626711111	4626773563
UNL-634	0A14082D69	Trotline	Hatchery pallid 2017	47163E0030	4627144425
UNL-643	3DD.003BA12008	Trotline	Wild Shovelnose	NA	NA
UNL-755	412C75796E	Trotline	Hatchery pallid 2007	471A2C1013	412C3D4B11
UNL-775	3DD.003BA1201E	Trotline	Triploid	NA	NA
UNL-793	3DD.003BA12051	Trotline	Hatchery pallid 2001	411D262C1F	34354689429
UNL-821	3DD.003BA1200A	Trotline	Wild Shovelnose	NA	NA
UNL-824	3DD.003BA1202F	Trotline	Hatchery pallid 2001	411D262C1F	411D0E2C5F
UNL-825	3DD.003D4E5B03	Trotline	Hatchery pallid 2018	4626641923	47191F7F39
UNL-832	3DD.003BA12005	Trotline	Wild backcross	NA	NA
UNL-858	3DD.003BA11FFB	Trotline	Hatchery pallid 2011	4626553E42	460E275D2B
UNL-864	3DD.003BA12040	Trotline	Hatchery pallid 2018	4715674971	434A582F17
UNL-868	3DD.003D4E5FA1	Trotline	Hatchery pallid 2018	4626641923	462711443D
UNL-885	3DD.003BA1203E	Trotline	Hatchery pallid 2007	471A2C1013	412C3D4B11
UNL-908	3DD.003D4E6001	Trotline	Triploid	NA	NA
UNL-941	3DD.003BA12012	Trotline	Hatchery pallid 2018	46270E6C3C	47041F697D
UNL-947	430F72522A	Trotline	Hatchery pallid 2002	116224546A	1F477B3A65
UNL-963	3DD.003BA1203C	Trotline	Hatchery pallid 2011	4868364835	412C5D2741
UNL-973	4A474A7836	Trotline	Hatchery pallid 2008	7F7F066452	1F4A13592B
MOS-843	1F4B225A1A	Fish Kill	Hatchery pallid 2013	462C7B2F49	1F4B225A1A
PLT-029	NA	Embryo	Shovelnose Embryo	NA	NA
PLT-076	NA	Egg	Unidentified Egg	NA	NA
PLT-079	NA	Embryo	Shovelnose Embryo	NA	NA

Table 3. Genetic assignments for 2023 sturgeon sample collected in the Platte River. Unshaded columns refer to NewHybrids assignments based on GTseq data with two pure species categories (pallid and shovelnose sturgeon) and four hybrid categories (F1 hybrid, F2 hybrid, backcross to pallid, and backcross to shovelnose sturgeon). Shaded columns refer to NewHybrids assignments based on microsatellite data for the two pure species categories with values from the four hybrid categories summed into a single hybrid (Hyb) value. NA: not assigned due to triploidy or insufficient DNA amplification. TBD: to be rerun for final assignment.

ID	PAL	SHO	F1	F2	BxP	BxS	Pal	Hyb	Sho
UNL-377	1.000	0.000	0.000	0.000	0.000	0.000	0.999	0.001	0.000
UNL-389	1.000	0.000	0.000	0.000	0.000	0.000	0.998	0.002	0.000
UNL-395	1.000	0.000	0.000	0.000	0.000	0.000	0.997	0.003	0.000
UNL-399	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-413	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-426	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-475	1.000	0.000	0.000	0.000	0.000	0.000	0.999	0.001	0.000
UNL-476	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-523	1.000	0.000	0.000	0.000	0.000	0.000	0.993	0.007	0.000
UNL-542	1.000	0.000	0.000	0.000	0.000	0.000	0.998	0.002	0.000
UNL-567	1.000	0.000	0.000	0.000	0.000	0.000	0.995	0.005	0.000
UNL-588	1.000	0.000	0.000	0.000	0.000	0.000	0.998	0.002	0.000
UNL-634	1.000	0.000	0.000	0.000	0.000	0.000	0.996	0.004	0.000
UNL-643	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.001	0.999
UNL-755	1.000	0.000	0.000	0.000	0.000	0.000	0.999	0.001	0.000
UNL-775	NA	NA	NA	NA	NA	NA	NA	NA	NA
UNL-793	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-821	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.001	0.999
UNL-824	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-825	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-832	0.000	0.573	0.000	0.000	0.000	0.426	0.000	0.000	1.000
UNL-858	1.000	0.000	0.000	0.000	0.000	0.000	0.998	0.002	0.000
UNL-864	1.000	0.000	0.000	0.000	0.000	0.000	0.999	0.001	0.000
UNL-868	1.000	0.000	0.000	0.000	0.000	0.000	0.999	0.001	0.000
UNL-885	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-908	NA	NA	NA	NA	NA	NA	NA	NA	NA
UNL-941	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
UNL-947	1.000	0.000	0.000	0.000	0.000	0.000	0.870	0.130	0.000
UNL-963	1.000	0.000	0.000	0.000	0.000	0.000	0.996	0.000	0.004
UNL-973	1.000	0.000	0.000	0.000	0.000	0.000	0.998	0.000	0.002
MOS-843	1.000	0.000	0.000	0.000	0.000	0.000	0.999	0.000	0.001
PLT-029	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
PLT-076	NA	NA	NA	NA	NA	NA	NA	NA	NA
PLT-079	TBD	TBD	TBD	TBD	TBD	TBD	0.000	0.000	1.000

Future work – We continue to genotype sturgeon fin clip samples collected from all pallid sturgeon management units to characterize the composition of these samples regarding the presence of pure and hybrid classes by management unit. Because our fin clip samples do not represent a random survey but are instead weighted in favor of individuals exhibiting pallid sturgeon morphologies we plan to perform a random survey of larvae collected in 2021 to assess something more similar to the actual distribution of sturgeon and hybrid classes produced by naturally spawning sturgeon in the CLMU. This work will complete the goal of assessing "**Population composition by species/hybrid**" by June 2025.

A large panel of unlinked markers can be used to assess effective population size (Waples and Do 2010). While Richard Flamio constructed a linkage map of some of the markers used for the GTseq panel, he was unable to map many of the markers due to a lack of polymorphism in the female sturgeon used to produce the map. We are collaborating with Aaron Delonay of the USGS laboratory in Columbia, Missouri and Trevor Krabbenholt of the University at Buffalo to construct a draft genome of pallid sturgeon. This genome will allow us to select a subset of independent markers that will allow us to estimate effective population size for the upper and lower Missouri River basins using as many pure wild pallid sturgeon GTseq genotypes as are available. This will complete the project goal of "**Demographics – pallid sturgeon Ne by population**" by December 2025.

Table 4. Anticipated timeline for completion of project goals

1. Marker development/validation – December 2022
2. Refined species ID and baselines – December 2023
3. Population structure/Redefine management units – June 2024
4. Population composition by species/hybrid – June 2025
5. Demographics – pallid sturgeon N_e by population – December 2025
6. Final report to PRRIP – June 2026

Literature cited

Anderson, E. C., and E. A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data Genetics 160:1217-1229.

Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources 15(4):855-867.

Campton, D. E., A. L. Bass, F. A. Chapman, and B. W. Bowen. 2000. Genetic distinction of pallid, shovelnose, and Alabama sturgeon: emerging species and the US Endangered Species Act. Conservation Genetics 1:17-32.

Flamio, R., D. G. Swift, D. Portnoy, A. DeLonay, K. A. Chojnacki, J. Powell, P. J. Braaten, and E. J. Heist. 2022. Disomic marker development in the paleotetraploid sturgeons, *Scaphirhynchus albus* and *S. platorynchus*, facilitated by sequencing functional haploids. Molecular Ecology Resources Online early.

Jordan, G., E. J. Heist, B. R. Kuhajda, G. R. Moyer, P. Hartfield, and M. Piteo. 2019. Morphological Identification Over-Estimates The Number Of Pallid Sturgeon (*Scaphirhynchus albus*) In The Lower Mississippi River Due To Extensive Introgressive Hybridization. Transactions of the American Fisheries Society 148:1004-1023.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16(5):1099-1106.

Kashiwagi, T., A. J. DeLonay, P. J. Braaten, K. A. Chojnacki, R. M. Gocker, and E. J. Heist. 2020. Improved genetic identification of acipenseriform embryos with application to the endangered pallid sturgeon *Scaphirhynchus albus*. Journal of Fish Biology 96(2):10.

Kuhajda, B. R., R. L. Mayden, and R. M. Wood. 2007. Morphologic comparisons of hatchery-reared specimens of Scaphirhynchus albus, Scaphirhynchus platorynchus, and S-albus x S-platorynchus hybrids (Acipenseriformes : Acipenseridae). Journal of Applied Ichthyology 23(4):324-347.

Metcalf, A. L. 1966. Fishes of the Kansas River System in Relation to Zoogeography of the Great Plains. University of Kansas Publications of the Museum of Natural History 17(3):23-189.

Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher, and H. E. Hoekstra. 2012. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS ONE 7(5):11.

Rousset, F. 2008. Genepop '007: a complete re-implementation of the GENEPOP software for Windows and Linux. . Molecular Ecology Resources 8:103-106.

Ruskamp, R. 2023. Central Lowlands and Interior Highlands Pallid Sturgeon Spawning and Stocking Summary: 1992-2022. Nebraska Game and Parks Commission, Lincoln, Ne.

Schreier, A. D., J. P. Van Eenennaam, P. Anders, S. Young, and J. Crossman. 2021. Spontaneous autopolyploidy in the Acipenseriformes, with recommendations for management. Reviews in Fish Biology and Fisheries 31(2):159-180.

Schrey, A. W., and E. J. Heist. 2007. Stock structure of pallid sturgeon analyzed with microsatellite loci. Journal of Applied Ichthyology 23:297-303.

Tranah, G. J., H. L. Kincaid, C. C. Krueger, D. E. Campton, and B. May. 2001. Reproductive isolation in sympatric populations of pallid and shovelnose sturgeon. North American Journal of Fisheries Management 21(2):367-373.

USFWS. 2014. U.S. Fish and Wildlife Service Revised Recovery Plan for the Pallid Sturgeon (*Scaphirhynchus albus*). U.S. Fish and Wildlife Service, Denver, CO.

Waples, R. S., and C. Do. 2010. Linkage disequilibrium estimates of contemporary N-e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications 3(3):244-262.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Wringe, B. F., R. R. E. Stanley, N. W. Jeffery, E. C. Anderson, and I. R. Bradbury. 2017. hybriddetective: A workflow and package to facilitate the detection of hybridization using genomic data in r. Molecular Ecology Resources 17(6):e275-e284.