



Updates on Pallid Sturgeon Research – Genetics

Ed Heist, Junman Huang and Amy Buhman
Southern Illinois University Carbondale

Objective 1: 2023 Platte Samples

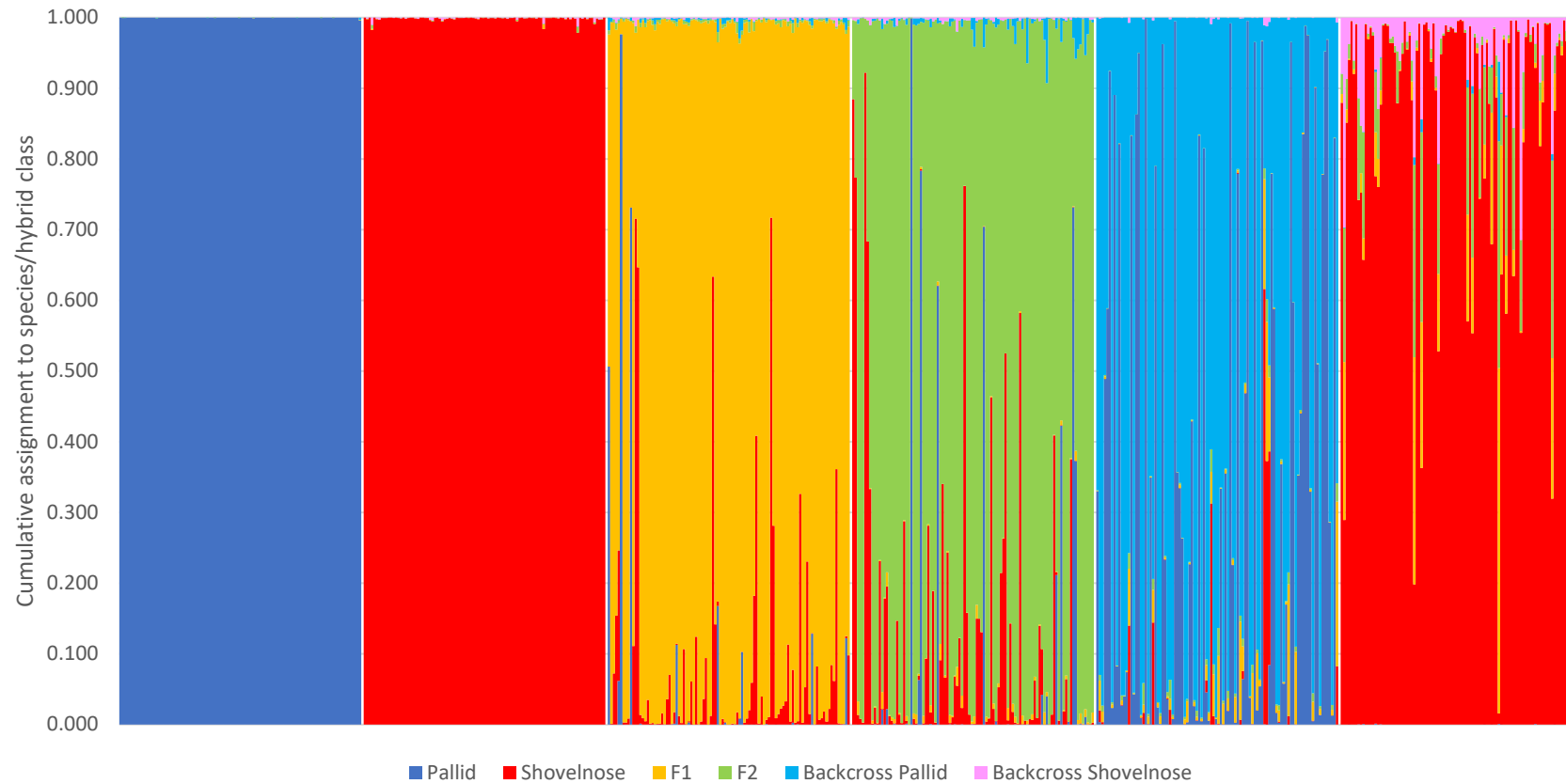
- WHY?
 - Genetic identification and origin of sturgeon using the Platte River
 - Identification of sturgeon eggs/embryos/larvae from the Platte River
 - Provide feedback to prioritize UNL active tracking
- 30 trotline fish
 - 28 hatchery-origin pallid sturgeon
 - 2 triploids
 - 2001-2018 year-classes
 - 1 wild hybrid
 - 1 wild shovelnose
- 1 fish-kill fish
 - Hatchery pallid
- 2 shovelnose embryos
- 1 unidentified egg
 - Unfertilized eggs and embryos <stage 14 can not be genetically identified

Objective 1: New GTseq Markers

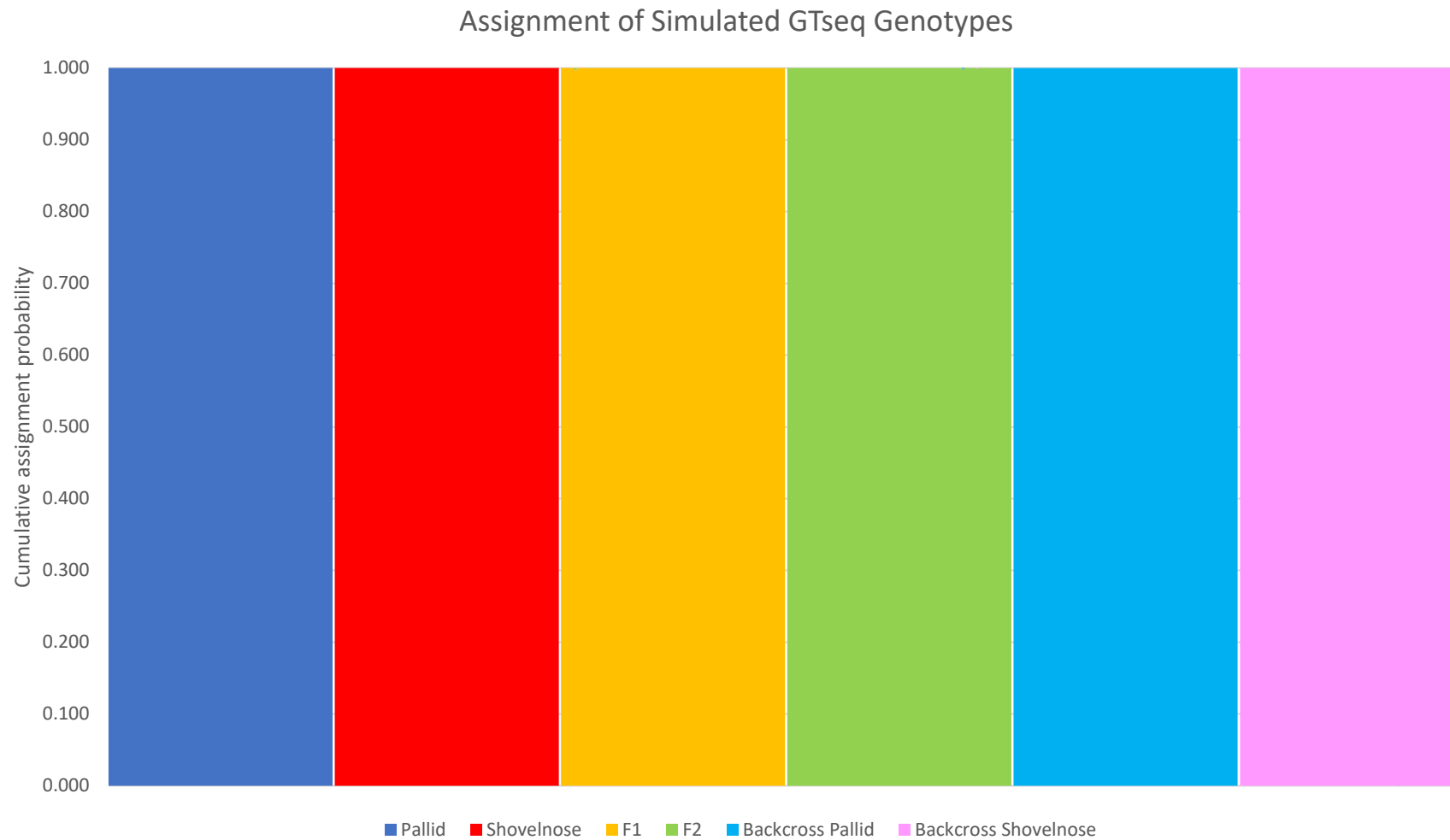
- WHY?: Stronger and more confident assignment of Individuals, Broodstock and Larval Sturgeon.



Assignment of Simulated Microsatellite Genotypes



Assignments for 600 simulated genotypes, 100 each for (left to right) pure pallid sturgeon, pure shovelnose sturgeon, F1, F2, backcross to pallid and backcross to shovelnose sturgeon.



- Gtseq markers far outperform microsatellites for species and hybrid ID for these categories
- Additional hybrid categories likely exist (e.g., backcross pallid x pallid).

Validating Marker Assignments

- WHY?: Validation of assignments using GTseq

DIFFICULTIES with validation

- No fixed differences occur between species
 - Pallid and shovelnose less distinct than many intraspecific populations
- Wild hybrids exist in all management units
- Hybrids are fertile and can backcross
- We can not be sure *a priori* that any fish is a pure pallid or shovelnose sturgeon.
- However: Full siblings should all be assigned to same species/hybrid class



Validating Marker Assignments: 1992 Year Class

- First year *Scaphirhynchus* were successfully spawned.
- 3 females and 4 males utilized
 - May not have all been successfully spawned
- No genetic ID attempted.
 - No tissues saved from parents for genetics.
- Some broodstock came from the Mississippi River.
 - Nearly all pallid morphotypes in the Mississippi River are hybrids.
- 4182 offspring stocked
 - Not adequately tagged
 - 99 in Platte River

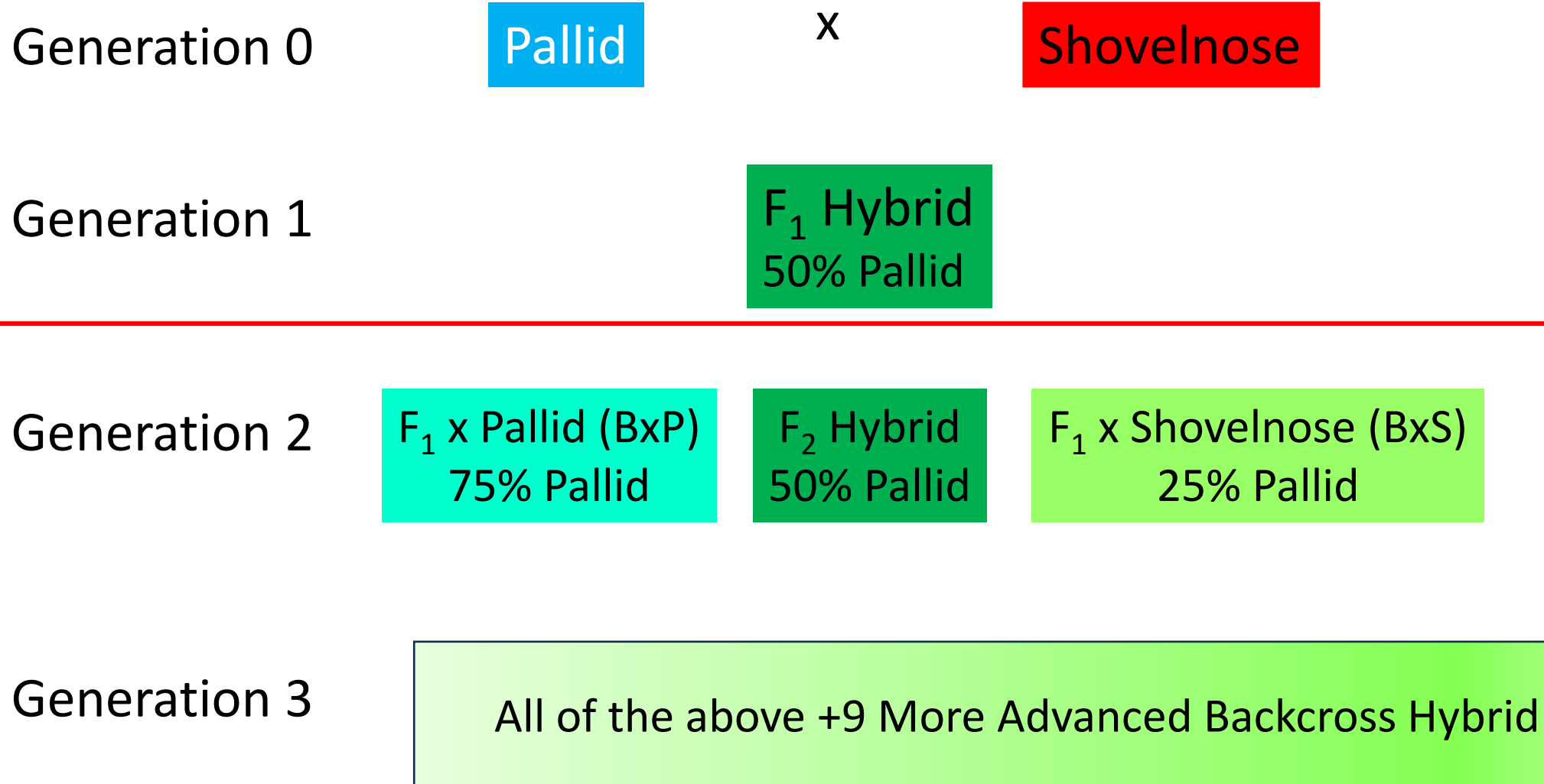
Reconstruct 1992 parents GTseq genotypes

- GTseq parent assignments (two hybrid generations) consistent with earlier microsatellites

GTseq assignments (two hybrid generations)

| SIU ID | Pal | Sho | F1 | F2 | BxP | BxS |
|--------|-------|-------|-------|-------|-------|-------|
| 1992A | 0.570 | 0.000 | 0.000 | 0.000 | 0.430 | 0.000 |
| 1992B | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 1992J | 0.000 | 0.000 | 0.000 | 0.001 | 0.999 | 0.000 |
| 1992K | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 1992L | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

Sturgeon Hybrid Categories



GTseq assignments (two hybrid generations)

| SIU ID | Pal | Sho | F1 | F2 | BxP | BxS |
|--------|-------|-------|-------|-------|-------|-------|
| 1992A | 0.570 | 0.000 | 0.000 | 0.000 | 0.430 | 0.000 |
| 1992B | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 1992J | 0.000 | 0.000 | 0.000 | 0.001 | 0.999 | 0.000 |
| 1992K | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 1992L | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

GTseq assignments (three hybrid generations)

[illegible]

Conclusions about 1992 parents

| SIU ID | Pal | Sho | F1 | BxP | BxS | F2 | (BxP)xP | (BxP)xS | (BxP)xF1 | BxP)x(BxP) | BxS)x(BxP) | (BxS)xS | (BxS)xF1 | BxS)x(BxS) | (BxS)xBxS) |
|--------|-------|-------|-------|-------|-------|-------|---------|---------|----------|------------|------------|---------|----------|------------|------------|
| 1992A | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.673 | 0.000 | 0.000 | 0.327 | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 |
| 1992B | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.791 | 0.000 | 0.000 | 0.209 | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 |
| 1992J | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 |
| 1992K | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 |
| 1992L | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 |

- 1992A and 1992B were both advanced backcrosses
- 1992J was likely a (BxP)x(BxP).
- 1992K and 1992L were likely pure pallid sturgeon.
- All 1992 year-class offspring are advanced hybrids.
 - Two generations model not sufficient for ID.

1992B x 1992K

Microsatellites

GTseq (three hybrid generations)

Parents

| Sample | Pal | Sho | F1 | F2 | BxP | BxS |
|--------|-------|-------|-------|-------|-------|-------|
| 1992B | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 1992K | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

| Pal | Sho | F1 | BxP | BxS | F2 | (BxP)xP | (BxP)xS | (BxP)xF1 | (BxP)x(BxP) | (BxS)x(BxP) | (BxS)xS | (BxS)xF1 | (BxS)x(BxS) | (BxS)xBxS) |
|-------|-------|-------|-------|-------|-------|---------|---------|----------|-------------|-------------|---------|----------|-------------|------------|
| 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.791 | 0.000 | 0.000 | 0.209 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

Offspring

Siblings

| Sample | Pal | Sho | F1 | F2 | BxP | BxS |
|---------|-------|-------|-------|-------|-------|-------|
| AA-05 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| AWS-107 | 0.289 | 0.000 | 0.000 | 0.000 | 0.711 | 0.000 |
| CMF-026 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| HAT-020 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| HAT-033 | 0.997 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 |
| LMB-004 | 0.994 | 0.000 | 0.000 | 0.000 | 0.005 | 0.000 |
| LMB-062 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| MOS-344 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| MOS-349 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| MOS-375 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

| Pal | Sho | F1 | BxP | BxS | F2 | (BxP)xP | (BxP)xS | (BxP)xF1 | (BxP)x(BxP) | (BxS)x(BxP) | (BxS)xS | (BxS)xF1 | (BxS)x(BxS) | (BxS)xBxS) |
|-------|-------|-------|-------|-------|-------|---------|---------|----------|-------------|-------------|---------|----------|-------------|------------|
| 0.792 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.208 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.993 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.007 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.997 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.017 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.983 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.046 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.954 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.724 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.276 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

- Offspring likely 87.5% Pallid (if 1992B is a BxP) or 93.4% pallid (if 1992B is a (BxP)xP.).
- Most assign as pure pallid sturgeon using 2 generations, most assign as (BxP)xP using 3 generations.
- Working on modeling to find out what fraction of pure pallid sturgeon would be mis-identified using the 3-generation model.

Conclusions from 1992 year-class

- All 1992 offspring and 3 of 5 parents were advanced back-crosses
- Offspring with greater than 75% pallid genes sometimes assign as pure pallids.
- The presence of advanced backcrosses in the Mississippi River here makes identification of pure pallid sturgeon very problematic.
 - Less of a problem for the Missouri River.
 - May need to adopt a “policy on hybrids” that accommodates some introgression in pallid sturgeon.

Cutthroat Trout



- Natural hybridization with rainbow trout where they co-occur.
 - Different policies for natural vs. anthropomorphic hybridization.
- Increased hybridization where rainbow trout are stocked on top of cutthroat trout.
- Cutthroat individuals with up to 20% rainbow trout genes indistinguishable from pure cutthroat trout.
- Populations of morphological cutthroat trout can be protected under ESA even if they are introgressed with rainbow trout genes.
 - Federal register 68:152 (2003).

Objective 3: 2021 Year-Class Larvae

- 15,325 sturgeon larvae collected in lower Missouri River
- 64 identified as wild-spawned pallid sturgeon
 - Most years we find 0-2 pallid larvae
 - Previous record was 7 larvae in one year
- Family assignments can provide unprecedented investigations into wild spawning in pallid sturgeon.
 - Larvae captured by different agencies in different segments can be tied to a single spawning event

Family “A”

Parents

♀ 4627545945
♂ Unknown male

♀ 4627545945
♂ 462646292E

Female 4627545945 spawned with at least 2 males.

Offspring

21-00188

Siblings

21-00190

21-00270

21-00328

21-01130

MHMP-3431

MOPSA-1442

Larvae captured by different agencies in different segments can be tied to a single spawning event

Family “D”

Female 434D30680D spawned with 2 males. Male 44235B7567 spawned with 2 females.

Parents ♀ 434D30680D
♂ Unknown male

♀ 434D30680D
♂ 44235B7567

♀ Unknown female
♂ 44235B7567

Offspring

21-00976

Siblings

21-50601

21-53945

MHMP-3359

Siblings

21-50608

MHMP-3549

Conclusions from Family Reconstruction

- 64 wild pallid larvae from 2021 were the offspring of 56 unique parents.
- Evidence of both polyandry and polygyny.
- Unusually large number of pallid sturgeon larvae from 2021 resulted from successful reproduction by many individual pallid sturgeon rather than a few very successful spawners.

Objective 4: Effective Population Size (N_e)

- Why?: Pallid Sturgeon Recovery Criteria

“Pallid Sturgeon will be considered for reclassification from endangered to threatened when the listing/recovery factor criteria are sufficiently addressed such that a self-sustaining genetically diverse population of 5,000 adult Pallid Sturgeon is realized and maintained within each management unit for 2 generations (20-30 years).”

– Pallid Sturgeon Recovery Plan

Objective 4: Effective Population Size (N_e)

- $N_e = 500$ is the target for a genetically healthy population.
- Averaged over many species studied, $N_e \approx 1/10$ the number of adults (Frankham 1995).
- 5000 adults per management unit needed for $N_e=500$

N_e Estimates – Great Plains Management Unit

- Measures N_e for the **parents of the sampled generation.**
- Ardren et al. (2022)
 - Based on 17 microsatellites
 - Pallid $N_e = 246$
 - (95% CI = 181-367)
- Heist pallid estimate $N_e = 233$
 - (95% CI = 189-299) based on 19 microsatellite loci.
- Estimates N_e of the last generation of wild pallid sturgeon with successful recruitment
 - Pre-dam population (pre 1960s)
 - Parents of current “wild” fish
 - Grandparents of stocked fish

N_e Estimate - Lower basin

Central Lowlands and Interior Highlands Management Units

- Based on wild-spawned adults and sub-adults only
- Microsatellites only $N_e = 62.3$ (95% CI = 58.7- 66.2)
 - 539 wild fish
- Microsatellites + GTseq = 68.3 (95% CI = 66.1 – 70.5)
 - 131 wild fish
- Both estimates are for **parents of current wild adults**.
 - Comparable estimate to upper basin estimate, but for a later generation
 - Does not include impact of stocked fish
 - Recovery criteria based on natural reproduction.
 - Any increase in recruitment due to habitat/stocking won't be evident until wild-spawned offspring recruit.

Estimating Current N_e based on larvae

- N_b = effective number of breeders in a single year
- N_e = average of N_b taken over multiple years in lake sturgeon
- 2021
 - $N_b = 37.2$ (95% CI = 32.1 – 43.5).
 - $N_b <$ number of successful spawners
 - Consistent with finding 56 unique parents for 2021 larvae.

Conclusions

- New GTseq markers provide greater resolution of species and hybrids.
 - Difficulty with advanced hybrids in Mississippi River.
 - May need to adopt a “policy on hybrids” that accommodates some introgression in pallid sturgeon.
- Family assignments of larvae provide additional insights about pallid sturgeon reproduction.
- Effective population size of lower basin fish well below what is needed for recovery.
 - Linkage based N_e using current adults may not reflect current levels of reproduction/recruitment.
 - Measuring N_b using larvae indicates current N_e
 - Does reproduction = recruitment?