Genetic Management Plan for Captive-Reared Pallid Sturgeon Broodstock

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Summary

Pallid sturgeon from the upper Missouri River basin are genetically distinct from those in the other parts of the species' range. This stock has not experienced successful recruitment in decades and future survival of this management unit is now dependent upon the collection and spawning of a diminishing number of remaining wild fish. Maintaining the offspring of wild broodstock to maturity in the hatchery (i.e. captive broodstock) is a potentially useful strategy, but one with some risk to the genetic health of future populations. In order to assess whether sufficient genetic variation has been retained in the captive broodstock program, we genetically evaluated the wild broodstock used to produce captive broodstock currently held at Gavins Point National Fish Hatchery as well as the surviving captive broodstock fish. Captive broodstock fish were assigned to parents using a combination of hatchery records and genetic (microsatellite) genotypes. Based on our findings we make the following recommendations:

1) The natural population from which the wild broodstock were drawn appears to have consisted of unrelated individuals from the remnants of a larger population and thus the current generation of captive broodstock fish does not appear to be inbred.

2) A sufficient amount of wild broodstock genetic variation is represented in the captive broodstock program at Gavins Point for the maintenance of genetic diversity, provided that multiple offspring descended from each wild fish can ultimately be spawned in the future.

3) Future crosses should be performed in a manner that includes offspring of as many of the wild broodstock individuals as possible. To ensure that the contribution of each parent is included in the next generation, each male should be mated to two different females and each female should be mated to two different males (after Hedrick et al. (1995)).

4) Should it become necessary to cull the captive broodstock fish, care should be taken to retain some offspring of all wild parents. Maintaining multiple offspring from every parent is more important than maintaining offspring from every family.

5) All fish should be PIT tag prior to release to retain pedigree information. Untagged fish caught in the wild and used for broodstock should first be genotyped and checked for hatchery origin, parentage, and relatedness.

6) Pedigree information should be checked to ensure that male and female mates are not either full sibs or half sibs. In checking, remember that eight wild-caught males were used in more than one year to produce captive progeny so that families from different years may be half sibships. Pedigree information obtained from PIT tag number, hatchery records, and the spreadsheet is included with this report. For those fish that have shed their original PIT tag, DNA microsatellite genotypes can be used to assign parentage and/or estimate relatedness for potential crosses.

7) Because there is no natural reproduction and the upper Missouri River propagation efforts appear to have been based on a sufficient effective population of wild adults,genetics concerns alone should not limit the number of progeny stocked. However, care should be taken to maximize effective population of every year's stocked fish and the cumulative effective population size over multiple years. A combination of stocking records and field studies should be used, if possible, to assess the representation of the offspring of each wild parent present in the wild to maximize effective population size of the wild fish. Our results should not be construed to imply that there will be no ecological effects (e.g. intra- and interspecific competition) associated with stocking large numbers of pallid sturgeon, and there may well be other reasons unrelated to genetics to limit the number of fish stocked.

8) Microsatellite genotypes from unsampled wild broodstock can be reconstructed using hatchery and PIT tag records and should be done to identify the parentage of wild-caught pallid sturgeon.

Introduction

Pallid sturgeon from the upper Missouri River are genetically distinct from those in other parts of the species' range (Campton et al. 2000; Schrey and Heist 2007; Tranah et al. 2001) and are threatened with extirpation due to several decades of failed spawning and/or recruitment (USFWS 2007). Hatchery rearing of offspring produced by wild-caught and hatchery-spawned broodstock has been seen as the only viable means of preventing extirpation of upper Missouri River pallid sturgeon (USFWS 1993). Attempts to obtain wild broodstock in the upper Missouri often recapture adult pallid sturgeon used as broodstock in previous years and it has been estimated that as few as 45 wild adult pallid sturgeon remain in Recovery Priority Management Area (RPMA) 1 and that approximately 136 wild pallid sturgeon remain in RPMA 2 (USFWS 2007). Because of the difficulty in collecting unique wild pallid sturgeon for spawning, the upper basin pallid sturgeon work group (UBPSWG) is considering using a captive broodstock program that spawns only the captive offspring of wild fish as future broodstock for stocking into the upper Missouri River.

This plan could pose two significant risks to the genetic integrity of upper Missouri pallid sturgeon: 1) if too few wild fish are represented among the parental generation, offspring of the captive broodstock may not be representative of the genetic diversity initially present in the wild stock, and 2) using too few parents and/or unintentional crosses among related captive broodstock may result in inbreeding depression, further eroding the fitness of the species in the wild. This management plan describes a strategy for producing a captive broodstock management plan that accomplishes the following:

1) Determines whether the captive broodstock currently housed at Gavins Point hatchery are an adequate sample of the genetic diversity present in wild pallid sturgeon from the upper Missouri and Yellowstone rivers (RPMA 1 and RPMA 2),

2) Identifies the familial relationship among all future brood fish as a means of designing future crosses to avoid inbreeding, and

3) Provides guidance on determining the conservation value of each future brood fish by identifying how many other future brood fish have the same male and/or female parents.

A major risk associated with captive broodstock programs is inbreeding depression, which is defined as a loss of fitness of individual fish that are the progeny of related parents. Inbreeding depression in fishes has been shown to impact many important traits including survival, fertility, growth rate, and developmental abnormalities (Gall 1987; Kincaid 1983). Inbreeding has two general causes: pairings between related individuals and reductions in effective population size (N_e). Crosses between related individuals can be avoided by using pedigree information or by estimating relatedness among potential broodstock using molecular markers (e.g. microsatellites). Inbreeding due to low N_e can be avoided by maximizing N_e by using as many unrelated broodstock fish as possible and by minimizing the reproductive variance among broodstock. The oft-cited 50/500 rule (Franklin 1980) states that the short term N_e should be no less than 50 to avoid close inbreeding and the long term N_e at least 500 in order to maintain adaptive genetic variation.

While microsatellite loci (which are presumably neutral and thus have no direct influence on phenotype or fitness) can be used to statistically estimate relatedness (as described above), knowing the actual (parametric) relationship among potential future broodfish is a superior indicator of the potential for inbreeding depression caused by excess homozygosity at functional loci. The familial relationships can be permanently retained via the PIT tag number of each fish. Knowing the number of offspring from each parent is important for determining the conservation value of each future brood fish. Offspring of parents that have only one or a few future brood offspring have a higher conservation value than those of parents with many offspring. This is because they may have unrepresented ancestry that may be necessary for the future survival of the species and to counteract inbreeding among more numerous offspring of over-represented parents. As the future broodfish stock grows there may not be sufficient facilities to spawn or house them all. Thus, those fish with lower conservation value should be the first to be released from the program while those with the higher conservation value should be preferentially chosen for spawning.

Methods

Our approach to developing a management plan utilized information available for fish in the future brood program and especially based on information provided by former hatchery manager Herb Bollig and current hatchery manager Keith McGilvray. GPNFH has maintained record of the numbers of broodstock parents, their PIT tag numbers, and parentage of half-sib families by year class (Appendix 1).

We attempted to obtain fin clips from all wild broodstock (Appendix 1). Most tissue samples were already present at SIUC and additional fin clips from fish not represented at SIUC were obtained from the Abernathy Fish Technology Center. All sampled wild broodfish were genotyped at sixteen microsatellite loci as described in Schrey et al. (2007). A matrix of pair-wise relatedness values among all pairs of sampled broodstock fish was computed using the Kinship software package of Goodnight and Queller (1999). Using the same software we generated an equal number of simulated relatedness values using the allele frequencies observed in the wild broodstock.

In November, 2007 Dr. Ed Heist and students Melody Saltzgiver and Josh Geltz visited GPNFH to enumerate and read PIT tags of the captive broodstock fish. Captive broodstock fish at GPNFH are segregated by year class. We started with the older year classes but could not finish in the time allotted. GPNFH personnel finished sampling on a later date. Currently we possess fin clips for every captive broodstock fish. Some captive broodstock fish shed their PIT tags prior to sampling and thus the parentage information, but not the year class, was lost. When we encountered a captive broodstock fish that lacked a PIT tag, a new PIT tag was inserted. When a PIT tag was shed, the parentage, but not the year class information was lost. For year classes 1997-2003 we genotyped the captive broodstock at 13 microsatellite loci and used the Cervus software package of Marshall et al. (1998) to assign parentage (Appendix 2). In assigning parentage we also relied on the known crosses for each year class (Appendix 3) as a means of eliminating some potential parental combinations. If Cervus identified a single male/female pair that combined could account for all of the alleles in the captive broodstock fish we inferred that the parentage had been accurately determined. We allowed potential

parent/offspring pairs to have unshared alleles at one locus to accommodate mutations and genotyping error. If two or more wild broodstock fish of the same sex were potential parents of a particular captive broodstock (i.e. they shared alleles at all loci), we genotyped the captive broodstock fish at three additional loci. Effective population size of the wild broodstock generation was estimated using equations 1 and 2 (below).

Results and Discussion

Hatchery records (Table 1) indicated that 86 wild broodstock (56 Males and 30 Females) were used to produce the captive broodstock fish. Of 86 PIT-tagged fish we were able to obtain clips from 73 (85%). Microsatellite genotypes at 16 loci were determined for all 73 sampled fish. Two male fish bearing PIT tag numbers 7F7F06583D and 7F7D3C555D had a very high relatedness score (r = 0.95) and possessed identical microsatellite genotypes at all 16 loci (the difference between r = 1 and r = 0.95 is due to a correction for the probability that unrelated individuals could share alleles at any particular locus). It appears that the male fish 7F7F06583D spawned in 1997 and 1999 and the male fish 7F7D3C555D spawned in 2004 are the same fish, and that its original PIT tag was lost some time between spawning in 1999 and recapture in 2004. This finding highlights the usefulness of "genetic tags" for identifying individuals that shed traditional tags (Feldheim et al. 2002). Collapsing these two PIT tag individuals into a single broodstock fish leaves us with 72 of 85 unique broodstock sampled and presumably 30 females and 55 males contributing to the captive broodstock. The number of surviving future broodstock fish were tabulated into a spreadsheet included with this report which includes parentage retained from original PIT tags and parentage inferred from microsatellite genotypes for year classes 1997-2003.

The distribution of relatedness values (r) among adult broodstock was unimodal with a mean of 0.001 (Figure 1). There was a very close fit between the distribution of observed relatedness scores and the simulated distribution based on microsatellite allele frequencies at the same 16 loci. This is the relationship that would be expected to occur in a sample drawn from a large randomly mating population. Had there been a number of related pairs of individuals in the wild broodstock population we would have expected to find a secondary peak or higher-than-expected numbers of individuals with positive relatedness scores at around $r = 0.25$ (half-siblings) or $r = 0.5$ (full siblings). Thus the wild broodstock fish appear to be unrelated survivors of a previously larger upper Missouri River stock and that they can all be considered to be as unrelated as a random sample from a large population.

In dioecious animals each sex contributes half of the gametes to the next generation and thus unbalanced sex ratios can reduce effective population size as follows:

$$N_e = \frac{4N_m N_f}{N_m + N_f}, \qquad (1)$$

where N_m is the number of males and N_f is the number of females used to produce the next generation. Based on equation 1 above, and assuming that $N_m = 30$ and $N_f = 50$, N_e is 77.6, well above the minimum N_e of 50 prescribed by Franklin (1980) to avoid initial inbreeding depression.

In the above theoretical definition of the effective population size, it is assumed that gametes are drawn randomly from all breeding individuals, and the probability of each adult

producing a particular gamete equal to 1/*N* where *N* is the number of breeding individuals. However, an actual population may greatly differ from this ideal. For example, there may be a non-random (non-Poisson) distribution of progeny (gametes) per parent because of genetic, environmental, or accidental factors. For example, some birds have strongly determined numbers of eggs in a clutch so the variance of egg number in a clutch may be near zero. On the other hand, if whole clutches or broods survive or perish as a group, then the variance of progeny number may be larger than Poisson. Even more extreme, in some organisms (e.g. sturgeon) with very high reproductive potential, a substantial proportion of the progeny may come from only a few highly successful parents (Hedrick, 2005).

In general, to include variance in the number of progeny, the effective population size is approximately

$$N_e = \frac{N\bar{k} - 1}{\bar{k} - 1 + \frac{V_k}{\bar{k}}}, \qquad (2)$$

where *N* is the number of parents, $\bar{k}$ is the mean number of progeny per parent and V_k is the variance in the number of progeny (Kimura and Crow, 1963; Crow and Denniston, 1988). Therefore, if V_k is kept low, the effects of finite population size causing genetic drift can be avoided to some extent, and the effective population size be maximized. In the proposed broodstock program, in general we will maximize the effective population size, as given in the

equation above, by increasing the number of unrelated parents and minimizing the variance in the number of progeny over parents.

First, we can estimate the effective population size of the wild-caught parents. From the pairwise calculation of relatedness values among these individuals, it appears that they are random sample of the wild population, that is, there is not a significant proportion of close relatives, such as full siblings, in this sample. Note that we estimated the effective population size of the wild-caught parents above but now we are using this estimate as part of a multigenerational broodstock program to conserve genetic variation.

From examination of the families, 30 wild-caught females produced captive progeny (N_f = 30) and 55 wild-caught males produced progeny (N_m = 55). Therefore, using expression 1, the effective number of founders for the broodstock is N_{e1} = 77.6 (where the subscript 1 indicates the founder generation). The expected proportion (P_1) of genetic variation (heterozygosity) of the wild population in the founders can be calculated as

$$P_1 = \frac{H_1}{H_0} = \left(1 - \frac{1}{2N_{e1}}\right) \qquad (3)$$

where H_0 and H_1 are the heterozygosities in the wild population and the founders, respectively. Therefore, assuming N_{e1} = 77.6, and using expression 3, then it is estimated that 99.36 % of the genetic variation in the wild population is present in the 85 wild-caught parents.

To evaluate the impact of using these wild-caught parents to initiate a captive broodstock, we can estimate the effective number of individuals at the same stage a generation later, that is, the effective number of parents in the progeny of the wild-caught parents. In this case, a number of parameters of these individuals are of potential influence, namely the number of female and male parents as before, the mean number of offspring per female ($\bar{k}_f$) and per male ($\bar{k}_m$), and the variance in the number of offspring per female (V_{kf}) and per male (V_{km}) offspring. With this information, the effective number of females (N_{ef}) and males (N_{em}) for this generation can be calculated from the following expressions

$$N_{ef} = \frac{N_f \bar{k}_f - 1}{\bar{k}_f - 1 + \frac{V_{kf}}{\bar{k}_f}} \qquad (4a)$$

$$N_{em} = \frac{N_m \bar{k}_m - 1}{\bar{k}_m - 1 + \frac{V_{km}}{\bar{k}_m}} \qquad (4b)$$

(Lande and Barrowclough 1987).

As an example, assume that the numbers of parents for generation 2 are about the same as the number of founder parents, and there are equal numbers of female and male parents, so that

$N_f = N_m = 43$. Because the total number of individuals in the two generations at this stage are the same, $\bar{k}_f = \bar{k}_m = 2$ (the mean number of progeny per parent in this case is actually 2.02). (In order for an individual to reproduce itself, each parent must have two progeny because each progeny represents only half of each parent). If we assume that the progeny are randomly reproduced from the parents, then the distribution of progeny from parents is Poisson and $V_{kf} = V_{km} = 2$. Using these values in expressions 4a and 4b, then $N_{ef} = N_{em} = 42.5$. The overall effective population size is

$$N_e = \frac{4N_{ef}N_{em}}{N_{ef} + N_{em}} \qquad (5)$$

so that the expected effective population size in generation 2 is $N_{e2} = 85$.

If the number of progeny per parent is more even than expected under random generation of progeny because of an effort to equalize contributions of parents, then the variance can be less than the Poisson variance, or less than 2. For example, if $\bar{k}_f = \bar{k}_m = 2, V_{kf} = V_{km} = 1$, then $N_{ef} = 56.7$, $N_{em} = 56.7$, and $N_{e2} = 113.3$.

Conservatively, let us assume that the distribution of progeny is Poisson, that is $V_{kf} = V_{km} = 2$, and ask what is the impact on the reduction of genetic variation from these two generations. In this case, we can expand expression 3 above as

$$P_2 = \frac{H_2}{H_0} = \left(1 - \frac{1}{2N_{e1}}\right)\left(1 - \frac{1}{2N_{e2}}\right) \qquad (6)$$

Therefore, the expected proportion of genetic variation remaining from the wild populations after this generation is P_2 = (0.9936) (0.9941) = (0.9877) so that nearly 99% of the variation in the wild population should be still remaining in the 86 progeny of the founders selected to parents of the next generation.

We can also calculate the average effective population size over these two generations from the following expression

$$\overline{N}_e = \frac{t}{\sum \frac{1}{N_{ei}}} \qquad (7)$$

(Hedrick 2005). For N_{e2} = 85 and 113.3, $\overline{N}_e$ = 2/[(1/77.6) + (1/85)] = 81.1 and 92.1. In other words, the average effective size per generation is slightly increased compared to that in the first generation.

Or, we can determine what single generation effective population size (N_{eS}) would have the same impact as these two generations using

$$N_{eS} = \frac{1}{2\left[1-\left(1-\frac{1}{2N_{e1}}\right)\left(1-\frac{1}{2N_{e2}}\right)\right]} \qquad (8)$$

Therefore, a single generation with an effective population size of 40.6 would have the same impact on the loss of genetic variation as two generations with N_{e1} = 77.6 and N_{e2} = 85.

In consideration of the difficulty in obtaining unique wild broodstock, the persistence of stocked fish in the upper Missouri that will ultimately reach maturity, and the sufficiently large N_e of the captive broodstock program we conclude that additional sampling of wild broodstock from the upper Missouri is unnecessary. Recent genetic analyses (Heist unpublished) indicate that wild pallid sturgeon from the Missouri River below Gavins Point Dam but above Kansas City Missouri are genetically similar to upper Missouri pallids and in the event of a catastrophic loss of the fish held at GPNFH could be used to supplement stockings in the upper Missouri, although we recommend against such a stock transfer unless absolutely necessary.

Fish managers often limit the numbers of stocked fish introduced into a population with natural reproduction to avoid the Ryman and Laikre (1991) effect. The Ryman and Laikre effect is a reduction of the effective population size in the wild population (N_w) through the introduction of too many offspring from two few parents from a captive population. Because the upper Missouri stock of pallid sturgeon exhibits no natural recruitment, N_w is effectively zero and hence no amount of stocking can reduce it further. However, care should be taken to maximize the captive population size (N_c) that is stocked into the wild through the application of the calculations described in this document. We recommend that in each year crosses should be made among unrelated captive broodstock and that larger families should be culled only as this results in an increase in N_c.

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Table 1. Number of surviving offspring by for each family held at Gavin's Point National Fish Hatchery as of October, 2008.

Female Pit Tag	Male Pit Tag	Spawn Date	Tagging Date	Number of Offspring per Crossing
220E345E09	1F4A111C6A	2001	11/15/2002	17
411D262C1F	17509415139	2001	11/15/2002	24
411D262C1F	411D0B4E09	2001	11/15/2002	11
411D262C1F	411D0E2C5F	2001	11/15/2002	5
411D262C1F	41476A0462	2001	11/15/2002	13
132319571A	7F7D461025	2002	12/7/2004	6
4310187B69	7F7D434B54	2002	12/7/2004	2
116224546A	1F477B3A65	2002	?/? /2004	23
116224546A	116167123A	2002	?/? /2004	33
116224546A	7F7D461025	2002	?/? /2004	27
116224546A	1F4A27214F	2002	?/? /2004	35
116224546A	220F107A6F	2002	?/? /2004	23
116224546A	Unknown	2002	?/? /2004	7
115679394A	1F47760123	2003	6/1/2005	35
132256586A	132114552A	2003	10/6/2004	31
132256586A	132157621A	2003	10/6/2004	27
132256586A	1F47760123	2003	10/6/2004	24
7B7B016070	1F4A13592B	2003	6/1/2005	10
7B7B016070	1F4A363031	2003	6/1/2005	57
7F7B016070	132313521A	2003	10/6/2004	7

7F7B016070	1F521B1E56	2003	10/6/2004	32
7F7B016070	41475D3C5D	2003	10/6/2004	31
7F7B016070	7F7D291A07	2003	10/6/2004	33
7F7F054855	115669540A	2003	10/6/2004	25
7F7F054855	115675486A	2003	10/6/2004	53
7F7F054855	132313521A	2003	6/1/2005	47
7F7B016070	Unknown	2003	?/? /200?	3
114476216A	116167123A	2004	7/20/2005	30
114476216A	1F477B3A65	2004	7/20/2005	30
114476216A	1F4A27214F	2004	7/20/2005	30
114476216A	1F4A312640	2004	7/20/2005	30
114476216A	1F4A4B5973	2004	7/20/2005	30
114476216A	220F107A6F	2004	7/21/2005	30
114476216A	430E452777	2004	7/20/2005	30
114476216A	431565767B	2004	7/20/2005	32
114476216A	7F7D487531	2004	7/20/2005	30
114476216A	7F7E55466D	2004	7/21/2005	30
115551683A	115552116A	2004	7/20/2005	29
115551683A	7F7D3C555D	2004	7/20/2005	29
115551683A	Unknown	2004	7/20/2005	1
115555495A	431565767B	2004	7/20/2005	30
132211792A	1F4A312640	2004	8/17/2005	29
132211792A	1F4A3E1445	2004	8/17/2005	28

132211792A	7F7E42795C	2004	8/17/2005	25
132211792A	132235554A	2004	8/17/2005	26
1F5330401E	MIX	2004	7/20/2005	25
1F5330401E	MIX2	2004	7/20/2005	24
454910202B	115679374A	2004	7/20/2005	23
454910202B	1F47606357	2004	7/20/2005	22
454910202B	220F0F7677	2004	7/20/2005	21
454B380D60	1F4A3E1445	2004	7/20/2005	20
454B380D60	7F7D376F73	2004	7/20/2005	19
454B380D60	7F7F065834	2004	7/20/2005	18
7F7F066452	114473737A	2004	7/20/2005	17
7F7F066452	1F4A3E1445	2004	7/20/2005	16
7F7F066452	7F7F065834	2004	7/20/2005	15
4443240458	115633183A	2005	8/8/2006	14
4443240458	444334021A	2005	8/8/2006	13
115557165A	1F50072169	2005	8/8/2006	12
115557165A	7F7B031F17	2005	8/8/2006	11
115557165A	7F7D2D723D	2005	8/8/2006	10
115676635A	1F50072169	2005	8/8/2006	9
115676635A	7F7B031F17	2005	8/8/2006	8
115676635A	7F7D2D723D	2005	8/8/2006	7
132213574A	1F482F3F2B	2005	8/8/2006	6

Figure 1. Distribution of observed and simulated relatedness values (*r*) among upper Missouri River wild pallid sturgeon broodstock. Two individuals with identical genotypes were removed.