Population Genetics Management Plan for Pallid Sturgeon in the Upper Missouri River Basin

January 30, 2013

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Final Report to: Western Area Power Authority

Introduction

Pallid sturgeon (*Scaphirhynchus albus*), are highly endangered throughout their range and were listed under the Endangered Species Act as endangered in 1990 (Dryer and Sandvol 1993). Pallid sturgeon are especially imperiled in the upper Missouri River basin where no natural recruitment has occurred for several decades and the few remaining wild pallid sturgeon are very old (Webb et al. 2005). Hatchery propagation was implemented in the upper Missouri River to prevent extinction of the species which was predicted to occur in the early decades of the 21st century as the remaining wild pallid sturgeon died off (Kapuscinski 2002). These efforts have been successful in increasing the numbers of upper Missouri River basin pallid sturgeon due to high survival of a large number of hatchery produced fish stocked into the wild. Hatchery reared offspring are also maintained in the Gavins Point National Fish Hatchery (GPNFH) Captive Broodstock Program. Thus, the upper Missouri River population of pallid sturgeon is no longer in imminent risk of demographic extinction. However, if a relatively small number of wild adults contributed large numbers of offspring to both the captive broodstock program and the wild stockings, the next generation of upper Missouri River pallid sturgeon may have reduced fitness and evolutionary potential due to a loss of genetic variation and the potential for inbreeding.

The use of genetic tools for monitoring and assessment of pallid sturgeon restoration has been well integrated into the recovery program for purposes such as identification of *Scaphirhynchus* species and their hybrids (Schrey et al. 2011; Schrey et al. 2007; Tranah et al. 2004; Tranah et al. 2001), identification of hatchery produced fish (DeHaan et al. 2008), and to evaluate hatchery practices (Saltzgiver et al. 2012). The availability of individually-based genetic data along with existing data on propagation, and stocking history for pallid sturgeon in the upper Missouri River basin recovery priority management areas (RPMAs) 1-3 allows for evaluation of reproductive contribution and determination of potential of inbreeding through artificial propagation. Integration of these genetic monitoring results can be used to guide the recovery program to achieve the goals of maintaining genetic diversity, and also provide guidance to continued stocking, hatchery, and management efforts within the upper Missouri River basin. However, it must be realized that the molecular markers used to make these assessments (chiefly microsatellites) presumably have no direct effect on the fitness of the sturgeon. Thus, it makes no sense for managers to focus on conserving microsatellite alleles per se. They are, however, indicators of how processes such as genetic drift, gene flow, mutation, and overall maintenance of diversity between generations are occurring in the surveyed populations. Because these markers are inherited from both parents, microsatellite data allow us to estimate levels of genetic diversity and relatedness and/or parentage to design hatchery crosses because patterns at such loci are expected to reflect levels of the loss of genetic variation and the amount of inbreeding that that may be occurring over the entire genome.

In this management plan, we provide guidance for the genetic management of upper Missouri River pallid sturgeon including the use of additional wild broodstock fish, offspring of wild broodstock fish in either the GPNFH Captive Broodstock Program or stocked into the upper Missouri River basin, cryopreserved sperm, and incorporation of hatchery activities in the pallid sturgeon recovery and

restoration program. This plan covers only the Great Plains Management Unit (GPMU) which comprises three recovery priority management areas (RPMAs). RPMA 1 includes the Missouri River above Fort Peck Dam, RPMA 2 includes the Yellowstone River and Missouri River below Fort Peck Dam and above Garrison Dam, and RPMA 3 includes a short stretch of riverine habitat above Lewis and Clark Lake (USFWS 2007). The regions formerly classified as RPMAs 4-6 are currently divided into three management units. The Central Lowlands Management Unit (CLMU) includes the Missouri River from Gavins Point Dam to the mouth of the Grand River. The Interior Highlands Management Unit (IHMU) includes the Missouri River below the mouth of the Grand River and the Mississippi River above the confluence with the Ohio River. The Coastal Plains Management Unit (CPMU) includes the Mississippi River below the confluence with the Ohio River and the Atchafalaya River. While this plan only covers the GPMU, we discuss the potential genetic effects of fish transfers among management units. To develop these guidelines, propagation and stocking records, and estimates of genetic diversity for pallid sturgeon in the upper Missouri River basin RPMAs 1-3 were evaluated with well-established principles for genetic management of hatchery propagation (Allendorf and Luikart 2007; George et al. 2009; Hallerman 2003). The recommendations provide guidance for decision making regarding the capture of wild broodstock, use of captive broodstock for reproduction, and spawning and stocking strategies, including which fish should have high priority for future spawning and which should not be spawned again. Recommendations are based on the goal of maintaining as much as possible of the natural genetic variation present in the native upper Missouri River basin pallid sturgeon so that future pallid sturgeon generations do not suffer from inbreeding depression, outbreeding depression, or domestication and retain sufficient genetic variation so that they can adapt to changing conditions.

For the purposes of this plan, in describing pallid sturgeon generations the remaining wild parental population is designated as the P_0 generation, their offspring as the F_1 generation, and subsequent generations as F_2, F_3 etc. (see Figure 1). Pallid sturgeon were first spawned and propagated in 1992. Because of the long generation length of pallid sturgeon, production of the F_1 generation, which has already been going on for two decades, may continue for years to come through the use of cryopreserved sperm, continued spawning of wild adults, and through the use of genetic parentage analysis to identify wild adults previously spawned but whose offspring have not been detected through subsequent surveys in the wild (or in the captive broodstock). Of particular concern is when and how to produce the F_2 generation. In this report we describe additional information needs required prior to spawning an F_2 generation and guidelines as to how to best accomplish broodstock identification to maintain the genetic diversity present in the F_1 generation and to achieve recovery program goals.

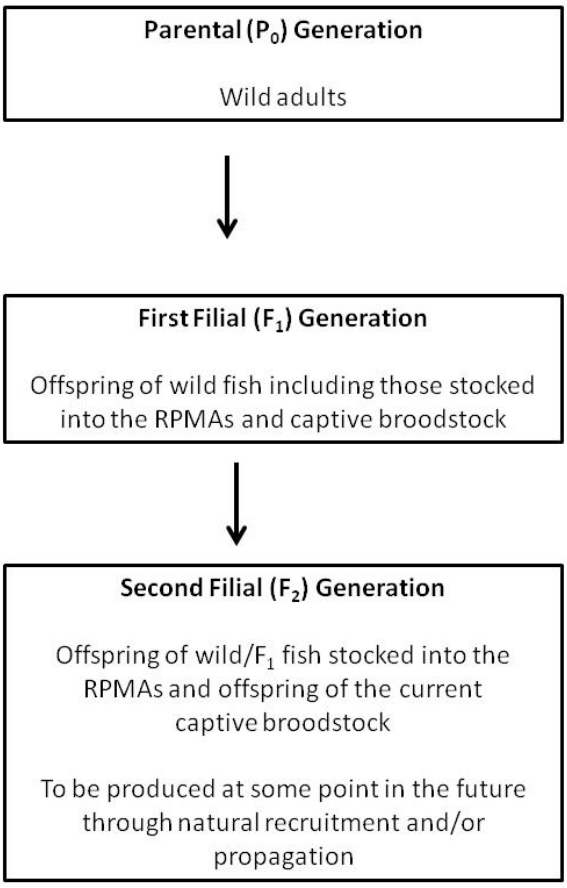

Figure 1. Upper basin pallid sturgeon generations referred to in this document.

1. Effective Population Size (N_e) , Drift, and Inbreeding

Genetic drift, inbreeding, and directional selection are the primary factors that result in the loss of genetic variation from populations. Genetic drift is the random change in allele (gene) frequencies between generations. This results because random sampling error causes the alleles of some parents to be passed to offspring more frequently than others and also because some parents produce more offspring than others. Inbreeding occurs when an individual's parents are related. Because the population of GPMU pallid sturgeon is closed, over time individuals are expected to become progressively more related and their offspring progressively more inbred and these processes are magnified when the population size is small.

Inbreeding not only results in a loss of genetic diversity but also possibly a reduction in fitness, which is known as inbreeding depression. Examples of traits associated with inbreeding depression in fishes include reduced hatching success, reduced survival rate, reduced growth rate, increased frequency of physical abnormalities, and impaired reproduction (Hallerman 2003; Waldman and McKinnon 1993). Directional selection occurs when an allele at one or more loci is deterministically favored over others. In captive broodstock, this not only results in a loss of genetic diversity but, also adaptation to the captive environment (domestication) through increased survival or reproduction by individuals that are more fit in the hatchery. While inbreeding coupled with artificial selection is a practice that animal breeders often use to alter commercially favorable traits in domesticated animals, this is not advisable for conservation and restoration programs. In contrast, such programs should strive to keep levels of genetic variation and phenotypic traits present in an established population as similar as possible to the native population from which it was established. Under this scenario, clearly genetic drift, inbreeding, and domestication must be avoided.

Geneticists use an index called "effective population size (N_e)" to estimate the rate at which a population loses genetic diversity and becomes inbred due to genetic drift. Effective population size can be defined as "the size of an ideal population (N) that will result in the same amount of genetic drift as the actual population being considered " (Allendorf and Luikart 2007). An ideal population is one in which all members are adults, the sex ratio is equal, and the variance in reproductive success among individuals is equal to the mean number of offspring produced per individual. The first two conditions, all members being adults and equal sex ratios reflect the fact that only adults contribute alleles to the next generation and that in the next generation half of the alleles come from the males and half from the females. Following these assumptions, N_e can be calculated by

$$N_e = \frac{4N_m N_f}{N_f + N_m} \qquad \text{Equation 1}$$

where N_m is the number of male parents and N_f is the number of female parents. When an equal number of males and females are used, $N_e = N_m + N_f$ = the number of breeders. For this equation to accurately describe the amount of genetic drift, the reproductive variance of both sexes must be equal to the number of offspring per parent. In a population of constant size, over multiple generations each parent has on average two offspring that survive to maturity. Figure 2 diagrams the expected number of adult offspring produced under this scenario with the variance in the number of offspring produced is also two as required by equation 1. Clearly this outcome would not be typical of pallid sturgeon, or most fish populations, where most adults probably have no offspring that survive to maturity and a few may have hundreds or perhaps thousands that survive to maturity. Thus, historically N_e was very likely less than the number of breeders. With captive breeding, however, this variance in reproductive success can be controlled to maximize N_e for a given number of parents.

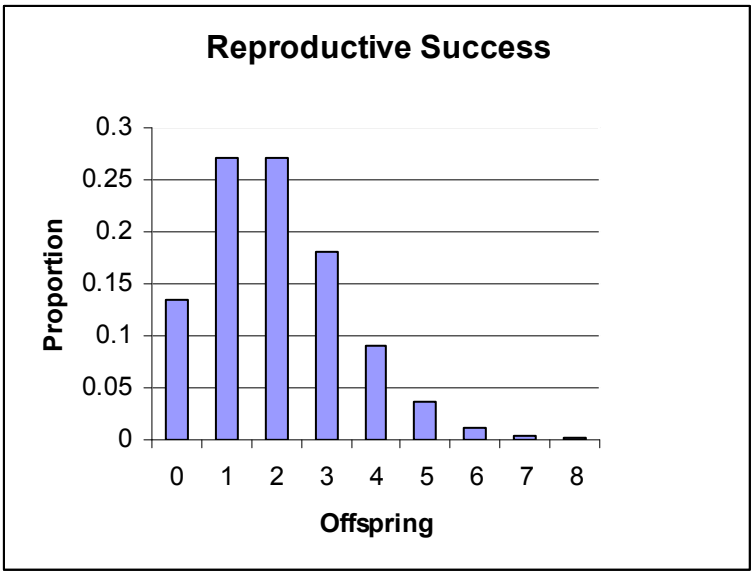

Figure 2: Distribution of the number of adult offspring produced assuming a mean and variance reproductive success of 2 as is required for equation 1 to accurately represent the amount of genetic drift.

If there is a great disparity in the number of offspring per parent that survive to reproduce, then the reproductive variance is high. In this case, the effective population size may be much smaller, perhaps orders of magnitude smaller, than the number of breeders. In the following equation N is the number of breeders, and variances in reproductive success of males and females are represented by V_{km} and V_{kf}, respectively.

$$N_e = \frac{8N}{4 + V_{km} + V_{kf}} \qquad \text{Equation 2}$$

Note that as the variances increase the value of N_e declines precipitously (Figure 3). However, if the variance terms are set to zero, meaning that every male and every female has an identical number of successfully reproducing offspring, N_e is actually twice the number of breeders. Species with low fecundities and low reproductive variances have N_e values similar to the number of breeders while those with high fecundities and high reproductive variance may have effective population sizes orders of magnitude smaller than the number of breeders. For example sandbar sharks, which typically produce no more than 12 well-developed offspring in a season, have N_e estimates that are very close to the number of breeders (Portnoy et al. 2009). Effective population sizes of female endangered razorback suckers were estimated at 29-38% of the number of female breeders (Turner et al. 2007). N_e for highly fecund red drum was approximately 1/1000th the number of breeders (Turner et al. 2002) while oysters have an N_e that is on the order of 1/1,000,000 the number of breeders (Hedgecock 1994) . Thus, depending on the reproductive variance, N_e can be slightly greater than the number of breeders but it is

typically smaller and sometimes only a tiny fraction of the number of breeders. However, in managed situations such as captive breeding, variance in reproductive success can be controlled to maximize N_e for a given and often limited number of breeders.

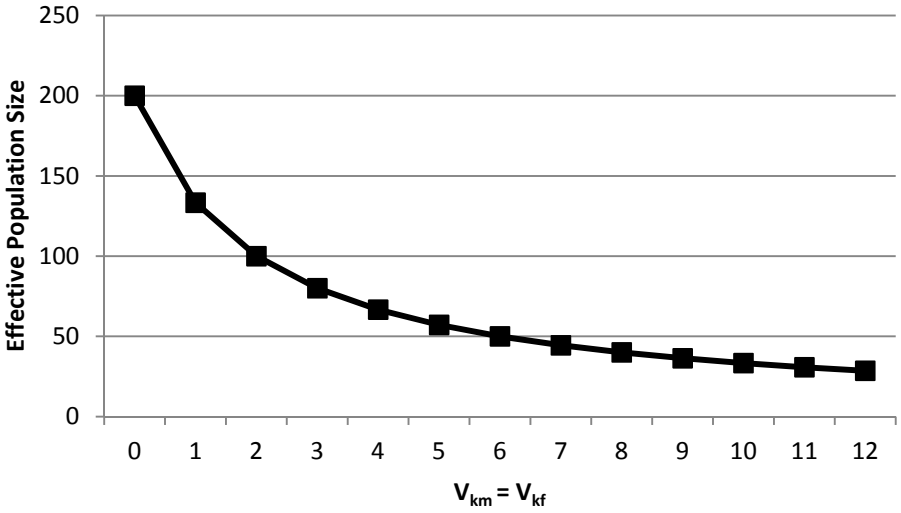

Figure 3. Relationship between effective population size (N_e) and reproductive variance assuming a parental population of 50 males and 50 females and equal reproductive variance for both sexes following equation 2 above. Note that N_e is twice the number of breeders when the variances are zero, equal to the number of breeders when the variances are 2, and less than the number of breeders for when the variances are greater than 2.

A goal of a conservation genetics program should be to reduce genetic drift and thus maintain allele frequencies as similar to the P_0 generation (the original wild fish) as possible. N_e is really just an index for estimating the amount of genetic drift occurring in a population. Estimates of N_e are sensitive to assumptions that may not be realized. Thus, it might be better in terms of managing upper basin pallid sturgeon to think in terms of reducing genetic drift, rather than trying to estimate N_e. What we really need to know is how much allele frequencies have changed between the P_0 and the F_1 generation which depends to some extent on how we define (or select) the F_1 generation. For example, only mature individuals count in estimates of N_e (see definition of an "ideal population above") yet very few of the F_1 wild fish are currently mature. Those that are mature are offspring of only a small number of P_0 parents spawned in the early years of the propagation program. Thus, N_e calculated based on them would be small and significant allele frequency changes would be observed between the mature F_1 fish and all potential P_O parents. As more and more F_1 fish become mature, N_e will increase and the amount of allele frequency change will decrease over time. Managers can produce a large N_e and hence little

allele frequency change if the F_1 and F_2 generations are produced by spawning a large number of unrelated adults and reducing variance in reproductive success among them. At this time, it appears a sufficient number of P_o parents were used to produce offspring for the captive broodstock program (Saltzgiver et al. 2012) and stocking into the RPMAS (this report) and that levels of genetic diversity are very similar between the P_o and potential F_1 generation. Following the guidelines in this report should maintain this level of genetic variation in successive generations of pallid sturgeon.

What should be the long-term N_e for upper basin pallid sturgeon? It is impossible to predict the level of inbreeding that a population can withstand before exhibiting the effects of inbreeding depression (Edmands 2007). Franklin (1980) recommended that for a single generation, a minimum N_e of 50 was sufficient to prevent significant inbreeding but, that long term N_es should be at least 500 to conserve allelic variation over many generations. Lande (1995) argued that an N_e of at least 5000 was needed to allow for natural selection to overcome genetic drift and purge deleterious alleles from populations over many generations. But populations with historically low N_e may have already purged deleterious mutations and may be more resilient to inbreeding depression than historically large populations (Sheffer et al. 1999) so Lande's estimate may be over conservative. Thus, there is no consensus on what N_e managers should strive to maintain.

If we assume that N_e in pallid sturgeon is about 10% of the adult population size, which is the approximate average taken over many species (Frankham 1995; Palstra and Ruzzante 2008), then 500 mature fish would be required to produce an N_e of 50 and 5000 an N_e of 500. Prior to the construction of dams it is likely that the pallid sturgeon in what are now recognized as RPMAs 1-2 constituted a single population. For purposes of conserving genetic variation of pallid sturgeon, therefore, we consider the wild adults in RPMA 1 and 2 to be a single population. Likewise, offspring of adults captured in RPMAS 1-2 stocked into RPMAs 1-3 are considered members of a single population. We discuss the small number of wild adults captured from RPMA 3 in the next section. We do not know what the carrying capacity of RPMAs 1-3 is but, feel it is certainly above 500 so it should be quite feasible to avoid significant loss of genetic variation and inbreeding in future sturgeon generations.

We recommend that when the F_2 generation is produced, adults of the F_1 generation are selected such that the N_e of the P_0 generation is at least 100 but ideally 250. Additionally, the F_1 generation represents individuals from multiple year classes over many years, so this estimate reflects multiple years of spawning both through artificial propagation and potentially in the wild. Either physical tags or genetic tags can be used to identify the parentage of each F_1 broodstock and the parentage of the F_1 broodstock can be used to calculate the N_e of the P_0 generation following the equations presented in Saltzgiver et al. (2012). For example, following equations 2a-2c in Saltzgiver et al. (2012), if the F_2 generation is produced by spawning 600 fish taken from the wild or the captive broodstock program that are of offspring of 110 wild males and 50 wild females with a reproductive variance of 4, the effective population size of the P_0 generation would be 145. Because of the longevity and long generation length of pallid sturgeon, this could be accomplished by spawning, on average, 20 unique males and 10 unique females each year for 20 years.

Recommendation 1 – Effective population size of pallid sturgeon in the upper basin Missouri River RPMAs (1-3) will depend on future spawning practices and potentially natural reproduction. Since the latter is likely to be minimal at best, it can be made sufficiently large provided that a large number of F_1 broodstock that are offspring of a large number of the P_0 parents are used to produce the F_2 generation. We recommend that when the F_2 generation is produced, F_1 broodstock are chosen such that the N_e of the P_0 generation is at least 100 but ideally 250. Offspring should be distributed to RPMAs 1-3 such that parental contributions are balanced to maximize N_e in the event of significant natural recruitment.

2. Outbreeding When fish are transferred to a different region or drainage from the one in which they are adapted, fitness in native populations may be lost through outbreeding depression which has two causes: loss of adaptation and loss of coadaptation (Templeton 1986). Loss of adaptation occurs when a fish is transferred to an environment that is ecologically different from the one to which it was evolutionarily adapted in terms of climate, flow, water quality, competitive interactions or other variables that have resulted in heritable selection. In such situations, a reduction in fitness may occur, if the introduced fish hybridize with the native fish. Differences in adaptation can develop over a relatively small geographic range especially if there is substantial ecological divergence. For example Philipp and Claussen (1995) found that reciprocally translocated largemouth bass stocks from northern and southern Illinois had significantly reduced growth rates compared to locally translocated stocks, while the performance of both were similar in central Illinois. Philipp and Claussen (1995) attributed these differences in performance to different climate regimes between southern and northern Illinois.

Coadaptation occurs when selection has favored certain combinations of alleles among loci in populations leading to increased fitness. When these combinations differ between populations, hybridization can result in a reduction in fitness regardless of the environment. Coadaptation can be difficult to demonstrate because when fish from different stocks are hybridized the first (F_1) generation hybrids have one half of their genome from the parentals and coadapted alleles may not be broken up. Furthermore, the F_1 may have enhanced fitness due to an increase in genetic diversity especially if the parentals are inbred. In the F_2 generation, alleles at particular genes from the different parental genomes begin to become mixed up and it may not be until this time that outbreeding becomes apparent. Pink salmon, which have a strict two-year life cycle and thus exist as genetically isolated odd-year and even-year stocks in the same river provide an elegant model species for the study of outbreeding depression. Gharrett et al. (1999) used cryopreserved sperm to produce F_1 hybrids between odd- and even-year pink salmon and then used returning F_1 salmon to produce F_2 hybrids. They found that F_1 salmon returned in similar numbers as controls, but F_2 salmon had very low return rates indicating that they were much less fit than either controls or F_1 fish adapted to the same river.

Pallid sturgeon exist across a vast latitudinal range and the environments vary greatly between the headwaters of the Missouri and the mouth of the Mississippi. Across that range pallid sturgeon differ in morphology (Murphy et al. 2007), growth (Killgore et al. 2007), fecundity (George et al. 2012), and genetics (Campton et al. 2000; Schrey and Heist 2007). Ed Heist, George Jordan and Kim Chojnacki

are involved in ongoing research of how allele frequencies vary geographically among pallid sturgeon. Microsatellite data were analyzed using the software package Structure (Pritchard et al. 2000). This algorithm identifies how many genetic groups (K) most parsimoniously describe a collection of genotypes without regard to the geographic location of the individuals sampled and simultaneously estimates what fraction of an individual's genotype is derived from each of the K populations. Structure identified pallid sturgeon collected from the GPMU, CLMU, and IHMU as most likely forming three groups (Figure 4). Nearly all fish from the GPMU assign strongly to the "green" group, the "yellow" group achieves prominence in the CLMU, while fish from the "blue" group are found in the extreme lower Missouri and middle Mississippi regions of the IHMU. The green fish in the CLMU and IHMU are likely hatchery releases from GPMU parents that have shed their physical tags. Not all hatchery fish can currently be identified using microsatellite loci because not all broodstock used to produce fish for propagation were fin-clipped and genotyped. The same investigators are also reconstructing as many genotypes of the unsampled broodstock as possible using known hatchery fish, and as genetic samples become available they are added to the database by the USFWS NEFC Conservation Genetics Lab.

The P_0 generation of adults, which were used in the analysis in Figure 4, are perhaps as old as or older than the dams that separate the upper and lower basins. The amount of genetic drift sufficient to produce the patterns evident in Figure 4 would take many sturgeon generations to develop. Thus, the observed population structure in pallid sturgeon most likely predates anthropogenic changes to the Missouri River. The genetic data indicate that pallid sturgeon populations were historically at least partially isolated while the morphological and life-history data indicate that pallid sturgeon may be locally adapted. Thus, mixing of pallid sturgeon stocks may result in outbreeding depression through loss of adaptation in the F_1 generation and through loss of coadaptation in the F_1 and subsequent generations. Therefore, transfers of pallid sturgeon into and out of the GPMU should be avoided. Wild fish from RPMA 3 and Lake Sharpe, which are geographically closer to the upper reaches of the CLMU than the lower limit of RPMA 2, deserve particular scrutiny. Heist (2007) found that of the several wild pallid sturgeon captured there, one was "yellow" and the rest were "green."

Inbreeding depression in managed populations due to low effective population size may be ameliorated if individuals are brought in from other populations resulting in a process known as genetic rescue (Hedrick and Fredrickson 2010). However, genetic rescue should only be attempted if inbreeding depression can be clearly demonstrated and the risks of outbreeding are small relative to the risks of continued inbreeding. Because there is no evidence of inbreeding in upper basin pallid sturgeon and there is a potential risk of outbreeding depression, genetic rescue is not warranted at this time.

Recommendation 2. Because a sufficient N_e can be maintained using only fish from the GPMU and because there is potential for outbreeding depression with stock transfers, pallid sturgeon should not be transferred out of or into the GPMU. We recommend that RPMA 3 continue to be stocked only with fish of upper basin origin, since that is what has already been stocked there, and that wild fish from RPMA 3 that are "green" be considered potential broodstock for the upper basin while those that are "yellow" be considered as broodstock for the CLMU.

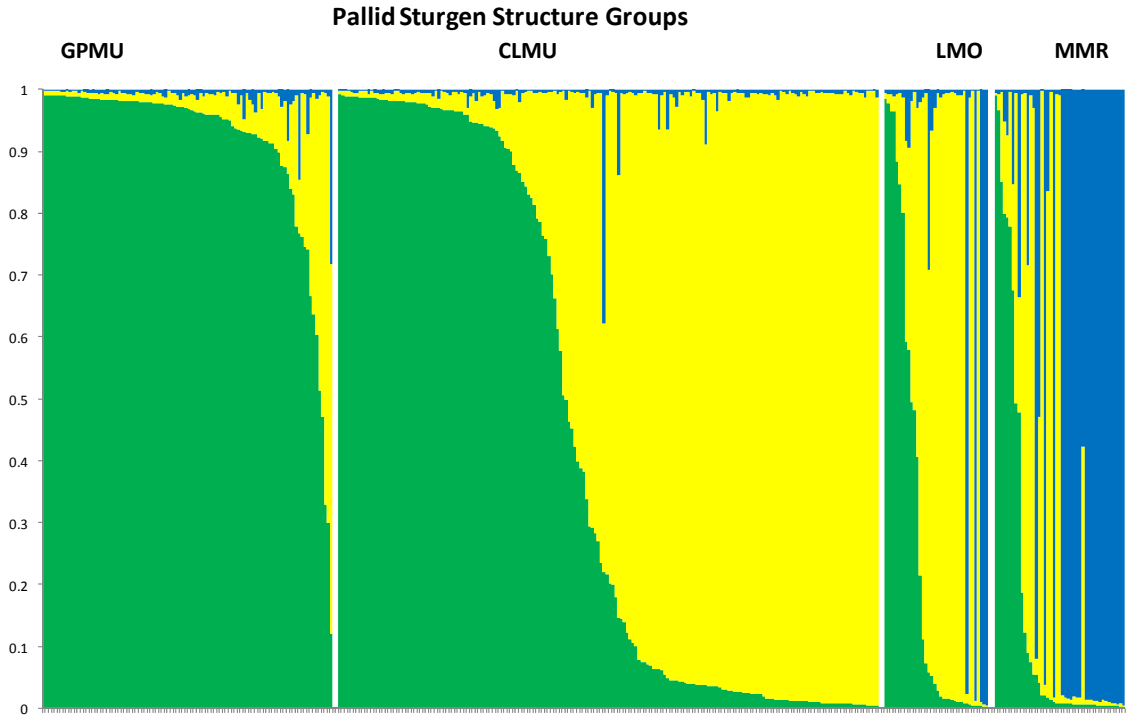

Figure 4. Structure assignments of 367 pallid sturgeon from the RPMAs 1 and 2 of the Great Plains Management Unit (GPMU), the Central Lowlands Management Unit (CLMU), the lower Missouri River (LMO) and middle Mississippi River (MMR) portions of the Interior Highlands Management Unit. Each of the 367 bars indicates the fraction of each individual's genotype attributed to the green, yellow, and blue structure groups. Many of the "green" fish in the CLMU, LMO, and MMR are likely untagged hatchery fish of upper basin origin. Data presented at the 2010 Pallid Sturgeon Recovery Team meeting by Ed Heist.

3. Domestication and Artificial Selection

Domestication is adaptation to the hatchery environment. While domestication can be beneficial to hatchery propagation of fishes for human consumption or the ornamental trade by altering reproductive traits including fecundity, timing, and frequency of spawning (Teletchea et al. 2009), domestication can have very negative effects in propagation to restore native stocks. Effects of domestication observed in Alaskan salmonids include reduced survival and reproduction in the wild, reduced aggression, reduced predator avoidance, impaired homing, and also changes in egg size, growth

rate, age at maturity, and timing of spawning (Reviewed in Grant (2012)). Christie et al. (2012) found that domestication in steelhead trout can occur within a single generation, with offspring of hatchery-reared parents outperforming those of wild parents in the hatchery, and a negative correlation between a family's reproductive success in the hatchery and its success in the wild. In situations where artificial propagation is necessary for conservation and management of small populations, efforts are necessary to minimize potential impacts of domestication to the population being maintained.

Guidelines to reduce domestication in hatchery stocks include spawning only wild fish, maintaining captive broodstock (if necessary) for no more than one generation, making rearing conditions as similar to wild conditions as possible, and releasing fish at the earliest stage possible to allow for natural selection, rather than hatchery selection, to better select which individuals survive to maturity and reproduce (Miller and Kapuscinski 2003). The pallid sturgeon recovery program currently stocks juvenile pallid sturgeon at a variety of life stages to minimize the time spent in captivity, and focuses reproductive efforts on river-caught spawners captured just prior to spawning in the hatchery environment. However, some management activities such as implementation of a captive broodstock program are necessary to retain genetic diversity within the population though potentially increasing the risk of domestication. We recommend that most of the pallid sturgeon used to produce the F_2 generation should come from F_1 parents that matured in the wild and represent as many P_O parents as possible. Furthermore, efforts should be taken to minimize as much as possible the reproductive variance among the individuals used to produce the F_2 generation. Only captive broodstock from those individuals that contain genes from under-represented P_0 adults should be spawned and stocked to continue to produce the F_1 generation. This does not mean that offspring of these parents should not be incorporated into producing the F_2 generation, only that sufficient wild broodstock from these families will be available and are preferable as described in section 9 below.

Below we identify which captive broodstock should not be spawned to continue to produce the F_1 generation. However, these may be used to produce offspring for research including improvements of hatchery practices. Managing for effective population size by reducing the reproductive variance will also help reduce domestication by limiting selection. There is a trade-off associated with releasing offspring at an early stage to reduce domestication and the need for fish to reach a minimum size before they can be physically tagged. Because it is important to keep track of the individuals as a means of reducing reproductive variance we suggest keeping most fish just long enough to physically tag them prior to release.

Recommendation 3. Captive broodstock from over-represented P_0 parents should not be spawned for continued F_1 production but may be spawned to produce fish for research. When the F_2 generation is produced, F_1 offspring that matured in the wild should be preferentially used over captive broodstock. Offspring should be reared under conditions as natural as possible and released at the earliest practical size to reduce domestication but allow for future identification.

4. Relatedness and Spawning Pair Selection

Annual spawning guidance for the upper basin pallid sturgeon program is conducted by the USFWS NEFC Conservation Genetics Lab, and is based on microsatellite data which is used to estimate r_{xy} following the approach of Queller and Goodnight (1989). Relatedness scores between fish "X" and fish "Y" (r_{xy}) are based on Hamilton's (1964) relatedness index (R) in which the R-value corresponds to the fraction of alleles two individuals share that are inherited from a recent common ancestor. Examples of R-values are 1.0 for clones or identical twins, 0.5 for parent/offspring pairs and full siblings, 0.25 for half-siblings, and 0.125 for first cousins. Because the number of alleles are finite in a population, two individuals that share an allele, especially a common allele, did not necessarily inherit that allele from a recent common ancestor. The algorithm considers not just the number of alleles shared, but also the population frequencies of the shared alleles and scales r_{xy} based on the expectations of background allele sharing (i.e. the fraction and frequency of alleles that are expected to be shared by unrelated individuals simply by chance). Thus, it is possible for two individuals to have a negative r_{xy} value if their level of allele sharing is less than the expected value and in a population of unrelated individuals we would expect about half to have negative r_{xy} values.

Current protocols for crossing wild pallid sturgeon require a microsatellite-derived relatedness score (r_{xy}) of 0.2 or less for a potential cross (Bartron and Kalie 2012). The $r_{xy} < 0.2$ criterion is applied to prevent the production of inbred offspring. While inbreeding depression is a well documented phenomenon, it is difficult to know what level of inbreeding will result in inbreeding depression in a particular species and population (Edmands 2007). However, there are numerous examples of inbreeding depression in fishes following a single generation of full-sibling mating (Waldman and McKinnon 1993). There are also many examples of no detectable inbreeding depression at higher levels of inbreeding (Waldman and McKinnon 1993). However, measuring inbreeding depression is especially challenging in captive populations in part because the conditions of husbandry may mask effects that may be more profound in the wild (Kalinowski and Hedrick 1999). Species that have experienced numerous bottlenecks in the past may purge deleterious genes and thus be more resistant to inbreeding depression (Sheffer et al. 1999).

Saltzgiver et al. (2012) used the program Kinship (Goodnight and Queller 1999) to compare the r_{xy} values among 100 wild adult P_0 pallid sturgeon from the upper basin with expected relatedness values based on the allele frequencies. If the original wild population contained large numbers of full- or half-siblings there would be modes in the distributions near $r_{xy} = 0.25$ and $r_{xy} = 0.5$, but none were seen. This was interpreted as indicating that the remaining wild fish are mostly unrelated (Saltzgiver et al. 2012). We modified those results using data from only the 17 loci currently employed by the USFWS Northeast Fishery Center (NEFC) and added the expected distributions given that the true relationships among fish were half-siblings (R = 0.25, blue) or full-siblings (R = 0.5, green). While the median values within the distribution are very close to the expected (median $r_{xy} = -0.001$ for unrelated, 0.249 for half-sibling, and 0.505 for full-sibling), there is considerable variance and the ranges of the estimated r_{xy} values for unrelated, half-sibling, and full-siblings overlap. The variation in the estimates indicates that with the current suite of microsatellite loci used that the estimates are not very precise. Even among unrelated individuals high r_{xy} values indicating close relatedness can occur. The precision could be

increased through the use of more loci, and especially through the use of additional loci with higher levels of variation (Blouin et al. 1996). Using the current criterion of $R_{xy} < 0.2$ and the simulations presented in Figure 5, 98% of full-sibling and 63% of half-sibling crosses would be avoided, while 9.1% of unrelated individual crosses would be spuriously prohibited because r_{xy} estimates would exceed 0.2. Based on the high power to avoid full- and half-sibling crosses balanced by the relatively low number of crosses among unrelated individuals that are erroneously excluded we believe that the r_{xy} <0.2 criterion is appropriate.

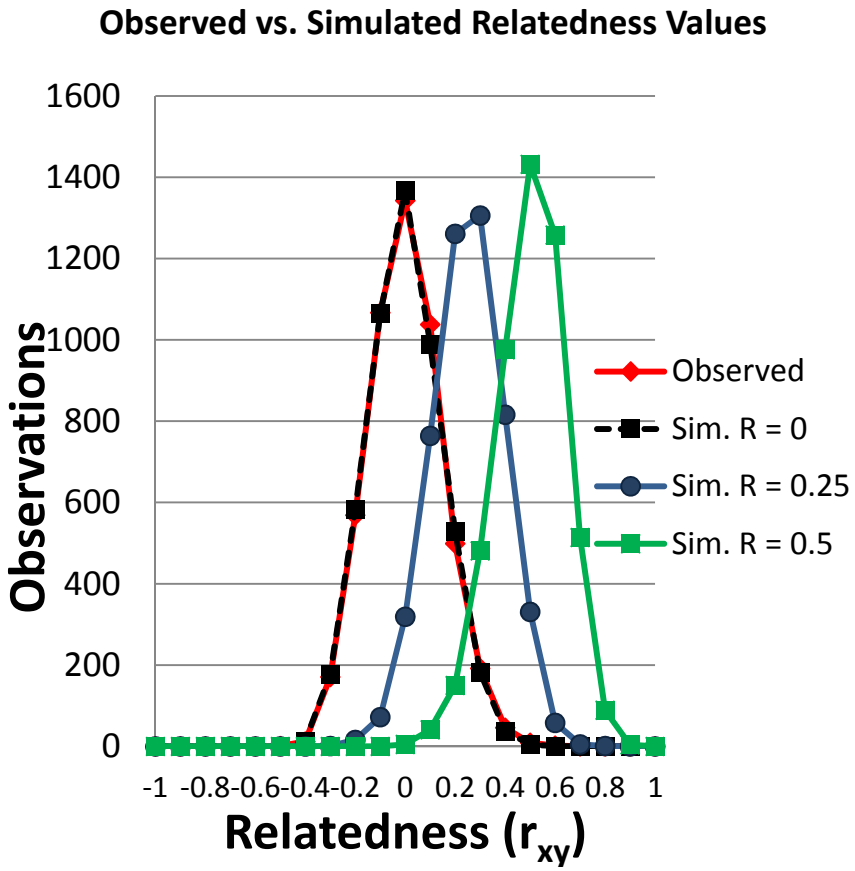

Figure 5. Distributions of observed r_{xy} values among 100 upper basin pallid sturgeon and simulated distributions of r_{xy} values assuming true relatedness scores of 0, 0.25, and 0.5 using allele frequencies at 17 microsatellite loci in upper basin pallid sturgeon.

Recommendation 4. The policy of not crossing two upper basin pallid sturgeon unless r_{xy} <0.2 is appropriate and should be continued.

5. Estimates of genetic diversity

Estimates of genetic diversity are generated annually using data resulting from the juvenile and adult pallid sturgeon monitoring and broodstock collection in the upper Missouri River basin. The genetic data are primarily used to determine species of origin (pallid or shovelnose sturgeon, or a hybrid thereof), to determine if unmarked individuals identified as pallid sturgeon are of hatchery origin through genetic determination of parentage, and to estimate relatedness to avoid crosses among related individuals (see Bartron and Kalie (2012) for a description of methods). These assessments have been conducted by the USFWS Abernathy Fish Technology Center, and since 2008, the NEFC (Bartron and Kalie 2012). In addition to species and origin determination, genotypic data from pallid sturgeon are used to generate estimates of genetic diversity. These estimates can be used to determine how much genetic diversity is being maintained within the pallid sturgeon progeny. The data could also be used to estimate effective population size by examining differences in allele frequencies due to genetic drift between the P_0 and F_1 generations although as discussed earlier actually defining the F_1 generation will be problematic.

Comparison of estimates of genetic diversity between the juvenile pallid sturgeon produced in the hatchery (F_1) and native adults captured for broodstock (P_O) indicate that genetic diversity is generally being retained between the adults and offspring (Table 1). Mean number of alleles per locus are identical between the groups. There is very little difference between the groups for average expected and observed heterozygosity. The inbreeding coefficient (f) which is expected to be zero in a non-inbred population and positive in an inbred population is slightly above zero, but probably not significantly so in both groups.

Table 1. Estimates of genetic diversity for all adults that have been sampled to date, and for all juveniles genotyped up to and including those sampled in 2011.

Sample group	Sample size	Mean number of alleles per locus	Expected Heterozygosity	Observed Heterozygosity	Inbreeding (f)
Adults	219	7.00	0.625	0.603	0.035
Juveniles	892	7.00	0.638	0.626	0.020

Although the number of reproductively mature adults is limited each year, spawning practices use multiple males per female to allow more males to contribute their gametes to the progeny generation. In terms of male contribution this practice is desirable but, it can also adversely impact effective population size by inflating variance in reproductive success among individuals. Furthermore, it can increase the potential for future inbreeding as the population contains many half-siblings. The available data suggest that the increased variance in reproductive success has not significantly reduced levels of genetic variation in the progeny. Physical tags, family monitoring at GPNFH, and when necessary genetic parentage analysis of both river-captured untagged juveniles or confirmation of captive family origin can be used to avoid mating related individuals in the future. In terms of the

estimates used, therefore, it appears that juveniles are a reasonable genetic representation of the adults.

Recommendation 5. NEFC should continue the annual monitoring program for the estimation of genetic diversity present in adult and juvenile pallid sturgeon in the upper basin.

6. Evaluation of past spawning and stocking practices and recapture of stocked juveniles

Past stocking efforts of pallid sturgeon for restoration have focused on increasing the number of individuals present in the wild to prevent demographic extinction. One method to evaluate the survival of stocked hatchery produced juvenile pallid sturgeon is through the annual monitoring efforts conducted throughout the basin. Juvenile sturgeon are sampled annually to assess biomass and distribution, and tissue samples from each individual are obtained for genetic analysis. Through tags and genetic parentage analysis of untagged fish conducted by NEFC, identification of individuals to family is usually possible. This cannot presently be accomplished for those individuals whose parents are lacking genotypic data. These assessments over time allow for determination of which P_O parents have produced progeny that have survived and persisted in the wild, and could represent future broodstock. Overall there has been good survival of stocked juveniles and offspring. Thus far, offspring from 46 females and 101 males have been recaptured (Table 2).

Evaluation of the parental origin of these recaptured individuals can be used to identify which should be spawned again to produce additional F_1 individuals due to no or low offspring recapture. This will increase the number of parents contributing to this and future generations and reduce variance in reproductive success among individuals thus increasing N_e and reducing genetic drift in production of the F_1 generation. For example, considering stocking data through 2011, 188 families were stocked into the upper Missouri River basin (RPMA 1-3). Only 178 of these families could be individually identified based on PIT tags. The others lacked recorded PIT tags or parents were identified as “mixtures” or “unknown”. Family origin of some these latter individuals was determined using genetic parentage analysis. As of spring 2011, 3178 individuals were recaptured (Ryan Wilson, USFWS, personal communication). This included 280 which were identified to family based on genetic parentage analysis, 2898 were identified to known family based on family specific tagging, and 456 remained unidentified due to either no physical tag or only a batch tag. We recommend that any untagged pallid sturgeon captured should be tagged and fin-clipped prior to release and that the fin clip be submitted to NEFC for family assignment as a means of making the data more complete.

Among the individuals recaptured up to and including 2011, based on family assignments offspring have been recaptured from 46 female and 101 male adult broodstock (Table 2), or 88.5% of the females and 91.0% of the males spawned and stocked (Table 2). The captive broodstock maintained at GPNFH is composed of 2418 offspring. These captive progeny came from 42 females and 87 males representing 80.8% of the female and 78.4% of the males spawned and stocked (Table 2). Offspring from four females were recaptured from the wild but were not present in the captive broodstock, and offspring from five females were present in the captive broodstock but not recaptured from the wild.

Offspring from 13 males were recaptured in the wild but were not present in the captive broodstock, and offspring from 12 males were present in the captive broodstock but not recaptured from the wild. Future F_1 stocking should emphasize males and females represented in captivity but have no known recaptures from the wild.

Recommendation 6. Continue to use physical tags and genetic parentage assignment (when necessary) to quantify the contributions of individual parents to pallid sturgeon in the RPMAs and captive broodstock program. Both parents for every family produced should be genotyped, if not already done, to allow genetic parentage assignment if necessary. Add offspring of un- and under-represented P0 parents to the captive broodstock program as space and circumstances permit.

Table 2. Summary of the total number of offspring recaptured or in captivity at Gavins Point National Fish Hatchery and number of females and males that produced them. Data are based on up to and including the 2011 spawning/stocking year.

			Offspring Recaptured		
Summary	Recaptured	Captive broodstock	RPMA 1	RPMA 2	RPMA 3
Total number offspring	3178	2418	812	1686	680
Number of female parents represented	46	42	33	43	37
Proportion female parents represented	0.885	0.808	0.635	0.827	0.712
Number of male parents represented	101	87	67	94	64
Proportion male parents represented	0.910	0.784	0.604	0.847	0.577

7. Cryo-preservation

Due to the often low number of pallid sturgeon broodstock collected from the wild annually, cryo-preservation is a useful management tool to maintain a repository of male gametes for use during spawning. Through cryo-preservation of milt, managers can ensure that a sufficient number of genetically suitable males are available to spawn in a given year. This better allows adherence to genetic spawning recommendations based on pairwise relatedness and past spawning history to avoid inbreeding. It also allows for increasing the number of males that produced the F_1 generation, which everything else being equal, will increase effective population size and the efficacy of maintaining genetic variation in future generations. Milt is collected annually from all or most males spawned, and cryo-preserved milt is maintained at Garrison Dam National Fish Hatchery (GDNFH) and Warm Springs National Fish Hatchery (WSNFH) for backup purposes.

Prior to the 2012 spawn, milt from 109 pallid sturgeon males had been cryo-preserved (Appendix 1). Some of these males have been preserved in multiple years due to repeated handling of males, or because of increased efficacy of more recently developed extenders for preservation (Dr. Bill Wayman, WSNFH, pers. comm.). Three cryo-preserved males, all of which have been spawned, were not genotyped. However, genotypes of these three males were recently determined by NEFC through genotyping of a sample of the cryopreserved sperm. Some of the extenders or methods used in the past for the cryo-preservation process may be of reduced quality compared to more recently used methods. If males are recaptured who have previously been cryo-preserved, Dr. Bill Wayman at WSNFH should be consulted to determine if preservation of the milt using more up-to-date methods is needed. While many of the males in the cryo-preserved sperm program have been spawned multiple times, 16 have never been spawned (Appendix 1). These unspawned P_0 generation males represent a valuable source of genetic material from the original wild population of upper basin pallid sturgeon and should be spawned as soon as possible, either with under-represented wild P_0 females or with high-priority females (see below) from the captive broodstock program. These unused cryopreserved pallid sturgeon represent the most efficient means by which effective population size of the F_1 generation of upper basin pallid sturgeon can be increased.

Recommendation 7. Cryopreserved sperm from high priority males should be used as soon as possible to incorporate their offspring represented in the wild and the captive broodstock program. If no wild-caught females are available, use of captive broodstock females, preferably those that are offspring of high priority males and females is recommended. Continue use of cryo-preservation to maintain storage repositories of pallid sturgeon milt is also recommended.

8. Hatchery capabilities

Three hatcheries are primarily responsible for spawning, rearing, and stocking pallid sturgeon in the upper Missouri River basin. Miles City State Fish Hatchery (MCSFH) is operated by Montana Fish Wildlife & Parks, and has capabilities to both spawn adult broodstock and rear juveniles for stocking. GDNFH in North Dakota, and GPNFH in South Dakota are operated by the USFWS. To assess hatchery

capacity and identify current production limitations, we conducted a survey of the hatcheries used for pallid sturgeon production in the upper Missouri River basin. Information was available only for GDNFH and GPNFH (Table 3).

Based on the results of the hatchery survey, hatchery production is currently limited only by the number of broodstock collected and genetic recommendations (Table 3). Since upper basin Missouri River production targets are easily met with the current number of broodstock, expansion of hatchery capacity is not needed at this time. Presently, each hatchery has adequate capacity to rear each family created separately prior to stocking or transfer to the captive broodstock program at GPNFH allowing efficient monitoring.

Table 3. Summary of hatchery production and holding capacity for two of the three hatcheries which spawn and produce pallid sturgeon for the upper Missouri River basin: Garrison Dam National Fish Hatchery (GDNFH) and Gavins Point National Fish Hatchery (GPNFH).

Facility	Maximum number of broodstock held	Current production	Juvenile capacity	Maximum number of families produced	Maximum number of families reared
GDNFH	24	60 @ 8"	9619 @ 10"	30	30
GPNFH	12,115 lbs.	1627 @ 10"	9504 @ 10"	8	54

Recommendation 8. Use capacity to incorporate the under-represented males and females into propagation and stocking. Continue to maintain families separately during rearing to track offspring survival and allow family-specific marking of stocked fish.

9. Spawning and Stocking Recommendations

The question of how much additional stocking should be done in the RPMAs is now to a large extent related to demographics and to a lesser degree genetics. Fish should be stocked in a quantity that will produce a mature F_1 generation that has a density similar to pre-imperilment pallid sturgeon and should not exceed carrying capacity. We recommend that the UBPSWG commission a study to estimate what the population size should be in terms of adult fish per river kilometer and compare that to what is expected based on the numbers of fish stocked and estimated mortality schedules. In the mean time, stocking should be done only to increase the genetic representation of alleles from P_0 parents that have not contributed sufficiently to the wild F_1 population. This can be done through the use of unused cryopreserved sperm, sexually mature captive broodstock from under-represented parents, and perhaps through the collection of additional unused or underutilized P_0 adults. Fish should be stocked at a size and number sufficient to approximate the median contribution of each parent that

has already contributed to the recaptures in the RPMAs. The actual stocking numbers depend on the size at stocking and mortality rates which are beyond the expertise of these authors and the scope of this project

To determine the contribution of each P_0 parent to the F_1 generation we tallied the numbers of offspring from each P_0 female (Appendix 2) and male (Appendix 3) present in the captive broodstock program and recaptured from the RPMAs. These data are graphically presented in Figures 6-7. We assigned each P_0 parent a priority score based on the number of recaptures and number of captive offspring (Table 4). We also examined the numbers of recaptures from of each of the captive broodstock families (Appendix 4). Captive broodstock families listed as "cull" in Appendix 4 should never be spawned for additional F_1 propagation. The parents of these families are already over-represented in the RPMA recaptures thus wild-caught fish form these families should eventually be available for F_2 production. The only pallid sturgeon that should be spawned for additional F_1 propagation and stocking in the near term are wild adults with priority scores 1-3, the 16 unspawned males present as cryopreserved sperm discussed in section 7, and perhaps some of the captive broodstock fish with Appendix 4 priority scores of "keep" as a means of spawning the males present only as cryopreserved sperm. The data present in Appendices 2, 3, and 4 should be updated annually using recapture data and the priorities of adult fish and captive broodstock families may shift as younger year classes recruit to the sampling gear.

Once the decision is made to produce the F_2 generation, which may be several decades in the future, most of the broodstock should be collected from the wild. Fish collected from the wild have the benefit of experiencing at least some natural selection and thus are expected to be less domesticated. We expect that future efforts to collect broodstock from the wild will obtain large numbers of the families marked "cull." Thus, members of these families can be spawned in representative numbers into the F_2 generation based solely on wild-caught fish. Provided that the parentage of the F_1 broodstock adults can be unambiguously determined, spawning should be based on pedigrees (i.e. relationships inferred from parentage) rather than microsatellite-derived r_{xy} scores which are less precise (Figure 4).

Recommendation 9 An assessment of future standing stock and carrying capacity is needed to determine how much additional stocking is needed for demographic purposes in the near term. Meanwhile, stocking should only be performed to increase genetic representation of under-represented P_0 adults through the collection of additional adults if possible, use of cryopreserved sperm, and captive broodstock offspring of high priority P_0 adults. The only pallid sturgeon that should be spawned for additional F_1 propagation and stocking in the near term are wild adults with priority scores 1-3, the 16 unspawned males present as cryopreserved sperm discussed in section 7, and perhaps some of the captive broodstock fish with Appendix 4 priority scores of "keep" as a means of spawning the males present only as cryopreserved sperm. Fish should be stocked at a size and number that approximates the mean contribution of the recaptures in the RPMAs.

Table 4. Individual adults to use as broodstock for additional F_1 production are prioritized into five different categories, listed in order of priority. Median recapture number is calculated annually, reflecting the median number of offspring recaptured per individual including both the tagged offspring and those genetically assigned. Family retention capacity is defined as the number of offspring per family group that can be maintained at GPNFH, which also may change annually. To calculate the priority score the median number of offspring recaptured per female was 56.5, based on data collected including the 2011 collection year, and the retention target was 25 offspring.

Priority	Action to be taken	Description	Recapture number	Captive number
1	Individuals should be spawned immediately	Individuals who have not been previously spawned in the hatchery (includes cryo-preserved males who have been collected but have not been spawned yet) and Individuals who have been spawned but whose offspring have not been recaptured in the wild AND whose offspring are not being maintained in the captive program.	Never spawned and none recaptured	None in captivity
2	Spawn wild adults if captured. Captive offspring could be used to spawn with Priority 1 or Priority 2 adults including those represented only by cryopreserved sperm.	Individuals whose offspring have been recaptured in the wild in limited numbers, AND have few offspring maintained in the captive program.	Below median	Below family retention capacity
3	Spawn wild adults if captured. Captive offspring could be used to spawn with Priority 1 or Priority 2 adults including those represented only by cryopreserved sperm.	Individuals who have offspring in captivity larger than the retention capacity number but who have no offspring recaptured in the wild.	Below median	Above family retention target
4	Do not spawn wild adults, but do not cull offspring in captivity	Individuals who have offspring in captivity in low number but who have a larger number of offspring recaptured in the wild.	Above median	Below family retention capacity
5	Do not spawn	Individuals who have an above median number of offspring recaptured in the wild and above the retention capacity in captivity.	Above median	Above family retention target

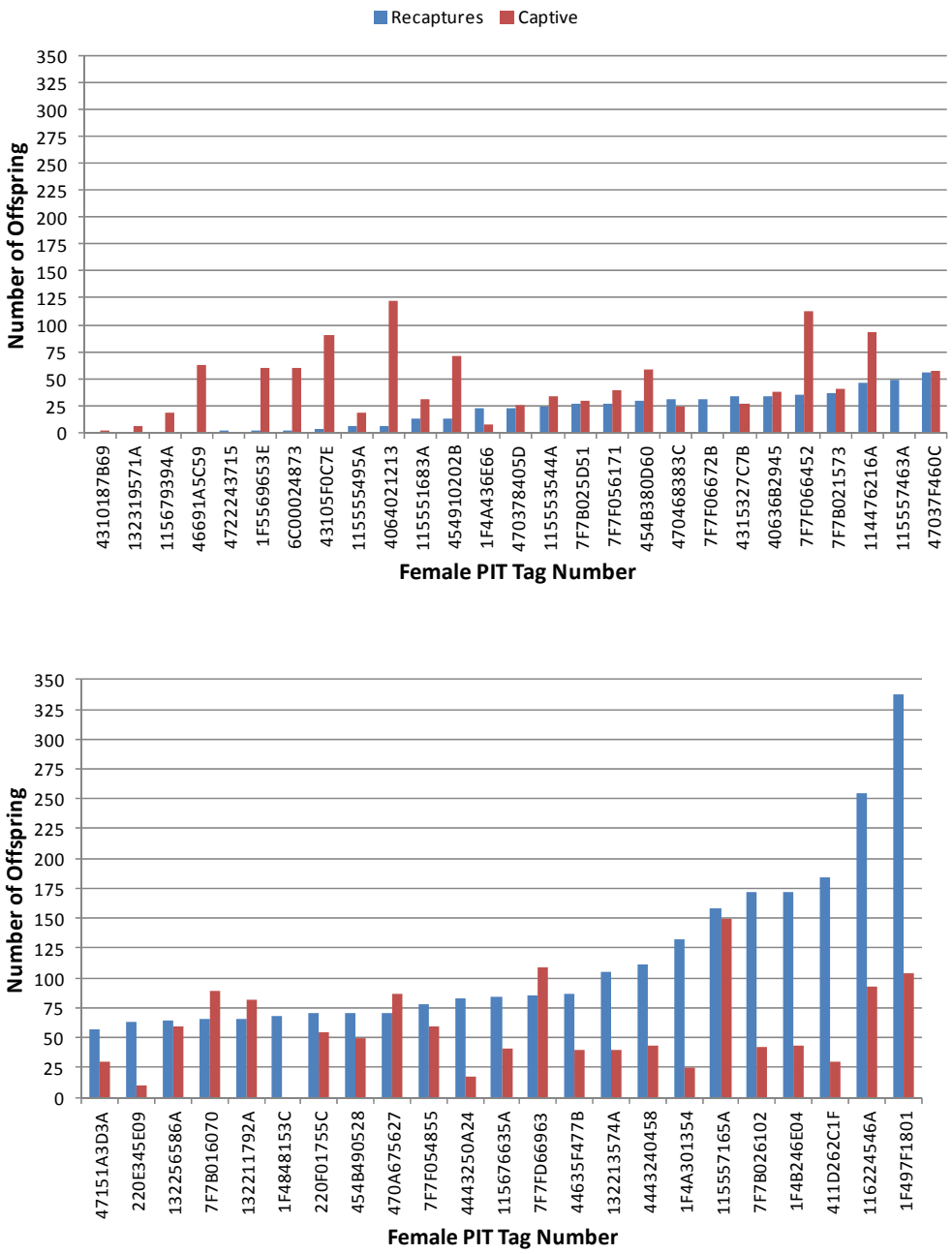

Figure 6. Numbers of offspring recaptured and numbers of offspring present in the captive broodstock program for each female P_0 parent. Those in the top figure are below median recapture. Those in the bottom figure are above median recapture. Retention for captive broodstock is 25.

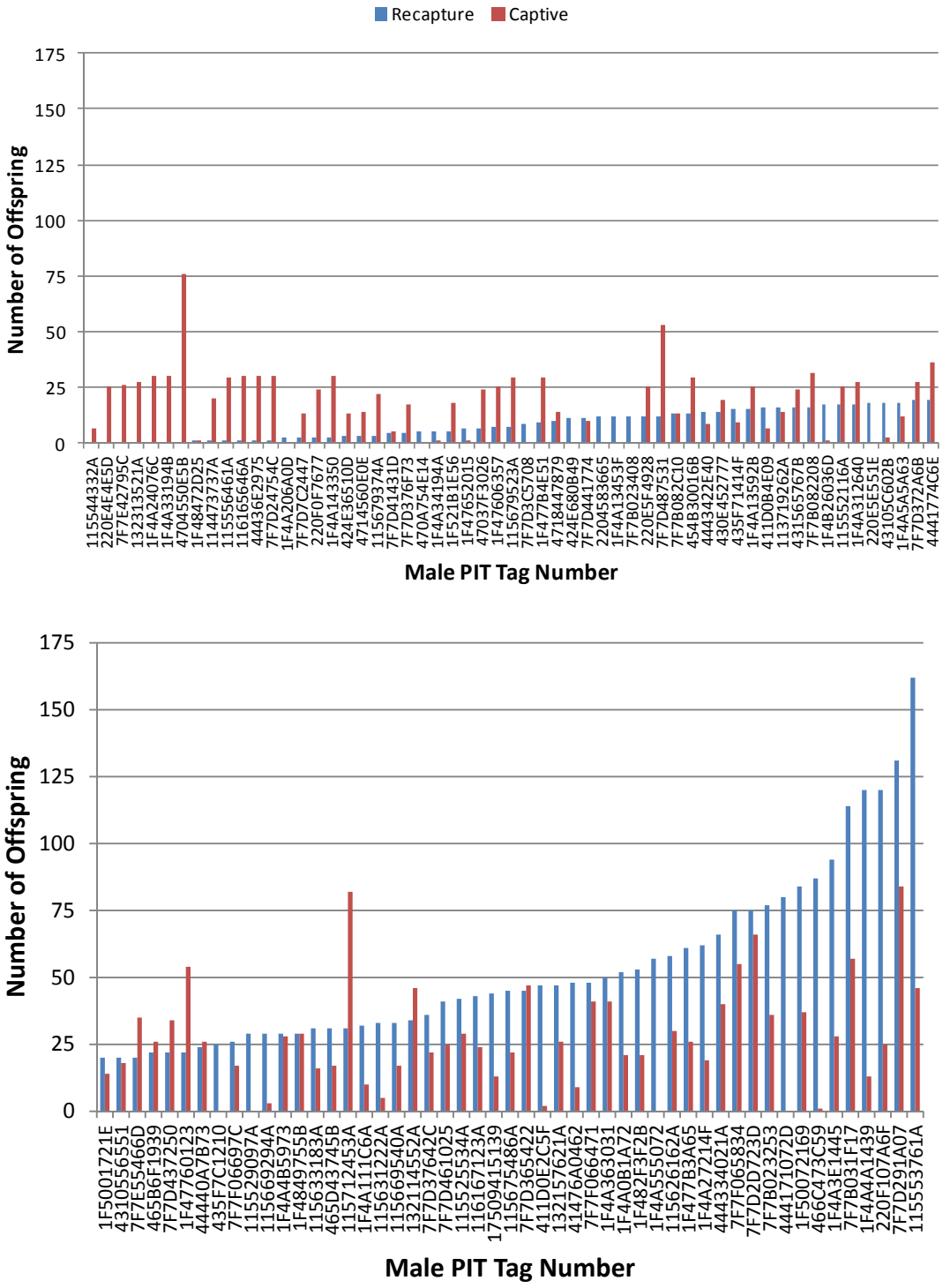

Figure 7. Numbers of offspring recaptured and numbers of offspring present in the captive broodstock program for each male P_0 parent. Those in the top figure are below median recapture. Those in the bottom figure are above median recapture. Retention for captive broodstock is 25.

Acknowledgements

We thank Ryan Wilson for access to and help with the data from the Rangewide Pallid Sturgeon Database and the Pallid Sturgeon Population Assessment Project. Steve Chipps, Rob Holm, Robert Klumb, Steve Krentz, Robert Snyder, Anne Tews, and Molly Webb provided useful criticisms on an earlier draft of this report. Bill Wayman was very helpful regarding cryopreservation of sperm. This project was funded by Western Area Power Authority agreement USDOE/WPA-GG65-11WB47955.

References Cited

Allendorf, F. W., and G. Luikart. 2007. Conservation and the Genetics of Populations. Blackwell Publishing, Malden, MA.

Bartron, M. L., and J. Kalie. 2012. Genotypic Analyses and Parental Identifications of Juvenile and Sub-adult Pallid Sturgeon in the Missouri River. Report to USFWS Missouri River Fish and Wildlife Conservation Office. April 3, 2012.

Blouin, M. S., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. Molecular Ecology 5(3):393-401.

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.

Christie, M. R., M. L. Marine, R. A. French, and M. S. Blouin. 2012. Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences of the United States of America 109(1):238-242.

DeHaan, P. W., G. R. Jordan, and W. R. Ardren. 2008. Use of genetic tags to identify captive-bred pallid sturgeon (*Scaphirhynchus albus*) in the wild: improving abundance estimates for an endangered species. Conservation Genetics 9(3):691-697.

Dryer, M. P., and A. J. Sandvol. 1993. Recovery plan for the pallid sturgeon (*Scaphirhynchus albus*). US Fish and Wildlife Service. Denver, CO.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16(3):463-475.

Frankham, R. 1995. Effective Population-Size Adult-Population Size Ratios In Wildlife - A Review. Genetical Research 66(2):95-107.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135-150 *in* M. E. Soule, and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, MA.

George, A. L., and coauthors. 2009. Guidelines for Propagation and Translocation for Freshwater Fish Conservation. Fisheries 34(11):529-545.

George, S. G., W. T. Slack, and J. J. Hoover. 2012. A note in the fecundity of pallid sturgeon. Journal of Applied Ichthyology 28:512-515.

Gharrett, A. J., W. W. Smoker, R. R. Reisenbichler, and S. G. Taylor. 1999. Outbreeding depression in hybrids between odd- and even-broodyear pink salmon. Aquaculture 173(1-4):117-129.

Goodnight, K. F., and D. C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. Molecular Ecology 8(7):1231-1234.

Grant, W. S. 2012. Understanding the adaptive consequences of hatchery-wild interactions in Alaska salmon. Environmental Biology of Fishes 94(1):325-342.

Hallerman, E. M. 2003. Population Genetics: Principles and Applications for Fisheries Scientists. American Fisheries Society, Bethesda, MD.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. Journal of Theoretical Biology 7:1-16.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population size of marine organisms? Pages 122-134 *in* A. Beaumont, editor. Genetics and evolution of aquatic organisms. Chapman and Hall, London.

Hedrick, P. W., and R. Fredrickson. 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Conservation Genetics 11(2):615-626.

Heist, E. J. 2007. Genetic Assignment Testing of Lake Sharpe Scaphirhynchus Sturgeon. US Fish and Wildlife Service, Billings, MT.

Kalinowski, S. T., and P. W. Hedrick. 1999. Detecting inbreeding depression is difficult in captive endangered species. Animal Conservation 2(2):131-136.

Kapuscinski, K. L. 2002. Population abundance estimation of wild pallid sturgeon in recovery priority management area #2 of the Missouri and Yellowstone Rivers during 1991–2001. Unpublished report, Montana Fish, Wildlife and Parks, Fort Peck, MT.

Killgore, K. J., and coauthors. 2007. Age and growth of pallid sturgeon in the free-flowing Mississippi River. Journal of Applied Ichthyology 23(4):452-456.

Lande, R. 1995. Mutation and conservation. Conservation Biology 9(4):782-791.

Miller, L. M., and A. R. Kapuscinski. 2003. Genetic guidelines for hatchery supplementation programs. Pages 329-355 *in* E. M. Hallerman, editor. Population Genetics: Principles and Applications for Fisheries Scientists. American Fisheries Society, Bethesda, MD.

Murphy, C. E., J. J. Hoover, S. G. George, and K. J. Killgore. 2007. Morphometric variation among river sturgeons (*Scaphirhynchus spp.*) of the Middle and Lower Mississippi River. Journal of Applied Ichthyology 23(4):313-323.

Palstra, F. P., and D. E. Ruzzante. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Molecular Ecology 17(15):3428-3447.

Philipp, D. P., and J. E. Claussen. 1995. Fitness and Performance Differences Between Two Stocks of Largemouth Bass from Different River Drainages Within Illinois. Uses and Effects of Cultured Fishes in Aquatic Ecosystems, volume Symposium 15:236-243. American Fisheries Society.

Portnoy, D. S., J. R. McDowell, C. T. McCandless, J. A. Musick, and J. E. Graves. 2009. Effective size closely approximates the census size in the heavily exploited western Atlantic population of the sandbar shark, Carcharhinus plumbeus. Conservation Genetics 10(6):1697-1705.

Pritchard, J. K., M. Stephens, and P. Donnely. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution 43:258-275.

Saltzgiver, M. J., E. J. Heist, and P. W. Hedrick. 2012. Genetic evaluation of the initiation of a captive population: the general approach and a case study in the endangered pallid sturgeon *(Scaphirhynchus albus*). Conservation Genetics 13(5):1381-1391.

Schrey, A. W., R. Boley, and E. J. Heist. 2011. Hybridization between pallid sturgeon *Scaphirhynchus albus* and shovelnose sturgeon *Scaphirhynchus platorynchus*. Journal of Fish Biology 79(7):1828-1850.

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

Schrey, A. W., B. L. Sloss, R. J. Sheehan, R. C. Heidinger, and E. J. Heist. 2007. Genetic discrimination of middle Mississippi River *Scaphirhynchus* sturgeon into pallid, shovelnose, and putative hybrids with multiple microsatellite loci. Conservation Genetics 8(3):683-693.

Sheffer, R. J., P. W. Hedrick, and A. L. Velasco. 1999. Testing for inbreeding and outbreeding depression in the endangered Gila topminnow. Animal Conservation 2(2):121-129.

Teletchea, F., and coauthors. 2009. Comparative analysis of reproductive traits in 65 freshwater fish species: application to the domestication of new fish species. Reviews in Fish Biology and Fisheries 19(4):403-430.

Templeton, A. 1986. Coadaptation and Outbreeding Depression. Pages 105-116 *in* M. E. Soule, editor. Conservation Biology: the Science of Scarcity and Diversity. Sinauer Associates Inc., Sunderland, MA.

Tranah, G., D. E. Campton, and B. May. 2004. Genetic evidence for hybridization of pallid and shovelnose sturgeon. Journal of Heredity 95(6):474-480.

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.

Turner, T. F., T. E. Dowling, P. C. Marsh, B. R. Kesner, and A. T. Kelsen. 2007. Effective size, census size, and genetic monitoring of the endangered razorback sucker, *Xyrauchen texanus*. Conservation Genetics 8(2):417-425.

Turner, T. F., J. P. Wares, and J. R. Gold. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162(3):1329-1339.

USFWS. 2007. Pallid sturgeon (*Scaphirhynchus albus*) five year review: summary and evaluation. Billings, MT.

Waldman, B., and J. S. McKinnon. 1993. Inbreeding and Outbreeding in Fishes, Amphibians, and Reptiles. Pages 250-282 *in* N. W. Thornhill, editor. The Natural History of Inbreeding and Outbreeding. University of Chicago Press, Chicago.

Webb, M. A. H., J. E. Williams, and L. R. Hildebrand. 2005. Recovery program review for endangered pallid sturgeon in the Upper Missouri River Basin. Reviews in Fisheries Science 13(3):165-176.

Appendix 1. Summary of cryo-preserved milt preserved to date, whether genetic samples have been obtained from the male, along with the year spawned and number of times each individual has been spawned. Hatchery source indicates from where the milt was obtained. Most milt, however, is maintained at Garrison Dam NFH or at Warm Springs NFH. Hatchery sources are Gavins Point NFH (GPNFH), Miles City SFH (MCSFH), Garrison Dam NFH (GDNFH), and Charlie M. Russell NWR (CMRNWR).

Pit Tag	Genotyped	Hatchery source	Year(s) cryopreserved			# times spawned
1F4B26036D	Yes	GPNFH	2007			1
115679374A	Yes	GDNFH	2004			1
430E452777	Yes	GDNFH	2004	2008	2010	1
7F7E55466D	Yes	GDNFH	2004	2008		3
220F0F7677	Yes	GDNFH	2004			1
7F7B082208	Yes	GDNFH	2009			1
7F7B031F17	Yes	GPNFH	2005			1
4704550E5B	Yes	GDNFH	2010			1
1F4838134E	Yes	GPNFH	2008			0
220E4E4E5D	Yes	GDNFH	2003	2009		1
7F7D365422	Yes	GDNFH	2009			2
470A754E14	Yes	GDNFH	2009			1
7F7F06697C	Yes	GDNFH	2009			1
1F477B3A65	Yes	GDNFH	2002	2004		3
115552116A	Yes	GDNFH	2004			2
7F7D3C5708	Yes	MCSFH	2001			1
115675486A	Yes	GDNFH	2003			1
411D0B4E09	Yes	MCSFH/CMRNWR	2001			1
1F521B1E56	Yes	GDNFH	2003			1
7F7D37642C	Yes	GDNFH	2009			1
4310556551	Yes	GDNFH	2006			3
4443422E34	Yes	GPNFH	2002	2006		1
7F7F066471	Yes	GPNFH	2006	2008		1
7F7D437250	Yes	MCSFH	2004	2008		2
1F5001721E	Yes	GPNFH	2007	2008		1
1F4A34194A	Yes	GPNFH	2007			1
1F48472D25	Yes	GPNFH	2007	2008		2
424E3E6127	Yes	GPNFH	2008			0
7F7D24754C	Yes	MCSFH	2010			1
1F47652015	Yes	GPNFH	2007			1
424E5A5314	Yes	GPNFH	2007	2008		0
7F7D41431D	Yes	GPNFH	2007	2008		1
425028583F	Yes	GPNFH	2008			0
4255534C5D	Yes	GPNFH	2008			0
470501194D	Yes	GPNFH	2008			0
1F4A143350	Yes	GDNFH	2000	2010		1

1F4A33194B	Yes	GDNFH	2000	2010		1
1F4849755B	Yes	GDNFH	2008			1
115712453A	Yes	GDNFH	2000	2006	2008	2
2202236E31	Yes	CMRNWR	2000			0
7F7F054773	Yes	GDNFH	2000			0
115525534A	Yes	GDNFH	2006			2
41476A0462	Yes	CMRNWR	2001			1
411D0E2C5F	Yes	CMRNWR	2001	2003		1
17509415139	Yes	CMRNWR	2001			1
115631222A	Yes	MCSFH	2001			2
1F4A27214F	Yes	MCSFH	2001			3
1F4A111C6A	Yes	MCSFH	2001			1
116167123A	Yes	GDNFH	2002	2008		2
1F4772396F	Yes	GDNFH	2002			0
1F4A3E1445	Yes	GDNFH	2002	2004	2007	3
115544332A	Yes	GDNFH	2002			1
220F107A6F	Yes	GDNFH	2002			2
7F7F065834	Yes	GDNFH	2002	2004		3
1F482F3F2B	Yes	GDNFH	2005			1
115556461A	Yes	GDNFH	2002			1
132157621A	Yes	GDNFH	2003	2006	2007	2
7F7D372A6B	Yes	GDNFH	2003	2006		1
132313521A	Yes	GDNFH	2003	2010		2
1F4A13592B	Yes	GDNFH	2003			1
7F7D291A07	Yes	GDNFH/MCSFH	2003	2006	2007	4
1F4A363031	Yes	GDNFH	2003	2007		3
435F71414F	Yes	GDNFH	2009			1
1F47760123	Yes	MCSFH	2003			3
132114552A	Yes	MCSFH	2003	2008		2
452A4E1F15	Yes	CMRNWR	2003			0
452738076E	Yes	CMRNWR	2003			0
115669540A	Yes	MCSFH	2003			1
115679523A	Yes	GDNFH	2009			1
444334021A	Yes	MCSFH	2008			2
115553761A	Yes	GDNFH	2005			1
7F7B023253	Yes	GDNFH	2005			1
7F7D2D723D	Yes	GDNFH	2007	2008		2
7F7E42795C	Yes	CMRNWR	2004			1
220E5F4928	Yes	GDNFH	2004			1
115529097A	Yes	GDNFH	2004	2006	2007	2
1F477B4E51	Yes	GDNFH	2004	2006		1
1F47606357	Yes	GDNFH	2004			1
114473737A	Yes	GDNFH	2004			1

1F4A312640	Yes	GDNFH	2004		2
7F7D376F73	Yes	GDNFH	2004		1
220F0E6207	Yes	MCSFH	2004		0
2204583665	Yes	GDNFH	2004	2006	1
4441774C6E	Yes	GDNFH	2006		1
431565767B	Yes	MCSFH	2007		2
4718447879	Yes	GDNFH	2009		1
466C473C59	Yes	MCSFH	2006		1
465D43745B	Yes	MCSFH	2006		1
220E5E551E	Yes	MCSFH	2008		1
43105C602B	Yes	GDNFH	2009		1
435F7C1210	Yes	GDNFH	2006		1
465B6F1939	Yes	GDNFH	2009		1
424E36510D	Yes	GPNFH	2007		1
115669294A	Yes	GDNFH	2008		1
44436E2975	Yes	GDNFH	2010		1
115626162A	Yes	GDNFH	2006	2008	2
44440A7B73	Yes	GDNFH	2007	2009	2
1F4A206A0D	Yes	GDNFH	2007		1
2224076523	Yes	MCSFH	2006		0
7F7B082C10	Yes	GDNFH	2009		1
454B30016B	Yes	GDNFH	2007		1
220E3F2578	Yes	GDNFH	2010		0
1F4A0C2E5D	Yes	GPNFH	2008		0
4714560E0E	Yes	GPNFH	2008		1
4625544F62	Yes	GPNFH	2008		0
1F4A4B5973	Yes	CMR	2001	2004	3
7F7D461025	Yes	CMR	2002		1
7F7D487531	Yes	CMR	2004		2
4310624556	Yes	MCSFH	2011		1

Appendix 2. Number of offspring from each female spawned and stocked that have been recaptured from the wild or maintained at Gavins Point National Fish Hatchery, the number of males each female was mated with, and the Priority Score for each female following the criteria in Table 4 based on data up to and including the 2011 spawning year.

Female PIT	Recaptured total	Captive brood	Number of males mated with	RPMA 1	RPMA 2	RPMA 3	Priority score
4722243715	1	0	1	1	0	0	2
115555495A	6	18	1	0	0	6	2
115557463A	49	0	4	28	21	0	2
115679394A	0	18	1	0	0	0	2
132319571A	0	5	1	0	0	0	2
1F4A436E66	22	7	3	6	14	2	2
4310187B69	0	2	1	0	0	0	2
470468383C	30	24	3	1	29	0	2
7F7F06672B	31	0	2	0	10	21	2
4064021213	6	122	4	0	0	6	3
114476216A	46	93	11	2	27	17	3
115551683A	12	31	2	0	6	6	3
115553544A	23	33	3	2	17	4	3
1F5569653E	1	60	3	0	1	0	3
40636B2945	33	37	6	0	32	1	3
43105F0C7E	3	90	3	0	3	0	3
4315327C7B	33	27	3	2	31	0	3
454910202B	12	71	4	0	8	4	3
454B380D60	29	58	9	7	9	13	3
46691A5C59	0	63	4	0	0	0	3
470378405D	22	25	3	8	13	1	3
6C00024873	1	60	2	0	1	0	3
7F7B021573	36	40	3	0	14	22	3
7F7B025D51	27	29	3	4	18	5	3
7F7F056171	27	39	2	0	11	16	3
7F7F066452	35	113	8	10	21	4	3
1F4848153C	68	0	2	9	59	0	4
220E345E09	63	10	2	0	12	51	4
4443250A24	83	17	3	30	51	2	4
4443240458	111	43	10	46	58	7	5
115557165A	158	150	10	52	84	22	5
115676635A	84	41	4	22	34	28	5
116224546A	254	93	5	0	144	110	5
132211792A	66	82	4	2	1	63	5

132213574A	105	39	2	82	14	9	5
132256586A	64	59	3	8	31	25	5
1F497F1801	337	104	9	149	177	11	5
1F4A301354	132	25	2	84	14	34	5
1F4B246E04	172	43	3	123	14	35	5
220F01755C	70	55	3	3	66	1	5
411D262C1F	184	30	5	12	104	68	5
44635F477B	87	40	2	3	72	12	5
454B490528	71	50	7	3	61	7	5
47037F460C	56	57	5	8	48	0	5
470A675627	71	86	3	20	47	4	5
47151A3D3A	57	30	3	12	44	1	5
7F7B016070	65	89	6	4	24	37	5
7F7B026102	172	42	2	47	112	13	5
7F7F054855	78	59	3	14	54	10	5
7F7FD66963	85	109	5	8	75	2	5
Sum	3178	2418		812	1686	680	

Appendix 3. Number of offspring from each male spawned and stocked that have been recaptured from the wild or maintained at Gavins Point National Fish Hatchery, the number of females each male was mated with, and the Priority Score for that individual following the criteria in Table 4 up to and including the 2011 spawning year.

Male PIT	Recaptured total	Captive brood	Number of females mated with	RPMA 1	RPMA 2	RPMA 3	Priority Score	Cryopreserved
2224076523	0	0	0	0	0	0	1	Yes
2202236E31	0	0	0	0	0	0	1	Yes
1F4772396F	0	0	0	0	0	0	1	Yes
1F4838134E	0	0	0	0	0	0	1	Yes
1F4A0C2E5D	0	0	0	0	0	0	1	Yes
220E3F2578	0	0	0	0	0	0	1	Yes
220F0E6207	0	0	0	0	0	0	1	Yes
424E3E6127	0	0	0	0	0	0	1	Yes
424E5A5314	0	0	0	0	0	0	1	Yes
425028583F	0	0	0	0	0	0	1	Yes
4255534C5D	0	0	0	0	0	0	1	Yes
452738076E	0	0	0	0	0	0	1	Yes
452A4E1F15	0	0	0	0	0	0	1	Yes
4625544F62	0	0	0	0	0	0	1	Yes
470501194D	0	0	0	0	0	0	1	Yes
7F7F054773	0	0	0	0	0	0	1	Yes
2204583665	12	0	1	7	5	0	2	Yes
4718447879	10	14	1	1	9	0	2	Yes
4443422E34	14	8	2	0	14	0	2	Yes
113719262A	16	14	1	0	7	9	2	No
114473737A	1	20	1	0	0	1	2	Yes
115544332A	0	6	1	0	0	0	2	Yes
115679374A	3	22	1	0	0	3	2	Yes
1F47652015	6	1	1	1	5	0	2	Yes
1F48472D25	1	1	2	1	0	0	2	Yes
1F4A13453F	12	0	1	1	11	0	2	No
1F4A206A0D	2	0	1	1	1	0	2	Yes
1F4A34194A	5	1	1	3	2	0	2	Yes
1F4A5A5A63	18	12	1	6	12	0	2	No
1F4B26036D	17	1	1	5	11	1	2	Yes
1F521B1E56	5	18	1	0	1	4	2	Yes
220E5E551E	18	0	1	7	11	0	2	Yes
220F0F7677	2	24	2	0	2	0	2	Yes
411D0B4E09	16	6	1	0	16	0	2	Yes
424E36510D	3	13	2	1	0	2	2	Yes

424E680B49	11	0	1	2	9	0	2	No
430E452777	14	19	1	1	10	3	2	Yes
43105C602B	18	2	1	1	16	1	2	Yes
431565767B	16	24	4	4	4	8	2	Yes
435F71414F	15	9	1	0	13	2	2	Yes
47037F3026	6	24	1	0	6	0	2	No
470A754E14	5	0	1	0	5	0	2	Yes
4714560E0E	3	14	2	1	1	1	2	Yes
7F7B023408	12	0	1	0	12	0	2	No
7F7B082C10	13	13	1	1	12	0	2	Yes
7F7D376F73	4	17	4	0	0	4	2	Yes
7F7D3C5708	8	0	1	0	3	5	2	Yes
7F7D41431D	4	5	1	1	3	0	2	Yes
7F7D441774	11	10	1	0	3	8	2	No
7F7D7C2447	2	13	1	0	2	0	2	No
115552116A	17	25	3	2	8	7	3	Yes
115556461A	1	29	2	0	0	1	3	Yes
115679523A	7	29	1	0	7	0	3	Yes
116165646A	1	30	1	0	1	0	3	No
132313521A	0	27	3	0	0	0	3	Yes
1F47606357	7	25	1	0	6	1	3	Yes
1F477B4E51	9	29	2	3	4	2	3	Yes
1F4A13592B	15	25	2	6	8	1	3	Yes
1F4A143350	2	30	1	0	2	0	3	Yes
1F4A24076C	0	30	1	0	0	0	3	No
1F4A312640	17	27	3	0	5	12	3	Yes
1F4A33194B	0	30	1	0	0	0	3	Yes
220E4E4E5D	0	25	1	0	0	0	3	Yes
220E5F4928	12	25	1	2	10	0	3	Yes
4441774C6E	19	36	2	2	13	4	3	Yes
44436E2975	1	30	1	0	1	0	3	Yes
454B30016B	13	29	1	0	13	0	3	Yes
4704550E5B	0	76	2	0	0	0	3	Yes
7F7B082208	16	31	1	1	15	0	3	Yes
7F7D24754C	1	30	1	0	1	0	3	Yes
7F7D372A6B	19	27	1	2	16	1	3	Yes
7F7D487531	12	53	4	0	0	12	3	Yes
7F7E42795C	0	26	2	0	0	0	3	Yes
4310556551	20	18	3	10	4	6	4	Yes
17509415139	44	13	1	3	17	24	4	Yes
115529097A	29	0	3	12	17	0	4	Yes
115631222A	33	5	2	2	15	16	4	Yes
115633183A	31	16	1	19	10	2	4	No

115669294A	29	3	1	6	23	0	4	Yes
115669540A	33	17	1	2	22	9	4	Yes
115675486A	45	22	1	12	32	1	4	Yes
116167123A	43	24	2	0	27	16	4	Yes
1F482F3F2B	53	21	1	42	9	2	4	Yes
1F4A0B1A72	52	21	1	33	15	4	4	No
1F4A111C6A	32	10	1	0	7	25	4	Yes
1F4A27214F	62	19	3	0	5	57	4	Yes
1F4A4A1439	120	13	2	87	4	29	4	No
1F4A555072	57	0	1	7	50	0	4	No
1F5001721E	20	14	1	2	14	4	4	Yes
411D0E2C5F	47	2	1	1	20	26	4	Yes
41476A0462	48	9	1	2	37	9	4	Yes
435F7C1210	25	0	1	6	19	0	4	Yes
444171072D	80	0	1	39	41	0	4	No
465D43745B	31	17	1	14	15	2	4	Yes
466C473C59	87	1	2	38	49	0	4	Yes
7F7D37642C	36	22	1	1	35	0	4	Yes
7F7F06697C	26	17	1	4	20	2	4	Yes
115525534A	42	29	1	3	39	0	5	Yes
115553761A	162	46	2	47	101	14	5	Yes
115626162A	58	30	3	13	41	4	5	Yes
115712453A	31	82	3	4	21	6	5	Yes
132114552A	34	46	2	0	26	8	5	Yes
132157621A	47	26	2	10	28	9	5	Yes
1F47760123	22	54	5	0	3	19	5	Yes
1F477B3A65	61	26	3	0	39	22	5	Yes
1F4849755B	29	29	1	12	16	1	5	Yes
1F4A363031	50	41	5	7	35	8	5	Yes
1F4A3E1445	94	28	6	17	36	41	5	Yes
1F4A4B5973	29	28	3	6	14	9	5	Yes
1F50072169	84	37	2	45	23	16	5	No
220F107A6F	120	25	2	0	93	27	5	Yes
444334021A	66	40	3	35	30	1	5	Yes
44440A7B73	24	26	2	1	22	1	5	Yes
465B6F1939	22	26	1	0	22	0	5	Yes
7F7B023253	77	36	2	40	19	18	5	Yes
7F7B031F17	114	57	3	3	93	18	5	Yes
7F7D291A07	131	84	5	81	19	31	5	Yes
7F7D2D723D	75	66	3	27	36	12	5	Yes
7F7D365422	45	47	2	1	13	31	5	Yes
7F7D437250	22	34	2	3	17	2	5	Yes
7F7D461025	41	25	2	0	15	26	5	Yes

7F7E55466D	20	35	4	1	17	2	5	Yes
7F7F065834	75	55	4	39	9	27	5	Yes
7F7F066471	48	41	3	12	36	0	5	Yes
Sum	3178	2418		812	1686	680		

Appendix 4. Priority list for captive broodstock at Gavins Point National Fish Hatchery. Families with priority "none" have no offspring in the captive broodstock program. Families with priority "Keep" have one or both parents with priority scores of 1-3 (see Appendices 4-5). Families with priority "Cull" have both parents with priority scores of 4 or 5 and should not be spawned for additional F_1 propagation but may be spawned for research purposes.

Family	Total Stocked	Total Recaptures	# Captive Fish	Female Priority	Male Priority	Priority
46691A5C59/115544332A	0	0	0	3	1	None
46691A5C59/4704550E5B	0	**0**	0	3	3	None
46691A5C59/7F7D376F73	0	0	0	3	2	None
46691A5C59/7F7E42795C	0	0	0	3	3	None
114476216A/116167123A	43	1	4	3	4	Keep
114476216A/1F477B3A65	382	4	6	3	5	Keep
114476216A/1F4A4B5973	475	0	24	3	5	Keep
114476216A/220F107A6F	27	3	11	3	5	Keep
114476216A/430E452777	23226	14	19	3	2	Keep
114476216A/431565767B	3	0	1	3	2	Keep
114476216A/7F7D487531	484	0	27	3	3	Keep
114476216A/7F7E55466D	22098	15	1	3	2	Keep
115551683A/115552116A	359	5	23	5	3	Keep
115551683A/7F7D437250	302	7	8	5	4	Cull
115553544A/115556461A	9190	1	6	3	3	Keep
115553544A/1F47652015	617	6	1	3	2	Keep
115553544A/4441774C6E	12051	16	26	3	3	Keep
115555495A/431565767B	26	6	18	2	2	Keep
115557165A/115679523A	26191	7	29	5	3	Keep
115557165A/1F47760123	10517	0	2	5	5	Cull
115557165A/1F50072169	1850	62	25	5	5	Cull
115557165A/431565767B	11561	6	3	5	2	Keep
115557165A/7F7B031F17	1501	20	23	5	5	Cull
115557165A/7F7D2D723D	1329	21	24	5	5	Keep
115557165A/7F7D365422	44582	10	27	5	5	Cull
115557165A/7F7F06697C	59977	26	17	5	4	Cull
115676635A/1F50072169	846	22	12	5	5	Cull
115676635A/7F7B031F17	1239	22	16	5	5	Cull
115676635A/7F7D2D723D	1203	40	13	5	5	Cull
115679394A/1F47760123	0	0	18	2	5	Keep
116224546A/116167123A	766	42	20	5	4	Cull
116224546A/1F477B3A65	773	25	20	5	5	Cull
116224546A/1F4A27214F	120	29	19	5	4	Cull
116224546A/220F107A6F	2155	117	14	5	5	Cull
116224546A/7F7D461025	773	41	20	5	5	Cull

132211792A/7F7D487531	136	12	25	5	3	Keep
132211792A/1F4A312640	90	10	26	5	3	Keep
132211792A/1F4A3E1445	668	44	10	5	5	Cull
132211792A/7F7E42795C	0	0	21	5	3	None
132213574A/1F482F3F2B	1564	53	21	5	4	Cull
132213574A/7F7B023253	3277	52	18	5	5	Cull
132256586A/132114552A	529	29	20	5	5	Cull
132256586A/132157621A	641	22	22	5	5	Cull
132256586A/1F47760123	151	13	17	5	5	Cull
132319571A/7F7D461025	0	0	5	2	5	None
1F497F1801/115712453A	12256	20	25	5	5	Cull
1F497F1801/1F4849755B	9614	29	29	5	5	Cull
1F497F1801/1F4A0B1A72	5058	52	21	5	4	Cull
1F497F1801/7F7D2D723D	10672	14	29	5	5	Cull
1F4A301354/1F4A4A1439	280	69	6	5	4	Cull
1F4A301354/7F7D291A07	312	63	19	5	5	Cull
1F4A436E66/115552116A	8041	10	2	2	3	Keep
1F4A436E66/115631222A	6179	10	5	2	4	Keep
1F4B246E04/1F4A4A1439	485	51	7	5	4	Cull
1F4B246E04/7F7D291A07	488	65	20	5	5	Cull
1F4B246E04/7F7F065834	386	56	16	5	5	Cull
1F5569653E/1F4A33194B	1587	0	30	3	3	Keep
1F5569653E/7F7D24754C	1577	1	30	3	3	Keep
220E345E09/1F4A111C6A	438	32	10	4	4	Cull
220F01755C/43105C602B	27679	18	2	5	2	Keep
220F01755C/7F7B082208	24710	16	31	5	3	Keep
220F01755C/7F7D37642C	35162	36	22	5	4	Cull
40636B2945/1F4A312640	71	0	1	3	3	Keep
40636B2945/1F4A4B5973	233	0	4	3	5	Keep
40636B2945/4443422E34	8002	14	6	3	2	Keep
40636B2945/44440A7B73	5309	8	26	3	5	Keep
4064021213/115712453A	0	0	57	3	5	None
4064021213/1F477B4E51	0	0	25	3	3	None
4064021213/4310556551	158	6	15	3	4	Keep
4064021213/7F7D291A07	33	0	25	3	5	Keep
411D262C1F/17509415139	1227	44	13	5	2	Keep
411D262C1F/411D0B4E09	367	16	6	5	2	Keep
411D262C1F/411D0E2C5F	679	47	2	5	4	Cull
411D262C1F/41476A0462	1047	48	9	5	4	Cull
4310187B69/4443422E34	0	0	2	2	2	None
43105F0C7E/1F4A143350	1549	2	30	3	3	Keep
43105F0C7E/44436E2975	1275	1	30	3	3	Keep
43105F0C7E/4704550E5B	1592	0	30	3	3	Keep

4315327C7B/4718447879	719	10	14	3	2	Keep
4315327C7B/7F7B082C10	1303	13	13	3	2	Keep
4443240458/115633183A	13147	31	16	5	4	Cull
4443240458/132157621A	104388	25	4	5	5	Cull
4443240458/1F4A206A0D	2511	2	0	5	2	None
4443240458/431565767B	223	4	2	5	2	Keep
4443240458/444334021A	24658	47	16	5	5	Cull
4443240458/466C473C59	0	0	1	5	4	None
4443240458/7F7E55466D	20	0	4	5	2	Keep
4443250A24/465D43745B	1341	31	17	4	4	Cull
44635F477B/115553761A	80	15	22	5	3	Keep
44635F477B/7F7B031F17	2594	72	18	5	5	Cull
454910202B/115679374A	13731	3	22	3	2	Keep
454910202B/1F47606357	43417	7	25	3	3	Keep
454910202B/220F0F7677	22555	2	24	3	2	Keep
454B380D60/132114552A	454	5	26	3	5	Keep
454B380D60/1F4A3E1445	2	0	9	3	5	Keep
454B380D60/4310556551	6959	0	3	3	4	Keep
454B380D60/7F7D376F73	119	4	11	3	2	Keep
454B380D60/7F7F065834	1017	8	9	3	2	Keep
454B490528/1F48472D25	7	0	1	5	2	Keep
454B490528/424E36510D	413	3	13	5	2	Keep
454B490528/435F71414F	3871	15	9	5	2	Keep
454B490528/44440A7B73	5783	16	0	5	5	None
454B490528/4714560E0E	526	3	14	5	2	Keep
454B490528/7F7D7C2447	194	2	13	5	2	Keep
470378405D/115556461A	10011	0	23	3	3	Keep
470378405D/1F4A34194A	8801	5	1	3	2	Keep
470378405D/1F4B26036D	2923	17	1	3	2	Keep
47037F460C/1F4A5A5A63	40553	18	12	5	2	Keep
47037F460C/454B30016B	70157	13	29	5	3	Keep
47037F460C/7F7D487531	2067	0	1	5	3	Keep
47037F460C/7F7F066471	38547	25	15	5	5	Cull
470468383C/47037F3026	5787	6	24	2	2	Keep
470A675627/115626162A	42681	47	30	5	5	Cull
470A675627/7F7E55466D	17497	5	30	5	2	Keep
470A675627/7F7F066471	47066	19	26	5	4	Cull
47151A3D3A/115669294A	28331	29	3	5	4	Cull
47151A3D3A/7F7D372A6B	16942	19	27	5	3	Keep
6C00024873/116165646A	1321	1	30	3	3	Keep
6C00024873/1F4A24076C	1340	0	30	3	3	Keep
7F7B016070/132313521A	0	0	7	3	3	None
7F7B016070/1F4A13592B	0	0	5	3	3	None

7F7B016070/1F4A363031	540	23	19	5	5	Cull
7F7B016070/1F521B1E56	60	5	18	3	2	Keep
7F7B016070/7F7D291A07	33	2	20	3	5	Keep
7F7B016070/7F7D365422	147	35	20	3	5	Keep
7F7B021573/113719262A	225	16	14	3	2	Keep
7F7B021573/7F7D441774	225	11	10	3	2	Keep
7F7B021573/7F7F06583D	209	9	16	3	3	Keep
7F7B025D51/1F5001721E	25386	20	14	3	4	Keep
7F7B025D51/4441774C6E	16698	3	10	3	3	Keep
7F7B025D51/7F7D41431D	33458	4	5	3	2	Keep
7F7B026102/115553761A	7346	147	24	5	3	Keep
7F7B026102/7F7B023253	912	25	18	5	5	Cull
7F7F054855/115669540A	2078	33	17	5	4	Cull
7F7F054855/115675486A	1738	45	22	5	4	Cull
7F7F054855/132313521A	0	0	20	5	3	None
7F7F056171/1F47760123	100	9	17	3	5	Keep
7F7F056171/1F4A363031	100	18	22	3	5	Keep
7F7F066452/114473737A	6	1	20	3	2	Keep
7F7F066452/1F4A13592B	5970	15	20	3	3	Keep
7F7F066452/1F4A3E1445	0	0	9	3	5	None
7F7F066452/444334021A	279	2	24	3	5	Keep
7F7F066452/7F7D437250	2192	15	26	3	4	Keep
7F7F066452/7F7F065834	135	2	14	3	2	Keep
7F7FD66963/115525534A	10228	42	29	5	5	Cull
7F7FD66963/1F477B4E51	6491	9	2	5	3	Keep
7F7FD66963/220E4E4E5D	2767	0	25	5	3	Keep
7F7FD66963/220E5F4928	11598	12	25	5	3	Keep
7F7FD66963/465B6F1939	3611	22	26	5	5	Cull