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ORIGINAL RESEARCH

Genetic evaluation of the initiation of a captive population: the general approach and a case study in the endangered pallid sturgeon (*Scaphirhynchus albus*)

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Abstract Because of recent environmental changes, a number of species have become critically endangered in the wild. To prevent extinction of these species, captive populations have been established and generally maintained in a manner that attempts to avoid detrimental genetic changes. In particular, wild founders should be unrelated and not inbred. The subsequent generations in captivity should make the effective population size as large as possible to retain the initial genetic variation. Pallid sturgeon (*Scaphirhynchus albus*) from the upper Missouri River USA have not successfully recruited in decades. Perpetuation of this stock is now accomplished through captive spawning of wild-caught fish with subsequent release of their offspring. In addition to the fish released into the wild, more than 2,000 offspring of the wild fish from 11 year-classes are captively housed. We examined the genetic risks associated with using these fish as a captive population for the future propagation of pallid sturgeon and concluded that the wild individuals are unrelated, non-inbred remnants of a formerly larger population. Further, there is a sufficiently large effective population size (N_e) present in the captive broodstock for propagation provided that the effective population size can be increased in subsequent generations. This study shows how genetic and evolutionary principles and applications can be used to evaluate the potential founders of a captive population and make recommendations for the long-term evolutionary success of an endangered species.

Keywords Broodstock · Effective population size · Heterozygosity · Inbreeding · Microsatellite loci · Relatedness · Sturgeon

Introduction

Captive reproduction and rearing of the offspring of wild-caught animals can be used to preserve a species that is no longer successfully reproducing in the wild (e.g. Hedrick et al. (2000a); Hedrick and Fredrickson (2008) with the goal of eventual re-establishment in a natural and appropriate habitat. While captive rearing may successfully perpetuate a species, if not performed with a consideration of maintaining the adaptive variation present in the population, the individuals produced may have reduced genetic variation or reduced fitness due to inbreeding, outbreeding, or domestication (adaptation to the captive environment). Reduction of fitness can then further exacerbate the lowered reproduction in the wild leading to an extinction vortex (Gilpin and Soule 1986). On the other hand, outbreeding may be desirable to genetically rescue a population that is suffering from inbreeding depression (e.g.Westemeier et al. 1998; Hedrick and Fredrickson 2010).

A number of species have been preserved in captivity before extinction in the wild or have been brought into captivity to preserve the species if it did go extinct in the wild. Some of these captive populations have been initiated with a very small number of founders, e.g., 6 in

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black-footed ferrets (*Mustela nigripes*) (Seal et al. 1989), 14 in California condors (*Gymnopyps californianus*) (Ralls and Ballou 2004), and 13 in Przewalski's horses (*Equus przewalskii*) (Boyd and Houpt 1994), raising concern about the initial level of genetic variation in the captive population. As an extreme example, the Mexican wolf population was originally initiated with only four founders but two of these were subsequently discovered to be first-degree relatives, mother and son, making only three unrelated founders (Hedrick et al. 1997). Two other captive lineages, both founded from two individuals, were eventually added to the population to make the total number of founders seven (Hedrick et al. 1997). Most of these species with small founder numbers have been mammals and birds maintained in zoos and other facilities and plants maintained in botanical gardens.

Captive breeding approaches have been used for a number of endangered fish species (Johnson and Jensen 1991), particularly for many salmonids (Fraser 2008) and for white sturgeon (Ireland et al. 2002; Drauch Schreier et al. 2012). An example of a captive broodstock program for an endangered fish is that for the bonytail chub for which the number of effective founders was estimated to be only 3.5 (Hedrick et al. 2000a).

Various aspects of evolutionary genetics have been examined in supplementation or supportive breeding programs in endangered fish which are important to consider in captive broodstock programs. These factors include inbreeding and its negative influence on fitness (Duchesne and Bernatchez 2002; Kalinowski et al. 2012), low effective population size and loss of genetic variation (Hedrick et al. 1995, 2000b; Turner et al. 2007; Araki et al. 2008), and domestication (Ford 2002; Araki et al. 2008; Christie et al. 2012); for general theoretical treatments, see Lynch and O'Hely (2001) and Wang and Ryman (2001). In general, genetic management to avoid inbreeding, retain genetic variation, or avoid adaptation to captivity overlap; efforts to retain genetic variation are expected to both reduce inbreeding and adaptation for domestication (Ballou et al. 1995; Frankham 2008).

The charismatic pallid sturgeon (*Scaphirhynchus albus*) is native to the Missouri River, Mississippi River, and several of their larger tributaries in North America. The pallid sturgeon was listed as an endangered species under the United States Endangered Species Act (ESA) in 1990 (USFWS 1990). Dams and channelization altered or eliminated a large portion of the preferred riverine habit used by the pallid sturgeon. On the Missouri River, 36 % of the pallid sturgeon's riverine habitat was destroyed by dams, 40 % has been channelized, and the remaining 24 % has been altered to some degree by modified flows associated with dams (Dryer and Sandvol 1993). As a result, there is no recruitment from the wild in the upper Missouri River. Less than 200 adult wild pallid sturgeon survive, and these adults are all very old (see discussion below). In other words, the status of upper Missouri River pallid sturgeon in the wild is extremely critical and extinction is likely in the near future without extraordinary management actions.

To prevent extinction of pallid sturgeon in the upper Missouri River, a captive breeding program has been initiated. The purpose of the captive breeding program is to ensure that there are broodstock available to propagate the next generation of upper Missouri River pallid sturgeon. Some of the progeny are stocked into the wild while others are retained in a captive broodstock program at Gavins Point national fish hatchery. Here we will examine the potential genetic effects of using only the captive broodstock program and predict how much genetic variation could be maintained under this management program. For example, a captive breeding program could potentially result in significant inbreeding if an insufficient number of unrelated, non-inbred adults are used to found the captive population or if crosses are performed among related individuals. Inbreeding as a result of an inadequate number of parents can be prevented by avoiding close mating between relatives and maximizing the effective population size. This can be done by founding the population with the offspring of as many unrelated, non-inbred parents as feasible and by minimizing the reproductive variance among those parents. Below we will evaluate the genetic effects of the initial matings in this potential captive broodstock program, examine the potential genetic impacts of their progeny on genetic variation, and predict further genetic effects into future generations. Genetic determination of relatedness, inbreeding, and parentage will be based on data from a number of highly variable microsatellite in the 129 potential wild founders and the approximately 2,300 hatchery progeny from these founders. Using family data from this investigation, the effective population size will be estimated and the expected impact on genetic variation predicted using population genetic models.

Materials and methods

Pallid sturgeon

Pallid sturgeon from the upper Missouri River (above Fort Peck Dam, see Fig. 1) are genetically distinct from those in other parts of the range (Schrey and Heist 2007), morphologically different from fish in the southernmost part of the range (Murphy et al. 2007), and geographically distant and disconnected from the southern pallid sturgeon. Dams impounded more than 1,200 km of former Missouri River channel, isolating upper Missouri River pallid sturgeon from the free-flowing river below Gavins Point Dam.

However, because pallid sturgeon are long-lived and slow to mature, genetic differences among stocks likely predate dams (Schrey and Heist 2007). The United States Fish and Wildlife Service (USFWS) currently recognizes four pallid sturgeon management units (Bergman et al. 2008) of which the northernmost and most upstream is the Great Plains Management Unit (GPMU) which includes the upper Missouri River and Yellowstone River. The GPMU contains three Recovery Priority Management Areas (RPMAs), RPMA 1 is in the Missouri River above Fort Peck Reservoir, RPMA 2 includes the Yellowstone River and the Missouri River between Fort Peck Dam and Lake Sakakawea, and RPMA 3 is a relatively short segment of riverine habitat above Lewis and Clark Lake into which pallid sturgeon have also been stocked (Fig. 1). The majority of the parents of the broodstock fish discussed below have come from RPMA 2, some from RPMA 1, and none from RPMA 3 (G. Jordan, personal communication).

Pallid sturgeon from the GPMU have not recruited successfully for several decades. Webb et al. (2005) estimated that fewer than 200 wild adult pallid sturgeon remain in the upper Missouri River basin with as few as 45 wild adult pallid sturgeon remaining in RPMA 1, approximately 136 wild pallid sturgeon remaining in RPMA 2, and no remaining wild pallid sturgeon in RPMA 3 (USFWS 2007). Recently Braaten et al. (2009) estimated the number of wild adult pallid sturgeon remaining in RPMA 2 at 159 with a 95 % confidence interval of 129–193.

Since extensive and effective sampling was implemented in 1990, only one immature pallid sturgeon has been collected and all other sturgeon were sexually mature and greater than 900 mm in length (USFWS 2007). Although it is difficult to precisely age older sturgeon by counting annuli in fin rays (Braaten et al. 2009), a fish collected in 1988 was estimated to be 37 years old and one collected in 1983 was estimated to be 41 years old (Keenlyne and Jenkins 1993). Pallid sturgeon appear to live at least as long as 50 years (USFWS 1993). The estimates are particularly significant because these fish appear to have been alive when Garrison Dam was closed in 1953, impounding Lake Sakakawea. In other words, there appears to have little or no natural recruitment for over 50 years and the wild population only continues to exist because of the extreme longevity of pallid sturgeon. The lack of recruitment is presumed to be the result of the interruption of riverine habitat caused by dams and reservoir (USFWS 2007). Specifically, in RPMA 2 in the Missouri River below Fort Peck Dam, pallid sturgeon are severely impacted by altered thermal and hydrological flow regimes resulting from dam activities (Braaten et al. 2009). The Yellowstone River has a more natural hydrograph but pallid sturgeon are restricted to a 114-river km section

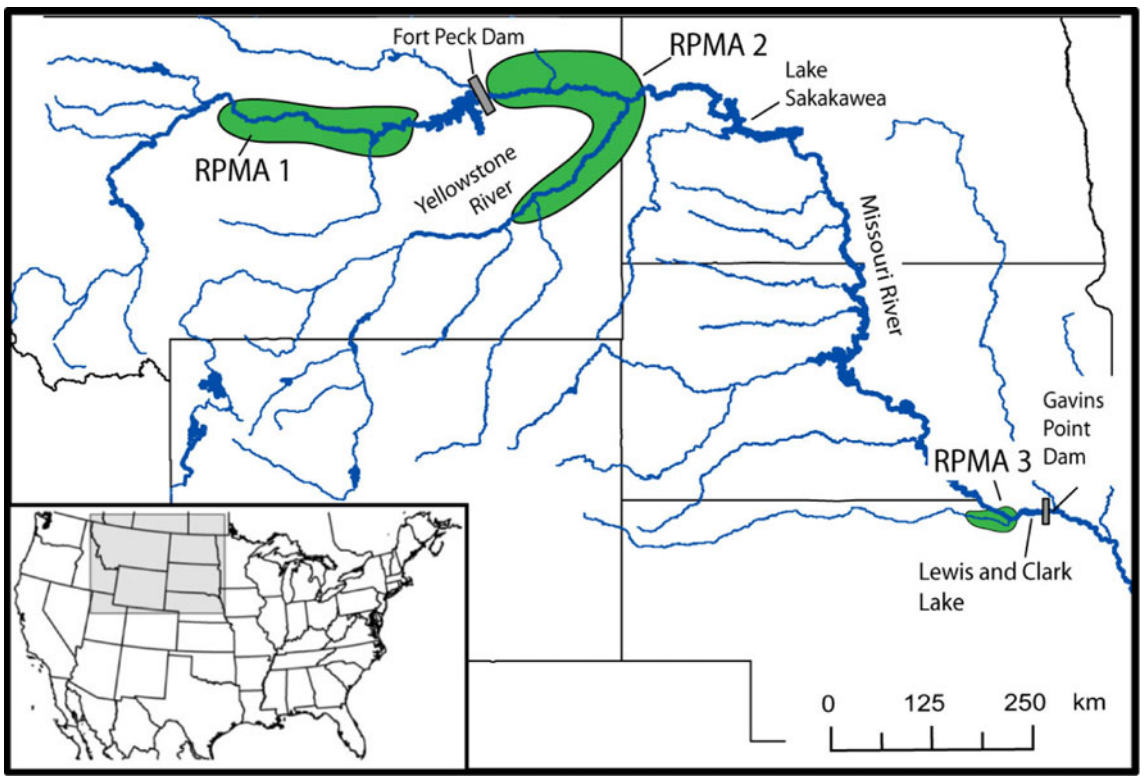

Fig. 1 Upper Missouri River basin showing the locations of three pallid sturgeon Resource Priority Management Areas (RPMAS) within the Great Plains Management Unit

bounded by an irrigation intake diversion dam constructed in 1905. Because larval pallid sturgeon drift between 245 and 530 km before settling to the benthos (Braaten et al. 2008), any pallid sturgeon presently spawned in the Yellowstone River will drift into unsuitable habitat in Lake Sakakawea. Currently a modification to the intake diversion dam is being constructed which will allow pallid sturgeon to ascend above the current location of the intake diversion dam, potentially providing pallid sturgeon larvae with enough drift distance to restore natural recruitment.

Captive reared pallid sturgeon released into the wild grow quickly at first, reaching an approximate length of 300 mm by year 2 with growth slowing to 70 mm per year by age 5 (Shuman et al. 2011). Annual survival of stocked pallid sturgeon in the Missouri River has been estimated at 0.05 for age-0 fish, 0.68 for age-1 fish, and 0.92 for older fish (Steffensen et al. 2010). Keenlyne and Jenkins (1993) reported that male pallid sturgeon reach sexual maturity by age 5–7 and females reach maturity by age 9–12 with first spawning by age 15. Some hatchery reared pallid sturgeon stocked into the wild in the 1990s and early 2000s are now mature (Steffensen et al. 2012). At this time persistence of the GPMU pallid sturgeon relies entirely on captive spawning of wild-caught individuals. Due to the scarcity of the remaining individuals and the difficulties associated with harvesting and spawning the wild fish, the Upper Basin Pallid Sturgeon Work Group (UBPSWG) has initiated a captive broodstock management plan. The plan would potentially discontinue the practice of capturing wild fish for spawning and stocking; instead they would rear the F_1 offspring of the wild fish to maturity at Gavin's Point National Fish Hatchery (GPNFH), make crosses between them, and use the resulting F_2 progeny to stock the upper Missouri River. As of January, 2010 nearly 2,300 pallid sturgeon offspring of wild adults from 11 different year classes were housed at GPNFH.

Data collection and analysis

Captive broodstock were produced at GPNFH from 1997 to 2009 and we obtained hatchery records regarding which crosses were used to produce these individuals. Table 1 summarizes the number of male and female parents spawned, the number of different families, and the total number of progeny produced each year. Overall, 86 male and 43 female parents were used in 129 families producing 2,296 progeny that were alive as of January, 2010.

Every parent was PIT (passive integrated tomography) tagged and the crosses performed were recorded, thus PIT tag numbers were available for all of the parents used to produce the F_1 generation housed at GPNFH. Family lots were kept separate until fish were large enough to receive their own PIT tag, at which time fish from the same year class are often pooled. If a captive fish sheds its PIT tag, knowing its year class could limit the number of possible parents based on hatchery records. We sampled every captive fish present at GPNFH up to year class 2005, noting its PIT tag number (if present) and collecting a fin clip, which was stored in 95 % ethanol. If we could not detect a PIT tag, a new one was inserted and the number was recorded. Numbers of remaining offspring from each family for year classes 2006–2009 were provided by GPNFH personnel. We obtained fin clips from as many of the wild parents as were available through the pallid sturgeon tissue repository then housed at Abernathy Fish Technology Center in Longview, Washington USA.

We genotyped all parental sturgeon and all wild adult pallid sturgeon from the upper Missouri River at 19 microsatellite loci developed by McQuown et al. (2000) as described by Schrey and Heist (2007). Briefly, we extracted genomic DNA from the fin clips using the DNeasy Tissue Kit (QIAGEN Inc. 2006). The resulting DNA was used in a 10 µl PCR reaction containing approximately 1–20 ng genomic DNA, 0.54 pMoles of each PCR primer, 5 µl of 2X Abgene master mix, and 2.4 µl of deionized water. The 2X Abgene master mix consists of 1.25 units of Thermoprime Plus DNA Polymerase, 75 mM Tris–HCl, 20 mM $(NH4)_2SO_4$ 2.0 mM $MgCl_2$, 0 0.01 % Tween 20, and 0.2 mM of each dATP, dCTP, dGTP, and dTTP. One primer was fluorescently labeled with either 6FAM, Hex, or Ned (Applied Biosystems) and we typically multiplexed

Table 1 The number of male parents and female parents spawned and the number of families produced each year, and the number of surviving progeny in the pallid sturgeon captive broodstock program at Gavin's Point National Fish Hatchery from each year as of January, 2010

Years	Number of			
	Male parents	Female parents	Families	Progeny
1997	3	2	5	77
1998	2	1	2	42
1999	3	1	3	41
2001	5	2	5	50
2002	6	3	7	122
2003	11	4	13	329
2004	20	7	24	369
2005	8	6	14	267
2006	6	3	6	162
2007	24	12	27	299
2008	9	3	9	240
2009	14	6	14	298
Total	86	43	129	2,296

Because some male and female parents were used in multiple years, the total number of male and female parents is less than the sum of the numbers for the individual years

three primer sets into a single PCR reaction. Thermal profiles for reactions were: 94 °C 2 min, 5 cycles of 94 °C 30 s, 54 °C 30 s, 70 °C 30 s, and 35 cycles of 95 °C for 30 s, 56 °C 30 s, and 70 °C for 30 s. PCR products were diluted (1:1) in loading buffer (deionized formamide, blue dextran EDTA, and Rox-400 sizestandard; The Gel Company), resolved on either an Applied Biosystems 377 gel-based automated DNA analyzer using a 5 % Long Ranger(Cambrex) 36 cm gel, and run at 2,500 scans per hour for 2.5 h or an Applied Biosystems 3730xl capillary-based analyzer using 36 cm capillaries and Pop-7 polymer. We initially used the 377 instrument to assign untagged offspring to potential parents from the same year class. All other data presented (i.e. genotypes of the 71 founders and 29 additional wild sturgeon) in this manuscript are based on data from the 3730xl instrument. We never attempted to standardize allele calls between the instruments because all analyses were based solely on data from one or the other instrument. Resultant images were analyzed with GENESCAN v 3.1.2 (Applied Biosystems) and GENOTYPER v 2.5 (Applied Biosystems 2000). We examined the microsatellite data for deviation from Hardy–Weinberg (HWE) and linkage disequilibrium (LE) using Genepop version 4 (Rousset 2008) and checked for the presence of genotyping errors due to stuttering, allelic dropout, and null alleles using MicroChecker version 2.2.3 (Van Oosterhout et al. 2004).

Estimating relatedness, inbreeding, and parentage

We used Kinship 1.3.1 software program (Goodnight and Queller 1999) to create a matrix of pair-wise relatedness (r_{xy}) values between all 100 pairs of wild adult pallid sturgeon sampled from the upper Missouri River including 71 parents of the captive broodstock and 29 additional wild pallid sturgeon. We used the simulation tool in Kinship to create an equal number of simulated r_{xy} values using the allele frequencies from the sampled sturgeon under the assumption that that there was no relatedness between all pairs of individuals.

We estimated the inbreeding coefficient for the wild adult pallid sturgeon using the maximum likelihood approach developed by Hall et al. (in press) and software provided by A. Anderson. Briefly, the likelihood function for locus k, L_k, gives the probability of the data as a function of the model parameters, here the estimated allele frequencies and the inbreeding coefficient f. Therefore, when the observed genotype is a homozygote A_iA_i, then the likelihood is

$$L_k = p_{ki}^2(1-f) + p_{ki}f \quad (1)$$

and when the observed genotype is a heterozygote A_iA_j, then the likelihood is

$$L_k = 2p_{ki}p_{kj}(1-f) \quad (2)$$

The likelihood for all m loci is

$$L = \prod_{k=1}^{m} L_k \quad (3)$$

The f value that maximizes the value of the likelihood is the maximum likelihood function and can be found by calculating the likelihood for all f values.

Effective population size estimates were based only on captive-reared sturgeon for which both parents were wild and were identified either through PIT tags or reconstructed. We attempted to assign captive broodstock fish that had lost their original PIT tag back to parents using a combination of hatchery records, 13 microsatellite loci, and the CERVUS computer software program (Kalinowski et al. 2007). We were able to eliminate most potential parent combinations by utilizing GPNFH's yearly cross records. We then used Cervus to compare the potential parent's microsatellite profiles for that year with the profiles of the offspring with missing tags. We constructed the profiles using our most polymorphic markers to expedite parental assignment. Generally 13 loci provided sufficient power to eliminate all but one dam or sire. However, if more than one dam or sire were consistent with parentage of a F_1, we genotyped that fish at three additional loci and then compared to the potential parents again. To allow for potential mismatches due to mutation and/or genotyping error, the presence of one unshared allele at one locus was allowed when it was assigned to its potential parent pairing.

Theoretical approach

We calculated the effective number of male parents (N_{em}) and the effective number of female parents (N_{ef}) using the following expressions

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

and

$$N_{em} = \frac{N_m\bar{k}_m - 1}{\bar{k}_m - 1 + \frac{V_{km}}{\bar{k}_m}} \quad (5)$$

where $\bar{k}_f$ and $\bar{k}_m$ are the mean number of offspring for female and male parents (also includes survival to counting), respectively, and V_{kf} and V_{km} are the variances in the number of offspring for female and male parents, respectively (Lande & Barrowclough 1987). The overall effective population size was then calculated using these values as

$$N_e = \frac{4N_{ef}N_{em}}{N_{ef} + N_{em}}. \quad (6)$$

Crow and Morton (1955) proposed that the variance in progeny number be standardized for a constant population size so that the mean number of progeny is two. When there is random survival of individuals, then the standardized variance in the number of offspring is

$$V_{k2} = s(1-s)\bar{k} + s^2 V_k \quad (7)$$

where s is the proportion of survival to adult or $s = 2/\bar{k}$ (see also Waples 2002).

The expected loss of genetic variation in a finite population is

$$H_{t+1} = H_t\left(1 - \frac{1}{2N_e}\right) \quad (8)$$

where H_t is the heterozygosity in generation t (e.g. Hedrick 2011). Using this expression and the estimated effective population size value in the parental generation, N_{e1}, the expected proportion of heterozygosity present in the original parental generation 0 that was retained in the F_1 generation (P_1) is

$$P_1 = \frac{H_1}{H_0} = \left(1 - \frac{1}{2N_{e1}}\right) \quad (9)$$

Similarly, the expected proportion of heterozygosity retained in F_2 generation is

$$P_2 = \frac{H_2}{H_0} = \left(1 - \frac{1}{2N_{e1}}\right)\left(1 - \frac{1}{2N_{e2}}\right) \quad (10)$$

The average effective population size over these two generations was calculated from the following expression

$$\bar{N}_e = \frac{t}{\sum_{i=1}^{2} \frac{1}{N_{ei}}} \quad (11)$$

Or, the single generation effective population size (N_{eS}) that would have the same impact as these two generations can be calculated as

$$N_{eS} = \frac{1}{2\left[1 - \left(1 - \frac{1}{2N_{e1}}\right)\left(1 - \frac{1}{2N_{e2}}\right)\right]} \quad (12)$$

These expressions can be expanded for more generations but this is not necessary here for the long-generation pallid sturgeon.

The above analysis assumes discrete generations but in the wild the long-lived pallid sturgeon have overlapping generations. However, in the first several generations using the captive broodstock, generations will be discrete, i.e., founders will be used to produce the F_1 captive broodstock and the F_1 fish will be used to produce the F_2, so that the above analysis is appropriate. Within the F_1 generation, attempts should be made to equalize contributions of parents so that the early cohorts are not overrepresented as parents. Because some 50 year old fish are now being spawned successfully, it should be possible to produce F_2 fish from the F_1 generation over several decades, and spawning between individuals from the early and late cohorts can approximate random mating among cohorts and allow for the equalization of contributions among cohorts. After the F_1 generation (several decades from now), modification of this analysis to allow overlapping generations would be useful to more precisely predict the long-term impact of the captive broodstock.

Results

Relatedness among broodstock parents

Hatchery records indicated that 129 wild fish, 43 females (N_f) and 86 males (N_m), were used to produce the captive broodstock fish. We were able to obtain fin clips from 71 of these plus an additional 29 wild pallid sturgeon from the upper Missouri River. Samples of only 71 broodstock fish were available because adult broodstock were released into the wild after they were spawned and tissue samples from the early years of the propagation program were not retained. The samples that we have used are from adults that were subsequently recaptured.

Summaries of the microsatellite data from these 100 wild individuals (71 broodstock and 29 wild sturgeon) used to calculate relatedness distributions are presented in Table S1. Loci exhibited 3–17 alleles with observed heterozygosities ranging from 0.02 to 0.863. Overall observed and expected heterozygosities were 0.663 and 0.651, respectively, with an overall F_{IS} of −0.003. The most variable locus (Spl26) deviated significantly from HWE following a Bonferroni correction for multiple comparisons, however, the estimate of inbreeding, F_{IS} is only −0.007, not significantly different from 0. One pair of loci (Spl56 and Spl101) deviated significantly from linkage equilibrium following a Bonferroni correction for multiple comparisons. Microchecker did not detect the presence of genotyping errors due to stuttering, allelic dropout, and null alleles at any of the 19 loci.

The distribution of relatedness values (r) among these adults was unimodal with a mean of 0.001 (Fig. 2). 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 19 loci. The simulated distribution was constructed by using the sample allele frequencies and creating new individuals by sampling independently across loci. This is the relationship that would be expected to occur in a sample drawn from a large

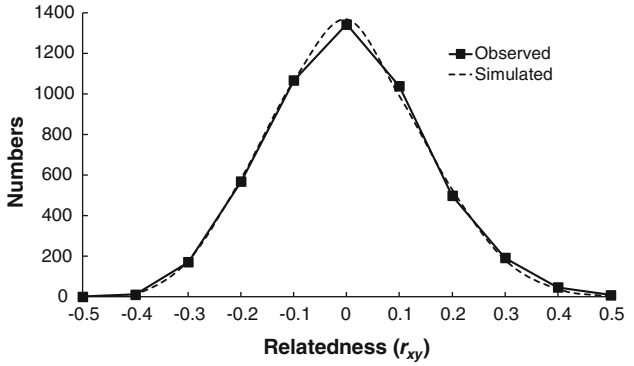

Fig. 2 Distribution of the observed and simulated relatedness values (r) among upper Missouri River wild pallid sturgeon

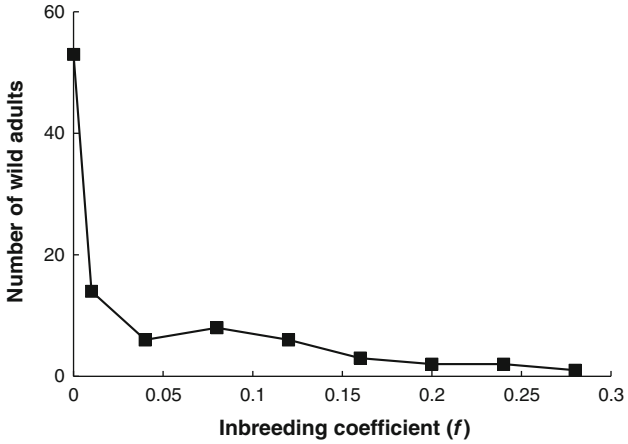

Fig. 3 Distribution of the observed estimated inbreeding coefficients (f) among among upper Missouri River wild pallid sturgeon

randomly mating population with any allele sharing occurring just by chance. Had there been a number of related pairs of individuals in the wild 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.

Estimated inbreeding coefficient of founders

The estimated distribution of inbreeding coefficients for these 100 wild adults is given in Fig. 3. The estimated inbreeding coefficient was not significantly greater than 0 (Hall et al. in press) for any of these adult fish. Only four fish had estimated inbreeding coefficients greater than 0.2 but none of these were significantly different from 0. The mean inbreeding coefficient for these fish was only 0.04 and 71 of the adults had inbreeding coefficients <0.02.

Effective population size and genetic variation

From the numbers of progeny produced by the 43 females and 86 males, the values needed to estimate effective population size were calculated (Table 2). For example, using the approach of Lande and Barrowclough (1987), the female and male effective population sizes were 27.5 and 63.6 or 64.0 % and 74.0 % of the total number of male and female parents, respectively. Combining these estimates, the overall effective population size is estimated to be 76.8. Therefore, given these variances the effective number of founders for the broodstock is $N_{e1} = 76.8$ (where the subscript 1 indicates the founder generation).

Because the fish were collected over 12 years, it is instructive to examine the temporal increase in the numbers of parents and the cumulative effective population size (Fig. 4). Obviously both values only slowly increased in the first 4 years but starting in 2001, the number of parents increased sharply in a linear fashion. The cumulative effective population size also increased substantially over this period but appeared to begin to asymptote in the last several years. By 2006, an N_e value of 50 was reached and at this time 85 different parents were used for propagation.

Table 2 The mean and variance of the number of progeny per female and male parents and the effective population size for females, males, and overall using the approach of Lande and Barrowclough (1987) and approach of Crow and Morton (1955) where the variance is standardized assuming that individuals randomly survive and the mean number of progeny is two

	Value	Lande and Barrowclough (1987)	Crow and Morton (1955)
Females	$\overline{k}_f$	53.4	2.0
	V_{kf}	1655.2	4.246
	$V_{kf}/\overline{k}_f$	31.00	2.123
	N_{ef}	27.51	27.72
	N_{ef}/N_f	0.640	0.645
Males	$\overline{k}_m$	26.70	2.0
	V_{km}	276.7	3.40
	$V_{km}/\overline{k}_m$	10.36	1.70
	N_{em}	63.64	63.33
	N_{em}/N_m	0.740	0.736
Overall	N_e	76.85	77.12
	N_e/N	0.596	0.598

Standardizing the variance of progeny number, using the approach of Crow and Morton (1955), gives very similar results (Table 2) with the overall estimate of the effective population size 77.1. This similarity is consistent with the finding of Waples (2002) who showed theoretically that this adjustment is not necessary for inbreeding estimates of effective population size. The standardized values of $V_{kf}/\overline{k}_f$

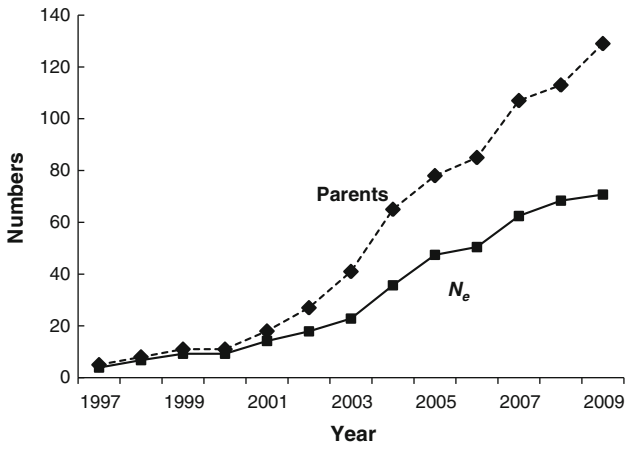

Fig. 4 Cumulative numbers of parents used (Parents) and effective population sizes (N_e) calculated for each year using the formula of Lande and Barrowclough (1987)

Table 3 The expected female (N_{ef}), male (N_{em}), and overall (N_e) effective population sizes in the progeny of the founders given that different variances in progeny (V_{kf}, for females and V_{km} for males) when $\bar{k} = 2$

V_{kf}, V_{km}	N_{ef}	N_{em}	N_e
0, 0	85.0	175.0	231.1
1, 1	56.7	114.0	151.4
2, 2	42.5	85.5	113.6
4.25, 4.95	27.7	49.2	70.9

and $V_{km}/\bar{k}_m$ calculated using this approach were similar to the $V_k/\bar{k}$ values observed in *Drosophila* and humans by Crow and Morton (1955).

Instead of choosing random individuals to survive and become parents in the next generation, the representation over female and male progeny can be made more equal. At the extreme, if $V_{kf} = V_{kf} = 0$ and $\bar{k}_f = \bar{k}_m = 2$ (to compare to a non-growing population), then Eqs. (4) and (5) become $N_{ef} = 2N_f - 1$ and $N_{em} = 2N_m - 1$ and Eq. (6) becomes

$$N_e = \frac{8N_fN_m}{N_f + N_m - 1} \tag{13}$$

Assuming that $N_f = 43$ and $N_m = 86$, then $N_e = 231.1$, 3.0 times as large as above when there is random selection of progeny and 1.8 times as large as the total number of female and male parents (Table 3). Or, 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 or equal to the Poisson variance of 2. To illustrate, Table 3 gives examples for $V_{kf} = V_{kf} = 1$ and 2 when $\bar{k}_f = \bar{k}_m = 2$, using expressions (4–6). When $V_{kf} = V_{kf} = 2$, Poisson distribution of progeny, then $N_{ef} = 42.5$, $N_{em} = 85.5$, and $N_e = 113.6$, 48 % higher than the 76.8 using the observed distribution of progeny.

To evaluate the impact of using these wild-caught parents to initiate a captive breeding population, we can estimate the effective number of individuals at the same stage a captive breeding generation later, that is, the effective number of parents in the progeny of the wild-caught parents. 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 $N = 128$ and $N_f = N_m = 64$. 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 1.98). Using these values in expressions (4) and (5), then $N_{ef} = N_{em} = 63$. The overall effective population size in generation 2 would then be $N_{e2} = 126$. Using Eq. (9) and $N_e = 76.8$, then it is estimated that 99.35 % of the genetic variation in the wild population is present in the progeny of the 129 wild-caught parents.

Assuming that the distribution of progeny in the next generation is Poisson, that is $V_{kf} = V_{km} = 2$, and $N_e = 126$, then what is the impact on the reduction of genetic variation from these two generations? In this case, using expression (10), the expected proportion of genetic variation remaining from the wild populations after this generation is $P_2 = (0.9935)\ (0.9960) = (0.989589)$ so that nearly 99 % of the variation in the wild population should be still remaining in the 126 progeny selected to be parents of the next generation.

We can also calculate the average effective population size over these two generations from expression (11). For $N_{e1} = 76.8$ and $N_{e2} = 126$, $\bar{N}_e = 2/[(1/70.7) + (1/126)] = 95.69$. In other words, the average effective size per generation is 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 expression (12). Therefore, a single generation with an effective population size of 47.62 would have the same impact on the loss of genetic variation as two generations with $N_{e1} = 76.8$ and $N_{e2} = 126$.

Discussion

For genetic considerations, in the initiation of a captive population it is important to have as many unrelated and non-inbred founders as possible. Fortunately, our evaluation of the potential broodstock for pallid sturgeon demonstrated that the situation in pallid sturgeon does not appear to have related or inbred founders. First, using estimates of relatedness from microsatellite loci, we found that the wild adults (71 of which were founders) were only

as related as individuals from a large random-mating population. As a comparison, Crossman et al. (2011) found significant relatedness in hatchery lake sturgeon using a similar approach. Second, also using estimates of inbreeding from microsatellite loci, we found that none of the wild adults had a significant inbreeding coefficient. If there were any inbred founders, their progeny could have an inbreeding coefficient of zero if the inbred founder was crossed with unrelated mates. Third, there was a large number of unrelated founders, 43 females and 86 males and a large expected initial effective population size (76.8) compared to a number of other species as discussed in the introduction.

Our evaluation demonstrates that the initiation of the pallid sturgeon captive breeding population is not expected to suffer from bottlenecks, high relatedness or inbreeding, observed when some other captive populations have been initiated. As a result of this large founder number and large initial effective population size, in consideration of the difficulty in obtaining unique wild broodstock of pallid sturgeon, and assuming that the natural population will increase in size in a few generations because of habitat restoration, we conclude that additional sampling of wild fish is unnecessary. Fortunately, the lifespan in sturgeon results in high retention of genetic variation for a given amount of time compared to short generation organisms (Quattro et al. 2002). It is possible that some catastrophic event could occur in the hatchery during the production of the F_1 generation. If so, this could potentially result in loss of broodstock randomly across families or in specific families. In this case, the expected effective population size would need to be recalculated based on the specific details of the loss over families to determine the expected reduction in effective population size. There are siblings of many of these fish in the river, given such a catastrophic event in the hatchery, these fish could be brought into the hatchery. They then could be identified genetically, and used to genetically shore up under-represented families to reduce the potential loss in effective population size.

Evolutionary applications to sturgeon

First, one concern in releasing fish from a captive population is that it will result in a reduction of the effective population size in the wild population (N_{ew}) through the introduction of too many offspring from too few parents from a captive population (Ryman and Laikre 1991). Because in this case the upper Missouri stock of pallid sturgeon exhibits no natural recruitment at this time, N_{ew} 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_{ec}) that is stocked into the wild through the application of the calculations described above. We recommend that each year crosses should be made among unrelated captive broodstock and that larger families should be managed to equalize contributions from different parents because high variance from parents results in a decrease in N_{ec}.

Second, the initial N_e is 76.8, well above the minimum N_e of 50 prescribed by Franklin (1980) to avoid initial inbreeding depression in a captive population. Even over two generations, the impact of small population size is only equivalent to a single generation of 38.5 (using expression 12), close to this guideline. N_e was also estimated using the standardization suggested by Crow and Morton (1955) and this estimate was 77.1, nearly identical to the general approach. Recent estimates of historical N_e for upper Missouri River pallid sturgeon range from 181 to 367 (W.R. Ardren, personal communication) and the long generation length of pallid sturgeon (females generally do not reproduce until they are 15 years old, (Dryer and Sandvol 1993) suggest that inbreeding depression and maintenance of genetic variation should not be of great concern for many years

Third, Hedrick et al. (1995) suggested that to ensure that each parent is included in subsequent generations that every male should be mated with two different females and that every female should be mated with two different males. This is done so that every individual has a chance to contribute even when crossed with an individual whose gametes are not viable. When the crosses for each year are created they should be done among unrelated captive individuals. All of these individuals are progeny of fish from the upper Missouri River so that outbreeding depression should not be a problem. If the number of captive individuals outstrip the accommodations it may become necessary to cull some individuals from the program by releasing them into the wild with the stipulation that offspring from each of the parents (not each family) are retained. Hatchery managers should choose the individuals that are to be released randomly from each family and not just retain the largest and most vigorous individuals.

Finally, avoiding crosses between related individuals requires estimates of the degree of relatedness among male–female dyads. This can be determined either using pedigrees for individuals of known ancestry or through estimates of relatedness produced by molecular markers (e.g. microsatellites). Knowing the actual family relationships between the potential broodstock is often preferable to marker-based estimates due to the inherent errors in estimating relatedness from even a large panel of highly polymorphic markers (Jones and Wang 2010). For captive sturgeon PIT tags are sometimes shed or broken requiring marker based estimates of relatedness or, where possible, matching of offspring back to parents via genetic tagging (DeHaan et al. 2008).

General lessons

More and more species are becoming endangered in the wild, necessitating captive breeding to maintain the species. Often there are only a few individuals to use as founders for the captive breeding population. The situation for the pallid sturgeon is quite extreme with no recruitment in many decades and with the remaining wild individuals reaching the end of their lifespan. Our genetic evaluation clearly demonstrates that these remaining individuals were not closely related but were random surviving individuals from a formerly large population and that they themselves were not inbred (for similar findings in an unrelated, long-lived endangered fish, see Lippe et al. 2006). These findings are very useful in evaluating the genetic and evolutionary impact over time of using captive individuals to set up matings and to produce progeny for release. Such a detailed examination of potential founders as we have carried out here can give an important evaluation of the expected long-term success of captive breeding program in other such endangered species. Given an adequate number of unrelated and non-inbred founders, management to maximize the effective population size can retain this initial genetic variation. Management for retention of genetic variation also generally results in a minimal increase in inbreeding and a reduction in selection for domestication. The message is in general a positive one, genetic applications can be used to evaluate founders and their long-term evolutionary potential, and if the number of unrelated founders is large and they are not inbred, then efforts can be focused on other aspects of conservation.

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