

**Genetic guidelines for robust redhorse *Moxostoma robustum* reintroduction and supplementation
in the Pee Dee River, North Carolina**

Prepared by

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The use of hatchery-reared fish to augment natural populations and the concomitant genetic effects on wild fish stocks have become subjects of controversy and debate in recent years (Meffe 1992; Myers et al. 2004). Campton (1995) summarized that the genetic risks of hatchery supplementation can be grouped into one of three categories: those associated with outbreeding depression, loss of genetic diversity and inbreeding depression, and artificial selection within the hatchery. Note that for detailed background information on population genetic theories and genetic risks associated with hatchery supplementation, conservation hatchery programs should consult Miller and Kapuscinski (2003) and Hallerman (2003).

Outbreeding depression

Outbreeding depression is reduced fitness, measured as reproductive success, resulting from the interbreeding between genetically distinct populations or species (Hallerman 2003, Edmands 2007). Reduction in fitness is due to a loss of genetic adaptations or a disruption of co-adapted gene complexes (several genes working together, resulting in the expression of a certain trait; Dobzhansky 1941) that were present in the original population (Lynch 1991). For example, outbreeding can result in the loss of genetic adaptations for life history migrations (Altukhov and Salmenkova 1987). The loss of adaptation may occur in either the first or second generation of offspring after the interbreeding between distinct populations, but the reduction in fitness will not be observed until the adaptation that was lost is needed for survival or reproduction. Unfortunately, it is difficult to predict the fitness effects of outbreeding depression because there are no reliable indicators for the effect that outbreeding will have on fitness (McClelland and Naish 2007).

Alternatively, interpopulation hybridization can result in hybrid vigor, or increased fitness in hybrids due to the masking of deleterious alleles (Remington and O'Malley 2000) and has been used as a conservation technique in populations suffering from severe inbreeding depression (termed genetic rescue; Hedrick and Fredrickson 2010). In populations that are highly inbred and experiencing detrimental fitness consequences, very low levels of immigration and subsequent hybridization between the local population and immigrants can improve the fitness of the population (Tallmon et al. 2004), but this increased fitness effect is often lost in subsequent generations (Edmunds 2007).

Robust redhorse populations along the Atlantic coast harbor substantial genetic diversity (Table 1; Wirgin, pers. comm.; T Darden, pers. comm.) and genetically mixing robust redhorse populations could disrupt co-adapted gene complexes. Several examples have demonstrated the occurrence of

outbreeding depression in aquatic organisms. Offspring from naturalized populations of rainbow trout (*Oncorhynchus mykiss*) had higher survival in Lake Superior tributary streams in Minnesota than offspring resulting from crosses between natural and hatchery strains (Miller et al. 2004). Hatchery hybrid offspring had a reduced chance of surviving the first winter. In this case, the effects of outbreeding may have been amplified when the offspring were faced with harsh environmental challenges. Similarly, reduced fitness, measured by changes in embryo development time and survival, was observed in progeny from crosses of three geographically separate populations of southeast Alaska coho salmon (*Oncorhynchus kisutch*), relative to control lines (Granath et al. 2004). In pink salmon (*Oncorhynchus gorbuscha*), decreased survival in the F₂ generation of crosses between genetically distinct populations provided evidence for outbreeding depression due to the breakdown of co-adapted gene complexes (Gilk et al. 2004). Outbreeding between largemouth bass populations with population genetic differentiation (F_{st}) values of 0.05, resulted in increased infectious disease susceptibility (Goldberg et al. 2005). Note that the F_{st} values for largemouth bass populations were less than values observed in robust redhorse studies (Table 1). Thus, outbreeding depression should be a primary concern for any robust redhorse reintroduction effort and the use of genetically divergent brood stock (i.e., any population originating from outside the Pee Dee River) should be avoided.

Loss of genetic diversity

Within-population genetic variation may be reduced when a representative sample is not obtained from the donor population during collection of the brood stock and/or propagation of gametes collected from adults. Whether gametes, larvae, or adults are collected, only a small portion of the population and its genetic diversity is represented. The allele frequencies in wild and captive populations can be different and some low-frequency alleles may be absent in captive populations (Allendorf and Ryman 1987). When collecting individuals from donor populations, it is important to randomly sample an adequate number of individuals to ensure that genetic diversity is fully represented. Collecting individuals across the spatial and temporal variation shown by a spawning population must be considered in making collections.

Inbreeding, or the mating of close relatives, is expected to decrease a population's viability (Mills and Smouse 1994) due to inbreeding depression, or reduced fitness measured by survival and reproductive success. Inbreeding depression can be caused by recessive deleterious genes that are expressed because of increased homozygosity, or by a decrease in heterozygotes, where heterozygotes have a fitness advantage (Charlesworth and Charlesworth 1987). The resultant decrease in

heterozygosity may not be observed for several generations and numerically depressed populations may have large numbers of related individuals without an apparent decrease in heterozygosity. High relatedness among spawning adults results in inbred progeny. If an inbred population is used as a donor stock for stocking, the resulting progeny will also be inbred.

Inbreeding may be increased in an artificial production setting through the mating of related adults and/or the release of large numbers of related offspring, possibly leading to inbreeding depression. Ryman (1970) observed decreased survival in inbred families of Atlantic salmon *Salmo salar* released from a hatchery compared to non-inbred families also released from the hatchery; thus demonstrating inbreeding depression is a primary concern for artificial propagation. Reduced survival and growth was also observed in hatchery-reared Pacific salmon *Oncorhynchus* spp. (Kincaid 1983).

The effective population size (N_e) is the size of a hypothetical ideal population that would experience the same amount of genetic change as the population under consideration (Wright 1931, 1938). It is inversely proportional to the rate of inbreeding and is therefore a measure of within-population genetic diversity. The parameter N_e is often smaller than the census population numbers (N) due to unequal sex ratios, variance in family sizes, and changes in population size over generations (Kimura and Crow 1963). In hatchery settings, the large number of eggs from robust redhorse and the difficulty in handling adult fish can encourage the use of small numbers of parents. However, these practices can decrease N_e . A trade-off exists, known as the Ryman-Laikre effect (Ryman and Laikre 1991), in which a gain in the total production of offspring through stocking is accompanied by a reduction in N_e and a loss of genetic diversity. If a wild population is selected to receive stocked fish (supplementation), then it is likely that the natural population has a small population size. The hatchery contribution could swamp the genetic contribution made by the natural population, resulting in a decreased N_e . This result was realized in simulations of proposed stocking of Gulf sturgeon, *Acipenser oxyrinchus desotoi*, into the Suwanee River, Florida (Tringali and Bert 1998).

Artificial selection

In theory, captive-reared organisms may accumulate deleterious alleles through artificial selection within the hatchery that could hinder the recovery of natural populations. The use of hatchery-reared fish as brood stock (parents of hatchery fish) for many generations has resulted in individuals that contribute less to the gene pool (are less fit), in comparison with wild fish, in natural environments (Araki et al. 2007). On the other hand, captive breeding programs that use local wild fish as brood stock are expected to produce hatchery fish having minimal differences in fitness from wild

fish. Nevertheless, such captive-reared fish can be genetically distinct from wild fish for a variety of traits (Theriault et al. 2010) and may influence the fitness of the receiving wild population (Araki et al. 2007; Theriault et al. 2011).

To minimize risks associated with artificial selection it is important to sample brood stock both temporally and spatially within the spawning site. Collecting at a single time period during the spawning run may inadvertently select for early or late spawning times. Collecting at a single location within the spawning site may inadvertently select for certain habitat preferences and, if genetically based, could result in the lack of representation of potential ecotypes in the resulting offspring. For example, sturgeon stocking within the Azov Sea basin (Russia) has been occurring since the late 1950s. Within this basin, the Kuban River has experienced a loss of ecotypes, demonstrated by a shortened spawning run and breeding season (Chebanov et al. 2002). The loss of diversity has been attributed to the rearing conditions and selective breeding of females.

In summary, stocking may result in increased risks of negative consequences to the remnant robust redhorse population in the Pee Dee River. Therefore, stocking plans should be established by and coordinated with other agencies that manage robust redhorse populations within a water body. Stocking should be implemented only when other actions will not accomplish management goals or objectives. Here, we propose numerical threshold guidelines for initiating hatchery propagation for the purpose of 1) reintroducing robust redhorse above Blewett Falls Dam on the Pee Dee River, NC., and 2) supplementing the existing natural population below Blewett Falls Dam. Reintroduction is defined as the establishment of robust redhorse into a spawning location where they are absent. Supplementation is defined as stocking at a location where a robust redhorse population currently exists but the population is below desired abundance. I am under the assumption that no wild population exists at or above Blewett Falls Dam and that the goal of the reintroduction is to provide a demographic increase in the total number of robust redhorse adults in the Pee Dee River and at the same time minimize both short and long-term potential losses of genetic variation due to small effective population sizes. Note that this reintroduction effort should be done with Pee Dee River origin fish as genetic differences have been found between Savannah, Altamaha and Pee Dee populations (Table 1). Guidelines and justifications are listed below.

A. Collection targets and mating design

- Gamete collection should target 60 females and 60 males over a period of 20 years (resulting in an $N_e \approx 120$, with a yearly $N_e \geq 6$). Individuals should be used as brood stock once during this

20 year period. An estimate of the number of eggs needed from each female can be determined *a priori* based on expected survival rates during incubation and rearing so that sufficient progeny are available to meet stocking targets (see section B, Stocking numbers) without encumbering propagation facilities with unusable excess.

- If possible, adult brood stock should be captured throughout the spawning season and at several locations from the spawning population in order to maximize genetic diversity in collected gametes.
- Tag all brood stock so that their use in propagation will be known if they are recaptured and collect tissue samples for genetic analysis to establish a baseline of parental genotypes. This is critical for the long-term success of the program
- Implement a complete factorial mating design that does not equalize parental contribution. Eggs from each female should be divided equally into three separate batches and each male fertilizes a batch from each female. If less than three females and three males are sampled then follow the same factorial design mating scheme. Do not pool sperm or reuse males. All spawning activities should be recorded including the mating history and tag number. A tissue clip should be taken from each individual used as brood stock.

Rationale for collection targets and mating design

The Robust Redhorse Conservation Committee (RRCC) stated that any reintroduction effort should attempt to establish a population with an N_e of 100-200 (Appendix 2 of the 2002 RRCC Policies). Recognizing that the goal requires at least 100-200 adult brood stock makes achieving it difficult in a 1-2 year period. Fortunately, this goal can be spread out over at least a generation (i.e., ca. 20 years) or longer if deemed necessary. Thus robust redhorse gamete collection, propagation, and stocking require a long and sustained program if a population is to be restored or enhanced.

In theory, based on RRCC recommendations to achieve an N_e of 200, a total of 5 males and 5 females per year should be artificially spawned for 20 consecutive years (i.e., $200/20$ years = 10 brood stock or 5 males and 5 females per year). Demographic data indicate that this minimum number may be unattainable; alternatively, a reasonable goal for the first generation might be an $N_e > 120$ since a breeding population does exist (albeit a small one with an estimated N_e of 10-20; T. Darden, SCDNR pers comm.). This would equate to $120/20 = 6$ or 3 males and 3 females per year over a 20 year period.

In other stocking plans, 500 parents over 50 years (10 parents each year; yearly $N_e = 10$) were recommended for the white sturgeon *Acipenser transmontanus* (Pollard 2002), 100 parents over 10+ years (yearly $N_e = 6$) for the Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* (Pierre et al. 1996), and 50 parents over 5 years for the paddlefish *Polyodon spathula* (yearly $N_e = 10$) (MICRA Paddlefish Sturgeon Committee 1998). The Kootenai River white sturgeon conservation aquaculture program recommends a minimum target annual N_e of 10 (KTOI 2007).

Spawning protocols should attempt to achieve a balance between maximizing offspring production and minimizing the loss of genetic variation and is best achieved by a complete factorial breeding design (Fiumera et al 2004). The recommended mating scheme for robust redhorse is a complete factorial design, where females are mated to all available males (Fig. 1a, b). There is the possibility of an increase in relatedness among hatchery offspring (Miller and Kapuscinski 2003) upon implementation of a complete factorial design; therefore it is recommended that brood stock are only used once over the 20-year period. If a female has to be used more than once due to low abundance in the donor population, it should be mated with a different male(s). Sperm from multiple individuals should not be pooled; otherwise, sperm competition may result in the overrepresentation of a few males (Campton 2004). Collection of a small tissue sample from each brood stock will allow for subsequent genetic monitoring and assessment of hatchery return rates using molecular tags.

B. Stocking numbers

- Table 2 serves as a guide for the development of stocking targets based on life stage to be stocked. It is recommended that the scenario with survival rates of 10, 50, and 90% for age-0, ages 1-4 and ages 5-20 (respectively) be used because stage-specific survival appears closest to that of other long-lived iteroporous fish species. Thus the conservation hatchery program should strive to stock approximately 15,500 age-0 individuals above and below Blewett Falls Dam.
- To achieve this stocking target approximately 8600-10000 eggs per female (three females a year) will be needed annually. This target assumes a 10% survival rate from egg to fry.
- Stocking numbers are based on age-specific survival rates; therefore estimating these parameters should be of high priority. Stocking numbers should be reevaluated based on any new life history data.

Rationale for Stocking Numbers

The minimum target goal is to establish a group of breeding individuals with an expected N_e of 100-200 per generation above Blewett Falls Dam where suitable spawning habitat exists (Fisk 2010) and to increase N_e (to approximately 100-200 per generation) of the breeding population below Blewett Falls Dam. Assuming that N_e is approximately 10-50% of the census size of the breeding population, then a minimum target goal is to establish a per generation adult population size of at least 200-2000 ($100/0.01 = 200$; $200/0.01 = 2000$; note that all other values would be between 200 and 2000). This would equate to approximately 10-100 individuals per adult age class over the course of a generation above and below Blewett Falls Dam (i.e., $200/20$ age classes = 10; $2000/20$ age classes = 100).

There are several ways to calculate the number of robust redhorse to stock to achieve this population goal. The first is to assess known populations having this abundance of mature fish that are supported by successful reproduction and recruitment, and estimate the number of larvae, fingerlings, or yearlings that are produced. Unfortunately, these numbers are unavailable in similar systems. Alternatively, survival rates at different life stages can be estimated and stocking numbers back calculated. For example, Table 2 provides stocking rates for robust redhorse assuming various stage-specific survival rates. Annual survival rates may vary considerably among years and among populations; thus survival rates should be a priority research goal. Examples of known survival rates of hatchery fish are limited, but survival rates for other long lived iteroporous species can be used for comparative purposes. For instance, survival of hatchery-reared white sturgeon in the Kootenai River is estimated at 64% during the year after release and approximately 90% during all subsequent years (KTOI 2004). For lake sturgeon in the Peshtigo River (Lake Michigan), survival of wild larvae to fall fingerling stages appear to be around 1.0% (Benson 2004, Caroffino et al. 2007). In Green Bay, total annual mortality estimates for fish ages 9-60 ranged from 6.1% to 7.0%, although this estimate was considered a maximum, given the apparent increase in recruitment over the time period of the data (Gunderman and Elliott 2004). Total annual mortality for Manistee River fish ages 10-50 was estimated at 4.5% (Lallaman 2008). Baker and Borgeson (1999) reported a 5% total annual mortality rate for adult lake sturgeon in Black Lake, MI. Priegel and Wirth (1975) reported a total annual mortality of 5.4% for the Lake Winnebago sturgeon population. Annual mortality estimates for the Lake Winnebago population have ranges from 9.8 – 22.1% since 1953, with fishing exploitation accounting for 1.0 - 11.5% (Bruch 1999). Total annual mortality for lake sturgeon in the St. Clair

system was estimated at 9.0% (Thomas and Haas 2004). From these studies a very general pattern emerges – survival from the larval to juvenile stage is low (1%) with increasing survival at the juvenile to adult (40-50%) and adult stages (>90%). Table 2 provides examples of stocking rates to guide establishment of a population of 200-2000 mature adults (5-20 years of age) given three levels of assumed survival for three life stages. For example, assuming annual survival rates of 10% for age-0, 50% ages 1-4 and 90% for ages 5-20, a minimum of 15,542 age-0 robust redhorse would need to be stocked per year for 20 years to attain an adult population of approximately 791 fish with age classes 5-20 being represented by approximately 10-100 individuals (Table 3). The survival rates in Table 2 are assumptions and can greatly affect the number of fish needed for stocking, particularly those for adult fish. Survival rates and habitat availability for all life stages will need to be determined and continually evaluated to adjust or modify appropriate stocking rates. Habitat, including water quality, must be capable of supporting the life stage stocked.

Outlined above is a range of ideal annual stocking targets, but stocking targets will also be dictated by the number of eggs produced in each brood year. It is recommended that three males and three females be used for brood stock annually. Thus the predicted number of eggs in a given year would be 258,000 assuming approximately 86,000 eggs per female (three females per year). A 10% survival from egg to fry would indicate that each female should produce approximately 8600 eggs for a predicted total of 25,800 fry annually. If half of the fry (representing all female and male crosses) are stocked above Blewett Falls and the remainder below, then there should be enough eggs to obtain almost all stocking strategies listed in Table 2 (the exception would be the first strategy, but the numbers of eggs would probably be close enough even for this stocking strategy).

There are trade-offs in any supplementation program (i.e., below Blewett Falls Dam) and one has to remember that the supplemented population will be comprised of wild and hatchery fish that will eventually interact. Unfortunately, the more successful the stocking, the more the genetic composition of the population will resemble that of the hatchery. For example, N_e of Pee Dee robust redhorse is presumed to be 10-20. Following the equation of Ryman and Laikre (1991)

$$\frac{1}{N_e} = \frac{x^2}{N_h} + \frac{(1-x)^2}{N_w}$$

(where N_h is the effective size of the hatchery population, N_w is the effective size of the wild fish breeding in the wild, and the third parameter (x) is the relative reproductive success (contribution) of hatchery compared with wild fish when breeding in the wild), if we remove one male and one female (effective population size in captivity is two and in the wild is approximately 18) then the only way that the population will maintain genetic diversity (N_e) is if the relative contribution from the hatchery stocking is 10% when compare to the wild. In all other circumstances, genetic diversity will be lost after one generation of stocking (see Fig. 2). This is not a likely scenario for the robust redhorse supplementation program because we expect that the relative contribution of hatchery fish over the course of a generation will be large (i.e., 791; see Table 3) in comparison to that of the wild (i.e., 60; R. Heise, NCDNR pers. comm.). One way to minimize the Ryman-Laikre effect is to attempt to capture all the genetic diversity in the wild population over a single generation (Ryman and Laikre 1991). As shown in Figure 1, when a larger portion of the wild population is used in the hatchery supplementation program (i.e., when 18 of 20 individuals are used in the hatchery supplementation program for the example above) and if the predicted relative hatchery contribution is greater than 80%, then the loss of genetic diversity in the wild will be minimized (see Fig. 2 for $N_c = 18$). Thus, the Ryman-Laikre effect should be minimized in the robust redhorse supplementation program described herein because most of the wild population will be used for hatchery supplementation over the course of 20 years. Still, there is the potential for the loss of genetic diversity if the relative hatchery contribution is low (< 80%); therefore the success of the hatchery program (i.e., assessing the relative contribution of individuals that are of hatchery origin) should be carefully monitored.

C. Release techniques

- Release propagated robust redhorse at the earliest life stage possible, considering the trade-off between survival, domestication or other culture effects, and genetic and demographic benefits and risks.
- Mark all fish stocked so that, at a minimum, stocking location and year-class can be determined for recaptured fish. Ideally, all stocked fish would be marked with PIT tags or other individual-specific internal tags.
- Release robust redhorse in locations where habitat is suitable for the life stage(s) being stocked.

- Use release techniques that increase chances for survival, such as acclimation pens, nighttime releases, and multiple releases over time.

Rationale for release techniques

Although releasing fish at the earliest life stage possible will likely minimize artificial selection for the hatchery environment (Allendorf 1993), fish may be held at hatchery facilities through much of the first growing season to reduce exposure to early mortality sources such as predation, and to facilitate tagging or marking of individuals prior to release. In an attempt to maximize survival, fish should be released into receiving waters at locations where wild fish are known or would be expected to reside at that period in their life history.

Marking should identify individuals and/or individual families of origin including stocking location, experimental unit, date of release, and year class. A marking technique should be used that can be universally interpreted. PIT tags afford this level of discrimination at a relatively small cost compared to the overall cost of implementing restoration stocking. The potential benefits that will be afforded during evaluation justify the initial expense of individual tagging. Genetic analysis can also afford some of the identification specificity needed, but costs and logistics are likely to be greater. However, for larval drift collections, wild egg collections, or families that have been commingled prior to stocking, tissue samples for genetic analysis should be collected from all fish stocked in order to evaluate final parental contribution.

D. Evaluation

- Robust redhorse conservation plans that involve stocking projects should contain detailed evaluation criteria that are explicitly linked to conservation objectives. Evaluation will provide the opportunity to learn over the considerable time periods of robust redhorse stocking program and to adapt programs to be more effective. Benchmarks to evaluate stocking success should be developed for various life stages, behaviors, and time periods (e.g., every five years). Assessment and monitoring need to be carried out to gauge progress toward benchmarks. Commitment to evaluation is essential if a stocking program is to contribute new information and be adaptive.
- Records of the number and sex of donors obtained each year must be kept. If the number of donors falls short of the yearly target for 5 consecutive years, it is unlikely the overall minimum

contributions from at least 200 parents will be met. A different donor population should be identified or rehabilitation/restoration efforts should be reevaluated.

- Assess larval and juvenile survival for a minimum of 5 years after the first stocking event. Compare measured survival rates with the assumed survival rates used to determine stocking numbers. Adjust stocking numbers accordingly.
- Ten years after the first stocking event, rigorous evaluations of spawner returns should begin. If ripe males are not detected in the target river during years 10-20, consider possible reasons and modify the management approach accordingly.
- Twenty years after the first stocking event, rigorous evaluations of recruitment should begin (if natural recruitment was a stocking goal). If successful reproduction and recruitment are not detected in the target river during years 25-30, or soon after mature females are detected on the spawning grounds, consider possible reasons and modify the management approach accordingly.

Rationale for evaluation

Due to the long-term commitment necessary for robust redhorse stocking (20+ years), an opportunity exists for learning from the stocking program and making necessary modifications while the program is being implemented. The cost and effort required for an effective stocking program makes the creation of a thorough evaluation program a good investment of resources. Defining clear criteria for the determination of success or failure reduces subjectivity in determining whether management actions need to be altered.

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Table 1. Pair-wise estimates of genetic divergence (F_{st}) among robust redhorse populations. Above the diagonal are mitochondrial DNA F_{st} estimates, and below are nuclear microsatellite F_{st} estimates (I. Wirgin, pers. comm.)

	Oconee River	Pee Dee River	Savannah River
Oconee River	-	0.948	0.8153
Pee Dee River	0.219	-	0.935
Savannah River	0.118	0.129	-

Table 2. Estimated annual stocking numbers of age-0 and age-1 robust redhorse necessary to achieve the recommended adult population based on a range of possible annual survival rates (S). Stocking numbers assume 20 yrs of stocking and a target adult population of 200-2000 fish (ages 5- 20) that constitutes 10-100 adult fish per age class.

%S age 0	% S age 1-4	% S age 5-20	# age-0 to stock for target of 10-100 in each age class	# age-1 to stock for target of 10-100 in each age class
10	50	90	15,542	1,554
		95	6,907	691
		98	4,333	433
10	75	90	3,070	307
		95	1,364	136
		98	856	86
10	90	90	1,481	148
		95	658	66
		98	413	41
25	50	90	6,217	1,554
		95	2,763	691
		98	1,733	433
25	75	90	1,228	307
		95	546	136
		98	342	86
25	90	90	592	148
		95	263	66
		98	165	41
50	50	90	3,108	1,554
		95	1,381	691
		98	867	433
50	75	90	614	307
		95	273	136
		98	171	86
50	90	90	296	148
		95	132	66
		98	83	41

Table 3. Back calculation of the number of robust redhorse progeny needed to obtain 15 year classes of approximately 10-100 adults/age class assuming differing age-specific survival rates (*S*). Note that the overall estimate of adults in this scenario is 791 (sum of # of individuals from age-5 to age-20).

Age	# individuals	<i>S</i>
0	15,542	0.1
1	1,554	0.5
2	777	0.5
3	388	0.5
4	194	0.5
5	97	0.9
6	87	0.9
7	78	0.9
8	70	0.9
9	63	0.9
10	57	0.9
11	51	0.9
12	46	0.9
13	41	0.9
14	37	0.9
15	33	0.9
16	30	0.9
17	27	0.9
18	24	0.9
19	22	0.9
20	20	0.9

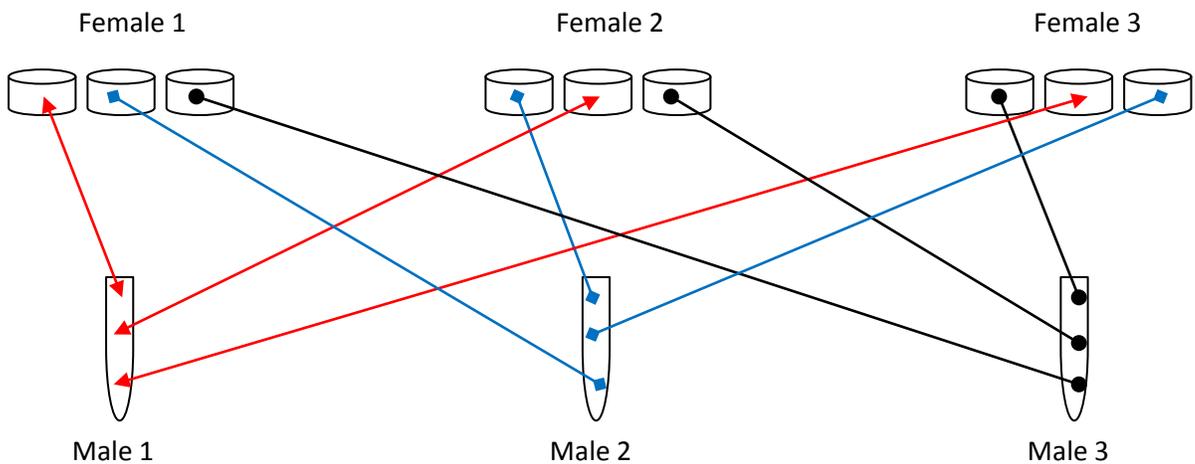
Figure 1. Full factorial mating design for robust redhorse based on three females and three males. A) Graphical representation of the factorial design. B) Pictorial representation of the factorial design. Each female's eggs will be collected and divided equally among three containers. Approximately one third of each male's collected sperm will be mixed with each female's eggs. Note that only one male's collected sperm fertilizes a container of eggs

A)

	male 1	male 2	male 3
female 1			
female 2			
female 3			

B)

- Each female's eggs divided into three containers:



- 1/3 of each male's sperm is used to fertilize each female's eggs:

Figure 2. Depiction of the Ryman-Laikre effect for various hatchery effective sizes and relative contributions. The maximum effective population size for the wild population is 20. The abbreviation N_c refers to the effective population size of the captive (hatchery) portion of the population. For small N_c (i.e., $N_c = 2$), the loss of genetic diversity can be great unless the relative hatchery contribution can be minimized. For large N_c (i.e., $N_c = 18$), the loss of genetic diversity can be minimized by maximizing the hatchery contribution compared to the wild population.

