Conservation in Practice

Maximizing Offspring Production While Maintaining Genetic Diversity in Supplemental Breeding Programs of Highly Fecund Managed Species

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Abstract: *Supplemental breeding is an intensive population management strategy wherein adults are captured from nature and spawned in controlled settings, and the resulting offspring are later released into the wild. To be effective, supplemental breeding programs require crossing strategies that maximize offspring production while maintaining genetic diversity within each supplemental year class. We used computer simulations to assess the efficacy of different mating designs to jointly maximize offspring production and maintain high levels of genetic diversity (as measured by the effective population size) under a variety of biological conditions particularly relevant to species with high fecundity and external fertilization, such as many fishes. We investigated four basic supplemental breeding designs involving either monogamous pairings or complete factorial designs (in which every female is mated to every male and vice versa), each with or without the added stipulation that all breeders contribute equally to the total reproductive output. In general, complete factorial designs that did not equalize parental contributions came closest to the goal of maximizing offspring production while still maintaining relatively large effective population sizes. Next, we estimated the effective population size of 10 different supplemental year classes within the breeding program of the robust redhorse (Moxostoma robustum). Two year classes failed to produce progeny, whereas successful year classes used partial factorial designs to realize effective sizes ranging from 2 to 26 individuals. On average, a complete factorial design could increase the effective size of each robust redhorse supplemental year class by 19%.*

Programas Reproductivos Suplementarios para Especies Bajo Manejo: Estrategias para Maximizar la Producción de Crías y Mantener la Diversidad Genética en Taxones Altamente Fecundos

Resumen: *La reproducción suplementaria es una estrategia de manejo intensivo de poblaciones en la que se capturan adultos silvestres, desovan en condiciones controladas y las crías resultantes son posteriormente liberadas al medio silvestre. Para ser efectivos, los programas de reproducción suplementaria requieren estrategias cruzadas que maximicen la producción de crías mientras se mantiene la diversidad genética dentro de cada clase suplementaria anual. Utilizamos simulaciones de computadora para evaluar la eficacia de diferentes diseños de apareamiento para conjuntamente maximizar la producción de crías y mantener niveles altos de diversidad genética (medida por el tamaño poblacional efectivo) bajo una variedad de condiciones biológicas particularmente relevantes para especies de alta fecundidad y fecundación externa, como muchos peces. Investigamos cuatro diseños básicos de reproducción suplementaria involucrando apareamientos monógamos o diseños completamente factoriales (en los que cada hembra es apareada con cada macho y viceversa), cada uno con o sin la estipulación de que todos los reproductores contribuyen equitativamente al total de la reproducción. En general, los diseños factoriales que no igualaron las contribuciones parentales fueron los más cercanos a la meta de maximizar la producción de crías y mantener tamaños poblacionales efectivos*

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relativamente grandes. A continuación, estimamos el tamaño poblacional efectivo de 10 clases suplementarias anuales en el programa de reproducción de Moxostoma robustum. Dos años dejaron de producir progenie, mientras que las clases anuales exitosas usaron diseños parcialmente factoriales para obtener tamaños efectivos que variaron de 2 a 26 individuos. En promedio, un diseño completamente factorial incrementaría el tamaño efectivo de cada clase suplemental anual de Moxostoma robustum en 19%.

Introduction

Many factors, including habitat degradation, overharvesting, exotic species introductions, and other anthropogenic influences, have severely reduced the range or population size of many species. One case in point is the robust redhorse (*Moxostoma robustum*), a native freshwater fish of Atlantic-coast drainages in the southeastern United States (Jenkins & Burkhead 1994). Formerly thought to have been extirpated following European settlement, five living specimens of the robust redhorse were discovered in 1991 (Bryant et al. 1996), and subsequent sampling efforts revealed extant populations in the Ocmulgee, Oconee, Pee Dee, and Savannah rivers. The Robust Redhorse Conservation Committee is implementing intensive management strategies to recover this species, reintroduce it to currently unoccupied rivers within its former range, and prevent it from formally being listed (Hendricks 1998). To help achieve these goals, a supplemental breeding program was established in 1993. Although the merits of supplemental breeding are debated (Snyder et al. 1996, 1997; Hutchins et al. 1997), this approach has become a common management technique for many fish species (e.g., Brown et al. 2000; Hedrick et al. 2000).

Supplemental breeding is an intensive population management strategy wherein adults (often assumed to be unrelated) are captured from nature and spawned in controlled settings, and the resulting offspring are later released into the wild. In the following year or cycle, breeding adults again are captured and the process is repeated. Supplemental breeding programs often attempt to jointly maximize the number of offspring produced and the genetic diversity present in the progeny. The high fecundity of many fishes leads to the possibility that only a small number of parents produce a large proportion of the supplemental progeny, such that subsequent reintroductions return only a limited amount of the natural genetic diversity. The effective size of a population (N_e) is a useful measure of the amount of genetic diversity expected to be retained within a population. Accordingly, issues related to N_e have figured prominently in conservation plans and in the design of captive breeding programs for many conserved species (Lacy 1989; Bartley et al. 1992; Allendorf 1993; Falconer & Mackay 1996; Montgomery et al. 1997; Fiumera et al. 1999).

We used computer simulations to investigate the effectiveness of various supplemental breeding designs with respect to preserving genetic diversity while maximizing offspring production under a variety of fitness scenarios. The results should apply to any species in which the gametes of both males and females can be collected, fertilization is external, and individual fecundities are high. Such is the case, for example, in many fishes, invertebrates, and plants. Additionally, we used estimates of the number of progeny produced to calculate the variance effective population size for the 10 year classes within the supplemental breeding program of the robust redhorse.

Methods

Effectiveness of Breeding Designs

If all adults are captured from the wild, only six discrete mating protocols (along a continuum of possibilities) potentially applicable to fishes need to be considered. Under the first protocol, as many females as possible are captured and the smallest number of males required to fertilize all the eggs are used as sires. Given a highly skewed sex ratio, this female-biased design yields a reduced effective population size (Wright 1931). Because this protocol does not meet the goal of maximizing genetic diversity, we do not consider it further. Nor do we consider the second, which is male-biased because sperm from as many males as possible are used to fertilize the eggs of only one or a few collected females. The practice of pooling gametes prior to fertilization can lead to high variance in reproductive success (Withler 1988; Gile & Ferguson 1995; Brown et al. 2000); thus, we did not include this in our simulations but discuss our findings in relation to this approach.

Here we summarize the remaining four discrete breeding designs for cases with equal numbers of sires and dams. In a monogamous design (MD), the eggs from each randomly chosen female are fertilized by sperm from only a single, randomly chosen male, and each male is mated to only a single female. Thus, all offspring from any parent are full-sibs. In a complete factorial design (FD), the eggs from each female are divided equally into different batches, and each male fertilizes one such batch from each female. Thus, each male is mated to every female and vice versa. Under both the MD and FD protocols, all the offspring generated are used in the supplemental breeding program, in which each parent contributes offspring in proportion to its reproductive success.

The final two discrete protocols—equalized-contribution monogamous design (ECMD) and equalized-contribution factorial design (ECFD)—are identical to MD and FD, respectively, except that not all the generated offspring are utilized in the supplemental breeding program. Instead, the offspring of overrepresented adults are purposefully culled, thereby resulting in an artificial equalization of the reproductive output (or genetic fitness) of all adults. This procedure, suggested by Allendorf (1993) to inhibit adaptation to captivity, is explicitly designed to reduce the variance in reproductive success and, in that respect, to maximize N_e.

Because complete factorial designs may not always be tractable in the field, we also investigated the performance of partial factorial designs (e.g., Hedrick et al. 2000). In these cases, each individual is crossed to the same number of multiple mates but not to all of them (without equalizing parental contributions). These protocols represent the sample space along the continuum from mating each individual with only a single partner (monogamous design) to mating every male with every female (complete factorial design).

We performed computer simulations with MatLab (The Math Works, Natick, Massachusetts), for the four basic protocols under varying numbers of breeding adults and fitness determinants (see Table 1 for details). Given that few empirical data exist on absolute fitness distributions in natural fish populations, we chose parameters to incorporate a variety of biological scenarios, each promising to generate a growing population (i.e., with a mean of more than two offspring per female) while maintaining similar overall reproductive outputs across conditions. We conducted simulations for populations with both equal numbers of males and females and sex ratios skewed toward males by two to one. In skewed "monogamous" cases, each female was mated to two males, but each male was mated to only one female.

Table 1. Conditions for each of the parameters in the computer simulations estimating the effective population size generated under different mating designs.

Parameter	*Conditions*
Number of breeding adults	
equal sex ratio	5, 10, 15, 20, 25 females and males
skewed sex ratio	5, 10, 15, 20, 25 females and twice as many males
Fitness determinants	
probability of sterility	0.01, 0.05, 0.10
fitness distributions	uniform (on 0.1,1.0), where a fitness of 1.0 produces 5000 offspring
	Poisson (mean = 2500 for additive, mean = 71 for multiplicative)
male X female interaction	additive or multiplicative

In each simulation, a specified number of adults (see Table 1) was generated, and some individuals were randomly designated as sterile (with a probability of 0.01, 0.05, or 0.10). Each sterile adult was assigned a fitness of 0. Nonsterile individuals were randomly assigned a "fitness value" according to either a uniform or a Poisson distribution (algorithm from Smyth 1999), as specified in Table 1. We regenerated the simulations in any instance where all adults of either sex were sterile. The fitness for each mated pair (i.e., the number of offspring produced) was determined according to either an additive or a multiplicative interaction between the fitness values of the parents (Table 1). Under the equalized-contribution designs, the reproductive output of each male (and female) was truncated to that of the least productive member of that gender. The variance in reproductive success among males and females was recorded and used to calculate N_e. For each set of parameters, the following were calculated across 5000 replicates: mean effective population size ($\overline{N_e}$), standardized variance in effective population size

$$\left(\frac{\mathrm{var}(N_e)}{\overline{N}_e^{\,2}}\right),$$

total offspring production, and standardized variance in offspring production. Standardized variances were recorded to determine the expected variability under each of the designs.

Finally, we investigated the potential benefit of a partial factorial breeding design, assuming a uniform fitness distribution and multiplicative fitness interactions for five parents of each sex. For each of the three probabilities of sterility (Table 1), we calculated the mean effective population size from 5000 replicates, in which each parent was mated to between one (monogamous) and five (factorial) partners of the opposite sex and parental contributions were not equalized. The cases in which each parent was mated to either two, three, or four members of the opposite sex represent the partial factorial design.

We calculated the variance effective population size for the growing populations as

$$N_e = \frac{4\,N_{\mathrm{em}}\,N_{\mathrm{ef}}}{N_{\mathrm{em}} + N_{\mathrm{ef}}},$$

where N_{em} and N_{ef} are the effective number of breeding males and females, respectively (Kimura & Crow 1963). Here, the effective number of males was computed as

$$N_{\mathrm{em}} = \frac{N_m \bar{k}_m - 1}{\bar{k}_m - 1 + \frac{V_{\mathrm{km}}}{\bar{k}_m}},$$

where N_m is the census number of breeding males, $\bar{k}_m$ is the mean number of offspring over all males, and V_{km} is the variance in male reproductive success; an analogous formula provides the effective number of breeding females, N_{ef} (Kimura & Crow 1963).

Effective Size of Robust Redhorse Supplemental Breeding Program

We estimated the effective population size from each year class within the actual supplemental breeding program of the robust redhorse using the total number of offspring from each cross at the time of fertilization (often the latest time point in which individual families were maintained separately). We excluded any crosses that experienced 100% mortality. To account for a growing population, we estimated N_e for each year class according to the formulas presented above (Kimura & Crow 1963). This supplemental breeding program used a partial factorial design and only wild individuals as breeders. We assumed that all breeders were unrelated, although we had no knowledge of relatedness. Therefore, it was not possible to purposely mate unrelated individuals to avoid the potential deleterious effects of inbreeding. We investigated the expected increase in N_e from using a complete factorial design by dividing the actual contribution of each female equally among all males.

Table 2. Average effective population size (mean N_e), standardized variance in effective population size [std. var(N_e)], mean relative production of offspring (mean prod.), and standardized variance in offspring production [std. var(Prod.)] for each of the four basic breeding designs.[a]

	Monogamous	*Factorial*	*Monogamous equalized*	*Factorial equalized*
Mean N_e				
UA	28.70	31.22	31.59	32.99
UM	22.13	26.87	31.97	33.41
PA	31.58	32.98	31.58	32.99
PM	30.82	32.53	31.59	32.99
Std. var(N_e)				
UA	0.0070	0.0024	0.0061	0.0023
UM	0.0153	0.0043	0.0061	0.0023
PA	0.0061	0.0022	0.0061	0.0022
PM	0.0061	0.0023	0.0061	0.0022
Mean prod.[b]				
UA	100	99.97	27.41	27.43
UM	99.95	100	7.75	7.76
PA	99.97	100	96.31	96.33
PM	100	99.97	62.20	62.21
Std. var(prod.)				
UA	0.0134	0.0129	0.0548	0.0564
UM	0.0377	0.0340	0.2481	0.2486
PA	0.0063	0.0061	0.0063	0.0061
PM	0.0080	0.0078	0.0182	0.0180

[a] *Results are given for a uniform fitness distribution with additive (UA) or multiplicative interactions (UM), and a Poisson distribution with additive (PA) or multiplicative interactions (PM). Results are averages across all parental numbers, sex ratios, and probabilities of adult sterility.*

[b] *Mean offspring production is presented as a percentage (relative to 100% for the mating design with the highest supplemental-breeding output for that fitness determinant).*

Results

Effectiveness of Breeding Designs

Numerical results from the computer simulations are summarized in Table 2, and specific points are illustrated in Figs. 1 and 2. The text below reflects the overall outcomes averaged across the various sets of fitness determinants tabulated in Table 2.

The breeding designs in order of increasing $\overline{N}_e$—and hence in their capacity to maintain genetic variation—were as follows (Fig. 1): monogamous designs (MD), complete factorial designs (FD), equalized-contributions monogamous (ECMD), and equalized-contributions factorial (ECFD). Across all conditions, MD and FD displayed mean effective population sizes that were, respectively, 85% and 93% that of ECFD. However, under a Poisson fitness distribution (with either additive or multiplicative fitness), FD had a slightly larger $\overline{N}_e$ than did ECMD (Table 2). The standardized variance in effective population size also differed among the four breeding designs (Table 2). In general (Fig. 1), the factorial designs (FD and ECFD) had lower standardized variances in effective population size than did the monogamous designs (MD and ECMD).

As expected, the two designs that equalized parental contributions (ECMD and ECFD) had a much lower mean production of offspring than did either one (MD or FD) with unequal contributions (Fig. 1). On average, the former protocols produced only 56% of the total number of offspring as the latter. Although rank order was concordant across all fitness determinants, the actual numerical values differed dramatically. For example, assuming a Poisson fitness distribution and additive interactions between male and female fitness, ECMD and ECFD produced 96% as many progeny as either MD or FD. Assuming a uniform fitness distribution and multiplicative interactions, ECMD and ECFD produced only about 8% as many progeny as MD and FD (Table 2). In addition to lower offspring production overall, ECMD and ECFD had higher standardized variances in offspring production levels than MD and FD (Fig. 1).

Given that factorial breeding designs had a larger $\overline{N}_e$ than the monogamous designs but still maintained high offspring production, we investigated the potential genetic benefits of partial factorial designs. With five parents of each sex and a uniform fitness distribution within the sexes and multiplicative interactions between the sexes, mating each parent with only two members of the opposite sex dramatically increased the effective population size of the supplemental breeding program compared with a complete monogamous design (Fig. 2). For example, under these conditions, mating each adult with two partners (a partial factorial design) increased N_e by 13% over the monogamous design (Fig. 2). Although a complete factorial design increased the effective population size by about 22%, the benefits from each

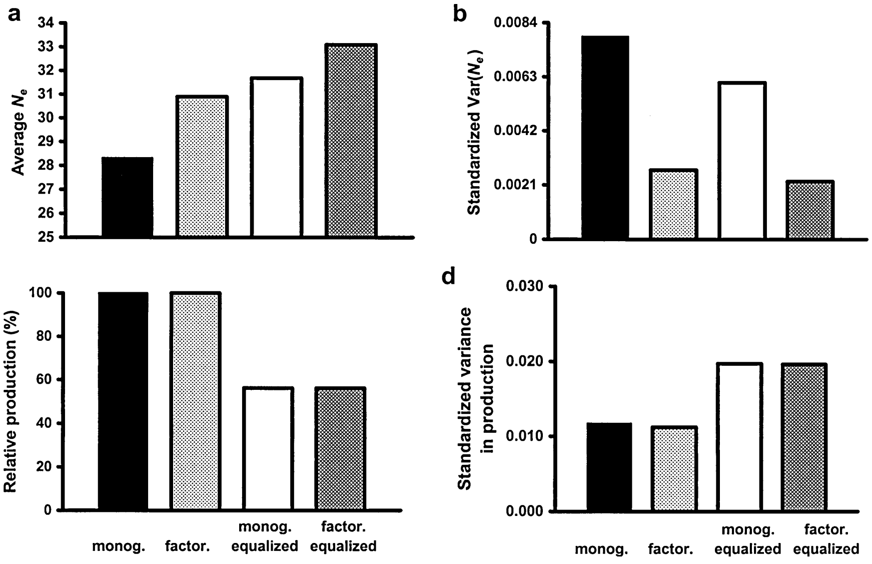

Figure 1. (a) Average effective population size, N_e; (b) standardized variance in effective population size; (c) average relative production of offspring (calculated as a percentage of the highest output in any of the breeding designs); and (d) standardized variance in offspring production for each of the four basic supplemental breeding designs: monogamous (monog.MD), factorial (factor.FD), monogamous with equalized contributions (monog.equalized ECMD) and factorial with equalized contributions (factor.equalized ECFD). Results are averages across all sets of 5000 replicated computer simulations involving varying combinations of fitness determinants, parental numbers, and probabilities of sterility.

additional mating declined sharply after two mates were used (Fig. 2).

Effective Size of Robust Redhorse Supplemental Breeding Program

The census number of parents contributing to each of the different supplemental year classes ranged from only 2 specimens in 1993 to 40 in 2000, for a total of 182 individuals (Table 3). In the actual supplemental breeding program for the robust redhorse, a partial factorial design was used. Females were mated to an average of 1-5 males in any year, whereas males had an average of 1-2.1 partners. The corresponding effective population sizes for each year ranged from 2 to approximately 26 between the different year classes (Table 3). Except in 1993, when only one parent of each sex was used, the effective population size of each year class was less than the census number of breeders. On average, a complete factorial design would have increased the effective

Figure 2. Relative effective population size (setting the complete factorial design to 100%) as a function of the number of matings per parent, assuming a uniform fitness distribution and multiplicative interactions between gametes from five dams and five sires. For each number of mates, ranging from one (monogamous design) to five (complete factorial design), results are averages across all 5000 replicates involving varying probabilities of adult sterility.

Table 3. Census number of spawners (N), number of mates, actual effective population size (actual N_e) given the realized crosses, and the potential effective population size under a complete factorial design (factorial N_e) in the robust redhorse supplemental breeding program.

		Mean no. mates per			
*Year class**	N	*female*	*male*	*Actual* N_e	*Factorial* N_e
1993	2	1	1	2	2
1995	32	1.4	1.4	20.7	24.9
1997	27	1.5	1.1	19.1	21.9
1998	14	2.5	1	7.7	9.5
1999	39	1.7	1.6	25.7	33.3
2000	40	4.3	1.9	20.0	27.0
2001	12	5	1	3.6	3.8
2002	16	4.6	2.1	7.3	8.9

**Year classes 1994 and 1996 experienced near 100% mortality and are not considered.*

population size by approximately 19% over that of the partial factorial design that was actually used (Table 3).

Discussion

Genetic Diversity versus Offspring Production

Maximizing offspring production and minimizing the loss of population genetic variation are often viewed as separate and sometimes competing goals. For example, equalizing family sizes has been promoted as a technique to reduce adaptation to captivity in controlled breeding programs (Allendorf 1993; but see Frankham et al. 2000), but this can greatly reduce total reproductive output. Likewise, population genetic theories underlying captive breeding programs often address the maintenance of genetic diversity but may neglect total offspring production (e.g., Kimura & Crow 1963; Ballou et al. 1995; Montgomery et al. 1997). The latter orientation is exemplified by the increasing number of studies that apply molecular markers to monitor the maintenance of genetic diversity in either captive or supplemental breeding programs (e.g., Pereira & Wajntal 1999; Schreiber & Hegel 1999; Fiumera et al. 2000).

Conversely, and at the other extreme, some breeding programs (particularly in fishery management) have focused primarily on gross progeny production but have neglected considerations of genetic variation and effective population size. For example, in attempts to lessen the immediate logistical hurdles of fish capture and husbandry, a common strategy has been to employ the minimum number of males required to fertilize the eggs of available females. This strategy may merely defer other real costs, however, including possible genetic difficulties such as inbreeding depression, promotion of adaptation to captivity, or loss of longer-term evolutionary potential.

Our purpose was to investigate the extent to which the maintenance of genetic diversity in supplemental breeding programs might inevitably come at the expense of reductions in immediate offspring production, or vice versa. Results of our computer simulations indicate that factorial designs, without equalizing parental contributions, can often provide a best-of-both-worlds strategy. Under this protocol, mean offspring production was maximized (at over twice the level that equalized-contribution designs achieved) whereas $\overline{N}_e$ was only 7% less than the maximum. In addition, this design realized a low standardized variance for both effective population size and offspring production, thus decreasing the chances of boom-and-bust years.

A complete factorial design is, in effect, a method that partially equalizes the reproductive contributions of breeding adults without eliminating any of the progeny produced. This may be especially beneficial when some of the potential breeders are sterile. For example, any clutch of eggs exposed exclusively to sperm from a sterile male will, by definition, fail to produce viable offspring. In a monogamous design, the entire genetic contribution of the male and his single mate are therefore lost. In a factorial design, however, that female would still produce offspring sired by other, fertile males. This same benefit likewise would be observed for any male mated to a female that produced nonviable eggs. Thus, a factorial breeding design is tantamount to an insurance policy against nonviable gametes in a sexual partner.

It is important to recognize that not all supplemental breeding programs have identical goals. In some cases, for example, the maintenance of genetic diversity may be the highest priority, and thus a complete factorial design with equalized family sizes may be appropriate. In other cases, extremely high variance in reproductive success may be partially due to the contribution of a single breeder, and limited culling could dramatically increase N_e. In short, although complete factorial designs can be an improvement over traditional methods, managers of each individual supplemental breeding program should fully investigate a variety of options before committing to a given breeding design.

Logistical and Other Constraints

With any field-oriented management approach, complications and logistical constraints likely will cause the actual execution of a supplemental breeding program to depart from the idealized scenario. For example, gametes stripped from breeding fish have a limited temporal viability (Bencic et al. 2000; Ritar & Campet 2000). In the case of the robust redhorse, where the primary spawning period can span 2 weeks, gametes collected early in the breeding season are often nonviable by the time ripe gametes from other individuals become available, thus making complete factorial designs impossible for field personnel. Despite the inherent difficulties of implementing a complete factorial design, large genetic benefits nonetheless can accrue from mating each individual to even as few as two members of the opposite sex (i.e., a partial factorial design).

Another important point relates to the common practice in some fish-breeding schemes of pooling stripped gametes from many individuals prior to fertilization or of exposing eggs to the milt serially stripped from multiple males. Large variances in reproductive success across adults often accompany these procedures (Withler 1988; Gile & Ferguson 1995; Brown et al. 2000). For example, Withler (1988) fertilized batches of chinook salmon eggs using milt pooled from three males. Across a series of replicates, the actual effective number of males was only 60% of what could have been achieved with an equalized-contribution complete factorial design. In our simulations, the complete factorial design without equalizing contributions recorded effective population sizes that were at least 80% of the maximum possible, much greater than if the gametes had been pooled. To ameliorate the effect of pooling milt, the crosses need to be separated completely, such that eggs from each female are first divided into multiple lots, which are each then fertilized by a different male. Although any such implementation of a complete factorial design requires added effort, these additional matings would be necessary to realize the predicted genetic benefits. The effect of pooling milt and the potential for true random mating in supplemental breeding programs should be investigated further, however, because they will likely provide insights into factors affecting selection and sperm competition as well.

Considerations for Release of Supplemental Offspring

Supplemental breeding programs ultimately aim to return captive-reared offspring to the wild. We focused on breeding designs that maximize the genetic diversity present in any single year's class of supplemental offspring, but genetic considerations also exist regarding appropriate stocking methods to maximize the expected genetic diversity present after supplementation. The effect of supplementation on the genetic diversity of wild populations is influenced by several factors. These factors include but are not limited to the effective size of the captive and wild populations and the relative contributions of each population to the total number of offspring produced. Several theoretical studies have investigated the effects of single- and multigeneration supplementation on the genetic diversity of managed populations (Waples & Do 1994; Ryman et al. 1995; Wang & Ryman 2001; Duchesne & Bernatchez 2002). In general, these studies demonstrate that the dynamics of multigeneration supplementation are complex, but one of the more important parameters appears to be the size of the captive component (Waples & Do 1994; Duchesne & Bernatchez 2002), hence our interest in designing mating strategies that maximize the effective size of each supplemental year class. Although genetic diversity can be effectively maintained within each year class by using factorial mating designs, the long-term effects of supplementation have not been investigated for the robust redhorse. Future studies need to investigate the genetic impact of supplementation on existing and reintroduced populations of this species.

Conclusions

We quantitatively demonstrated the benefits of using factorial mating designs to maintain genetic diversity in supplemental breeding programs. Mating each breeder with even as few as two members of the opposite sex can dramatically increase the effective population size and decrease the chance of boom-and-bust years. Additionally, we calculated N_e across 10 different year classes in the actual supplemental breeding program of the robust redhorse and demonstrated that a complete factorial design (if logistically feasible) could increase the effective population size of each year class by nearly 20%.

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Literature Cited

Allendorf, F. W. 1993. Delay of adaptation to captive breeding by equalizing family size. Conservation Biology **7**:416–419.

Ballou J. D., M. Gilpin, and T. J. Foose, editors. 1995. Population management for survival and recovery: analytical methods and strategies in small population conservation. Columbia University Press, New York.

Bartley, D., M. Bagley, G. Gall, and B. Bently. 1992. Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. Conservation Biology **6**:365–375.

Bencic, D. C., M. Krisfalusi, J. G. Cloud, and R. L. Ingermann. 2000. Short-term storage of salmonid sperm in air versus oxygen. North American Journal of Aquaculture **62**:19–25.

Brown, B. L., T. P. Gunter, J. M. Waters, and J. M. Epifanio. 2000. Evaluating genetic diversity associated with propagation-assisted restoration of American shad. Conservation Biology **14**:294–303.

Bryant, R. T., J. W. Evans, R. E. Jenkins, and B. J. Freeman. 1996. The mystery fish. Southern Wildlife **1**:26–35.

Duchesne, P., and L. Bernatchez. 2002. An analytical investigation of the dynamics of inbreeding in multi-generation supportive breeding. Conservation Genetics **3**:47–60.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th edition. Longman, Harlow, United Kingdom.

Fiumera, A. C., L. Wu, P. G. Parker, and P. A. Fuerst. 1999. Effective population size in the captive breeding program of the Lake Victoria cichlid *Paralabidochromis chilotes*. Zoo Biology **18**:215–222.

Fiumera, A. C., P. G. Parker, and P. A. Fuerst. 2000. Effective population size and maintenance of genetic diversity in captive-bred populations of a Lake Victoria cichlid. Conservation Biology **14**:886–892.

Frankham, R., H. Manning, S. H. Margan, and D. A. Briscoe. 2000. Does equalization of family sizes reduce genetic adaptation to captivity? Animal Conservation **4**:357–363.

Gile, S. R., and M. M. Ferguson. 1995. Factors affecting male potency in pooled gamete crosses of rainbow trout, *Oncorhynchus mykiss*. Environmental Biology of Fishes **42**:267–275.

Hedrick, P. W., D. Hedgecock, S. Hamelberg, and S. J. Croci. 2000. The impact of supplementation in winter-run chinook salmon on effective population size. Journal of Heredity **91**:112–116.

Hendricks, A. S. 1998. The conservation and restoration of the robust redhorse, *Moxostoma robustum*. Volume 1. Georgia Power Company, Environmental Laboratory, Smyrna.

Hutchins, M., R. J. Weise, and K. Willis. 1997. Captive breeding and conservation. Conservation Biology **11**:3–5.

Jenkins, R. E., and N. M. Burkhead. 1994. Freshwater Fishes of Virginia. American Fisheries Society, Bethesda, Maryland.

Kimura, M., and J. F. Crow. 1963. The measurement of effective population numbers. Evolution **17:**279–288.

Lacy, R. C. 1989. Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. Zoo Biology **8:**111–123.

Montgomery, M. E., J. B. Ballou, R. K. Nurthen, P. R. England, D. A. Briscoe, and R. Frankham. 1997. Minimizing kinship in captive breeding programs. Zoo Biology **16:**377–389.

Pereira, S. L., and A. Wajntal. 1999. Reintroduction of guans of the genus *Penelope* (Cracidau, Aves) in reforested areas in Brazil: assessment by DNA fingerprinting. Biological Conservation **87:**31–38.

Ritar, A. J., and M. Campet. 2000. Sperm survival during short-term storage and after cryopreservation of semen from striped trumpeter (*Latris lineata*). Theriogenology **54:**467–480.

Ryman, N., P. E. Jorde, and L. Laikre. 1995. Supportive breeding and variance effective population size. Conservation Biology **9:**1619–1628.

Schreiber, A., and G. von Hegel. 1999. Genetic variability in goitred gazelle *Gazella subgutturosa subgutturosa*: allozymes and karyotypes. Acta Theriologica **44:**55–66.

Smyth, G. K. (1999). StatBox 4.1: a statistics toolbox for Matlab. Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia. Available from www.statsci.org/matlab/statbox.html (accessed July 2003).

Snyder, N. F. R., S. R. Derrickson, S. R. Beissinger, J. W. Wiley, T. B. Smith, W. D. Toone, and B. Miller. 1996. Limitations of captive breeding in endangered species recovery. Conservation Biology **10:**338–348.

Snyder, N. F. R., S. R. Derrickson, S. R. Beissinger, J. W. Wiley, T. B. Smith, W. D. Toone, and B. Miller. 1997. Limitations of captive breeding in endangered species recovery: reply to Gippoliti and Carpaneto. Conservation Biology **11:**808–810.

Wang, J., and N. Ryman. 2001. Genetic effects of multiple generations of supportive breeding. Conservation Biology **15:**1619–1631.

Waples, R., and C. Do. 1994. Genetic risk associated with supplementation of Pacific salmonids: captive broodstock programs. Canadian Journal of Fisheries and Aquatic Sciences **51**(supplement 1):310–329.

Withler, R. E. 1988. Genetic consequences of fertilizing chinook salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. Aquaculture **68:**15–25.

Wright, S. 1931. Evolution in Mendelian populations. Genetics **16:**97–159.