Transactions of the American Fisheries Society 137:1327–1334, 2008

DOI: 10.1577/T07-192.1

[Note]

Genetic Effective Size in Populations of Hatchery-Raised Red Drum Released for Stock Enhancement

JOHN R. GOLD,* LIANG MA, AND ERIC SAILLANT
Center for Biosystematics and Biodiversity, Texas A&M University, College Station, Texas 77843-2258, USA

PAUL S. SILVA AND R. R. VEGA
Texas Parks and Wildlife Department, Coastal Conservation Association/ Central Power and Light Marine Development Center, Flour Bluff, Texas 78418, USA

Abstract.—Genetic analysis of progeny from 13 spawning events occurring over a 2-week period in a Texas Parks and Wildlife Department (TPWD) hatchery for red drum *Sciaenops ocellatus* during the spring of 2002 and hatchery spawning and release records over the 2003 spawning season were used to estimate the average genetic effective size of an average spawn and an average hatchery-released population. The purpose of this study was to assess the potential for a Ryman–Laikre effect in the TPWD red drum stock enhancement program. Genetic analysis revealed that 16 of 27 dams (59.2%) and 16 of 18 sires (88.9%) spawned at least once. The average effective size (N_e) for a single spawn was 2.59, approximately 43% less than the maximum N_e (4.55) predicted if all possible mating (dam × sire) combinations had occurred and family size per mating combination had been equivalent. The reduction in N_e stemming from the actual number of mating combinations was approximately 34% and appeared to be due primarily to nonspawning dams; the reduction in N_e generated by the actual variation in family size was approximately 9%. Spawning and release records at the TPWD hatchery indicate that in 2003 the number of released populations per bay or estuary ranged from 7 to 27. Using the average effective size (N_e) estimate for a single spawn (2.59), the estimated average effective size of all released fish per bay or estuary (N_{eR}) in 2003 ranged from about 28.5 to about 46.6. These values of N_{eR} are less than the averages estimates of about 272 and 263 for the long-term (N_{el}) and contemporaneous (N_{eV}) effective size, respectively, of red drum in bays and estuaries in the northern Gulf of Mexico and indicate a reasonable potential for a Ryman–Laikre effect. Approaches that might be employed to increase the N_{eR} of TPWD-released fish and decrease the probability of a Ryman–Laikre effect are discussed.

Hatchery-reared fish used in stock enhancement are often produced by a small number of breeders relative to the hypothesized number of breeders in natural populations (Ryman and Laikre 1991). Consequently, a small number of broodfish may contribute disproportionately to the overall ("wild" plus hatchery releases) juvenile pool, thereby increasing significantly the variance in family size in the overall population. This can lead to a reduction in the genetic effective size (N_e) of the wild population (Ryman and Laikre 1991; Tringali and Bert 1998) and result in inbreeding, an accumulation of deleterious genotypes, and a reduction in fitness within the fishery (Frankham 1995; Higgins and Lynch 2001). The negative aspects, of course, are based on the assumption that the hatchery-released or "stocked" fish reproduce and contribute significantly to subsequent wild generations (Ryman and Laikre 1991). This potential reduction in effective size of the wild population has been termed the Ryman–Laikre effect (Tringali and Bert 1998).

In this paper, we present the results of a study on the effective size of a "simulated" release population of red drum *Sciaenops ocellatus*. Briefly, in response to substantial declines in red drum abundance and recruitment, the Texas Parks and Wildlife Department (TPWD) implemented in the 1980s a stock enhancement program that now supports the wild fishery in Texas waters through annual releases of hatchery-produced red drum fingerlings (McEachron et al. 1995). At present, the program releases between 20 and 30 million hatchery-raised fingerlings annually into eight different Texas bays and estuaries (Vega et al. 2003) and represents one of the most visible (certainly the largest) marine stock enhancement program in the country. To ensure the maintenance of wild genotypes among released fish and maximize genetic diversity, the TPWD program utilizes randomly sampled adult fish from the wild as broodstock and replaces at least 25% of the broodfish (both sexes) each year (McEachron et al. 1995). In addition, both dams and sires are alternated among spawning tanks (typically three dams and two sires per tank) across years, and no broodfish are kept in the program for more than 4 years (McEachron et al. 1995). The effective size of a released population, however, is a function of the number of dam × sire combinations actually contributing to a released population and of the variance in reproductive success among individual

* Corresponding author: goldfish@tamu.edu

Received September 11, 2007; accepted February 23, 2008
Published online August 25, 2008

dams and sires. These parameters have not been assessed to date and are essential in order to determine the potential for a Ryman–Laikre effect on the wild red drum population in Texas waters.

In this study, we used (1) parentage data based on progeny produced from 13 separate spawning events occurring over a 2-week period in a TPWD hatchery during the spring of 2002 and (2) hatchery spawning and release records over the 2003 spawning season to estimate the average effective size of a single spawn and of a release of hatchery-reared fingerlings into the wild. The average reduction in effective size per spawn was estimated by identifying genetically the number of dams and sires and the number of offspring generated by each dam × sire combination contributing to a spawn. We then used simulation to estimate the average effective size of a released population comprised of mixes of progeny from different spawns.

Methods

A total of 45 adult red drum broodfish (27 dams and 18 sires) maintained in nine 13-m^3 spawning tanks at the TPWD Marine Development Center (MDC) in Flour Bluff were used in the study. Broodfish (dams and sires) were obtained by TPWD personnel from the wild red drum population offshore of the south Texas coast. Each spawning tank putatively contained three dams and two sires. Temperature and photoperiod were manipulated following a 150-d maturation cycle (McCarty 1987) in order to achieve spontaneous spawning. Approximately 15,000–30,000 offspring were sampled randomly from each of 13 separate spawning events occurring at night over a 2-week period (11–24 April) in the spring of 2002. Fertilized (buoyant) eggs were collected at the effluent of each spawning tank and incubated separately for about 72 h under conditions described in Henderson-Arzapalo (1987). Newly hatched larvae from each spawn were transferred to separate 1- or 2-acre, prefertilized ponds (Colura 1987). Harvest of ponds was conducted 45–60 d postfertilization, when fingerlings had reached an average length of approximately 30mm. A random sample of 125 fingerlings from each of the 13 spawning events were placed individually into labeled cryopreservation (Nunc) tubes and frozen in liquid nitrogen for subsequent genetic analysis.

Genomic DNA was extracted from caudal fin tissue of all broodfish via standard phenol–chloroform procedures, as described in Gold and Richardson (1991). Genomic DNA from all experimental progeny also was extracted from caudal fin tissue but using an alkaline lysis method (Saillant et al. 2002). A small piece (~2 mm^3) of fin from each individual was digested for 2 h at 65°C in 50 μL of a sodium hydroxide solution (200 mM). The final pH of the solution was adjusted to 8 by adding 50 μL tris–HCl (200 mM, pH 8.0).

Genotypes at four microsatellite loci (*Soc19*, *Soc85*, *Soc402*, and *Soc428*) were obtained via polymerase chain reaction (PCR) amplification of genomic DNA from all broodfish and all experimental offspring. Details, including PCR primer sequences, annealing temperatures, and observed or expected heterozygosity for these four microsatellites may be found in Saillant et al. (2004). One of each pair of PCR primers was fluorescently labeled with one fluorescent dye of set D (Applied Biosystems, Foster City, California): 6-Fam for *Soc19* and *Soc85* and Hex for *Soc402* and *Soc428*. Polymerase chain reactions were performed in 10-μL volumes containing 1 μL (25 ng) of genomic DNA, 1 μL of 10× reaction buffer (500 mM KCl, 100 mM tris–HCl [pH 9.0], 1% TritonX-100), 1.5 mM $MgCl_2$, 2.0 mM of each deoxynucleotide triphosphate, 5 pM of each primer, 0.5 units of *Taq* DNA polymerase (Gibco BRL). Thermal cycling was carried out as follows: initial denaturation at 94°C for 3 min followed by 35 cycles consisting of 30-s denaturation at 94°C, 45-s annealing at the optimized annealing temperature, 1-min extension at 72°C, with a final extension of 10 min at 72°C. Polymerase chain reaction products were loaded on 5% Long Ranger (Cambrex) single-pack gels and electrophoresed on an ABI PRISM 377 DNA automatic sequencer (Applied Biosystems). All gels were analyzed using Genescan Analysis 3.1.2 (Applied Biosystems); allele-calling was performed with Genotyper software, version 2.5 (Applied Biosystems). Genotypes at each microsatellite for each individual (broodfish and offspring) were scored and entered into a database. Assignment of offspring to an individual dam and sire based on the genotypes at the four microsatellites was implemented via the program Probmax version 1.2 (Danzmann 1997; available at http://www.uoguelph.ca/~rdanzman/software/PROBMAX/). Parentage assignment was unequivocal in all cases.

The genetic effective population size (N_e) of a released population is influenced in part by (1) the number of dams and sires in a spawning tank contributing to a spawn and (2) the variation in family size among mating (dam × sire) combinations. Following Crow and Kimura (1973), the value of N_e for the former was estimated from the equation

$$N_e = \frac{4N_dN_s}{(N_d + N_s)}, \quad (1)$$

where N_e is the genetic effective population size and N_d and N_s represent the number of dams and sires,

respectively, contributing to a spawn. The latter is based on the number of offspring (family size) produced from each dam × sire combination and accounts for unequal contributions of parents to offspring. This value of N_e was estimated from the equation

$$N_e = \frac{4N_{ed}N_{es}}{N_{ed} + N_{es}}, \quad (2)$$

where N_{ed} and N_{es} are the effective numbers of dams and sires, respectively, contributing to a spawn. Values for N_{ed} and N_{es} were estimated from the equations (Lacy 1989)

$$N_{ed} = \frac{1}{\sum_{k=1}^{n_f} q_k^2} \quad (3a)$$

$$N_{es} = \frac{1}{\sum_{k=1}^{n_m} q_k^2}, \quad (3b)$$

where n_f and n_m are the number of dams and sires, respectively, that contributed to a spawn, and q represents the proportion of progeny contributed by each dam or sire to that spawn.

The genetic effective size of a released population also is influenced by variation in the number of progeny from different spawning tanks when multiple spawns contribute to a released population. To examine this, we used records at the TPWD MDC for the year 2003 to generate an empirical distribution of the mixtures of spawns (including the number of spawns mixed and the number of progeny from each spawn) that were transferred to separate prefertilized ponds for prerelease grow out. Red drum at the MDC typically spawn during the night and, on average, approximately 400,000 progeny (measured by volume) from each spawning tank are collected, incubated, and transferred to prefertilized grow-out ponds. Released fish, typically about 150,000 per released population, generally come from individual grow-out ponds, meaning that released populations are derived essentially from a single night's spawn. During 2003, the number of spawns from individual spawning tanks that were mixed and transferred to individual ponds before release varied from one to seven; the number of progeny per spawn varied from about 20,000 to about 1,200,000. We used a bootstrap resampling approach (Efron 1979) to estimate the distribution of the overall genetic effective size of 10,000 released populations based on the empirical distributions of mixtures of spawns and number of progeny per spawn. The effective size (N_e) of each spawn was assigned randomly and varied from 2.00 to 4.18, based on empirical observations (see Results). The overall genetic effective size (N_{eR}) was estimated from the equation (Ryman and Laikre 1991)

$$N_{eR} = \frac{1}{\sum_{k=1}^{n_f} \frac{x_i^2}{N_{ei}}}, \quad (4)$$

where x_i is the proportion of the ith spawning tank's contribution to the final population and N_{ei} is the genetic effective population size of the ith spawning tank. We also generated via simulation estimates of the overall genetic effective size (N_{eR}) of 10,000 released populations when (1) the number of progeny from different spawns that contributed to a released population was equalized and (2) when spawns contributing less than 10% of the progeny in a mixture were included as is and the contribution of the remaining spawns in the mixture were equalized. The latter ("pseudoequalized" mixture) represents a more realistic situation for a stock enhancement program as equalizing spawn contributions to match a low volume spawn could mean discarding large volumes of hatched progeny.

Results and Discussion

Genotypes at four microsatellites (*Soc19*, *Soc85*, *Soc402*, and *Soc428*) were acquired for all 45 broodfish (dams and sires) and for 1,597 offspring (the genotypes of individual fish may be found at http://wfsc.tamu.edu/doc under the file name Appendix 1). Summary data for the 13 spawns are presented in Table 1; data for each spawning tank include number of spawns, dams and sires contributing to a spawn, and number of offspring genotyped from each spawning tank and from each dam × sire combination. The number of spawns per spawning tank over the time interval sampled was as follows: one (six tanks), two (two tanks), and three (one tank). The number of offspring genotyped per spawn ranged from 111 to 125. In one spawning tank (BB-2), genotype data indicated a mismatch in the sex identification (some offspring were assigned to two breeders putatively of the same sex), while in another brood tank (BB-11), genotype data indicated that although all six broodfish had contributed to the spawn, only two of the three fish tentatively identified as females were the same sex. Personnel at TPWD had tentatively identified each spawning tank as containing three females and two males. Assuming identification by TPWD personnel was mostly accurate, further analysis proceeded on the hypothesis that spawning tank BB-2 contained four

TABLE 1.—Summary data from 13 spawns in a study of genetic effective size in hatchery red drum populations in Texas in 2002.

Tank	Spawn (date)	Parent	Dam 1	Dam 2	Dam 3	Dam 4	Sires
BB-1	Apr 14	Sire 1	0	0	0		0
		Sire 2	0	0	123		123
		Dams	0	0	123		123
BB-2	Apr 17	Sire 1	0	77	0	48	125
		Dams	0	77	0	48	125
	Apr 18	Sire 1	0	47	77	0	124
		Dams	0	47	77	0	124
BB-7	Apr 13	Sire 1	61	1	59		121
		Sire 2	1	1	2		4
		Dams	62	2	61		125
BB-8	Apr 11	Sire 1	0	0	52		52
		Sire 2	0	0	64		64
		Dams	0	0	116		116
	Apr 13	Sire 1	87	0	0		87
		Sire 2	34	0	0		34
		Dams	121	0	0		121
	Apr 16	Sire 1	72	0	0		72
		Sire 2	51	0	0		51
		Dams	123	0	0		123
BB-11	Apr 21	Sire 1	33	0			33
		Sire 2	75	0			75
		Sire 3	16	0			16
		Dams	124	0			124
	Apr 23	Sire 1	32	10			42
		Sire 2	9	56			65
		Sire 3	1	17			18
		Dams	42	83			125
BB-12	Apr 20	Sire 1	105	0	0		105
		Sire 2	18	0	0		18
		Dams	123	0	0		123
VB3-1	Apr 24	Sire 1	0	0	101		101
		Sire 2	0	0	10		10
		Dams	0	0	111		111
VB3-3	Apr 19	Sire 1	0	0	0		0
		Sire 2	0	0	124		124
		Dams	0	0	124		124
VB4-1	Apr 24	Sire 1	0	4	0		4
		Sire 2	0	12	109		121
		Dams	0	16	108		125

females and one male, while spawning tank BB-11 contained two females and three males.

Based on parentage analysis, 16 of the 27 dams (59.2%) and 16 of the 18 sires (88.9%) spawned at least once. On average, each of the 13 spawns involved 1.46 dams and 1.85 sires. The number of spawning combinations (families) generated per brood tank varied from one (one dam × one sire) to six (three dams × two sires in one tank and two dams × three sires in a second tank). On average, more families were generated per spawning tank if multiple spawns in the same tank occurred over the time interval (Table 1).

Except for tank BB-2, where there were four dams and one sire, the maximum expected genetic effective size (N_e) for each spawn, based on equation (1) and assuming that all dams mated with all sires, was 4.8 (Table 2, column A). The maximum expected N_e for tank BB-2 was 3.2. The average maximum expected N_e over the 13 spawns was 4.55. Parental assignments

TABLE 2.—Estimates of effective size per spawn assuming (A) that all possible pairwise matings occur and that all families have an equal number of offspring; (B) the observed number of pairwise matings but that all families have an equal number of offspring; and (C) the observed number of pairwise matings and the observed size of each family.

	Scenario		
Tank (spawn)	A	B	C
BB-1 (1)	4.80	2.00	2.00
BB-2 (1)	3.20	2.67	2.62
BB-2 (2)	3.20	2.67	2.62
BB-7 (1)	4.80	4.80	2.81
BB-8 (1)	4.80	2.67	2.64
BB-8 (2)	4.80	2.67	2.66
BB-8 (3)	4.80	2.67	2.51
BB-11 (1)	4.80	3.00	4.18
BB-11 (2)	4.80	4.80	2.75
BB-12 (1)	4.80	2.67	2.29
VB3-1 (1)	4.80	2.67	2.18
VB3-3 (1)	4.80	2.00	2.00
VB4-1 (1)	4.80	4.00	2.33
Average	4.55	3.02	2.59

based on genotype data, however, revealed that only a subset of the breeders present in a spawning tank contributed to the offspring produced. Considering only the number of dams and sires that actually spawned, and assuming that family size per spawning pair was distributed binomially across all spawning pairs, the observed N_e for each of the 13 spawns ranged from 2.0 to 4.8 and averaged 3.02 (Table 2, column B), approximately 34% less than the predicted maximum expected average N_e of 4.55.

Parental assignments also revealed considerable variation in family size among spawning pairs (Table 1). As an example, while all six dam × sire combinations in tank BB-7 contributed to the spawn, 98% of the sampled progeny came from two of the three dams, while 97% came from one of the two sires. Similarly, in tank VB4-1, 84% of the sampled progeny came from one of two dams (the third dam did not contribute to the sampled progeny), while 97% of the sampled progeny came from one of two sires. In both of these examples, the actual effective size per spawn was over 40% less than that expected had family size per mating combination been equivalent. Estimates of N_e derived by considering the variation in family size per mating pair per spawn (equation 2) ranged from 2.00 to 4.18 per spawn and averaged 2.59; Table 2, column C). The average estimate of N_e (2.59) based on actual family size per spawning pair is approximately 43% less than the predicted maximum expected average N_e of 4.55.

The above results lead to four not-unexpected generalizations regarding the effective size of an offspring population generated in a single spawning event. First, the expected maximum N_e is not strictly a function of the number of fish in a spawning tank (given a space limitation in this case of five broodfish per tank) but rather of the number of dam × sire combinations (irrespective of sex) that contribute to a spawn. As examples, compare tank BB-2 (which had four females and one male and a maximum N_e of 3.2) with most other spawning tanks (three females and two males; maximum N_e, 4.8) and tank BB-11 (two females and three males), for which the expected maximum N_e was the same (4.8) as for most other spawning tanks (three females and two males). Second, assuming that the expected family sizes per mating pair follow a binomial distribution, N_e is a function of the number of dam × sire combinations (families) in a spawning event. Examples here include spawn 1 in tank BB-7 (three females × two males = six families; $N_e = 4.8$) versus spawn 1 in tank VB4–1 (two females × two males = four families; $N_e = 4.00$) or spawn 1 in tank BB-11 (one female × three males = three families; $N_e = 3.00$). Third, N_e is inversely correlated to the variation in size among different families generated within a spawning event. Examples include spawn 1 in tank BB-2 (two females × one male = two families in the proportions 61.6% and 38.4%; $N_e = 2.62$) versus spawn 1 in tank BB-12 (one female × two males = two families in the proportions 83.7% and 16.3%; $N_e = 2.31$) or spawn 1 in tank VB3–1 (one female × two males = two families in the proportions of 91% and 9%; $N_e = 2.00$). Finally, the number of actual dam × sire combinations had a proportionally greater effect on reducing N_e than did the variation in family size per spawning pair. The reduction in N_e stemming from the actual number of mating combinations was approximately 34%, while the reduction in N_e further generated by variation in family size was approximately 9%.

The bootstrap resampling simulations to assess the effect on N_{eR} (effective size of a released population) when progeny from different spawning tanks (spawns) were mixed to generate a release population demonstrated, as expected, that N_{eR} was reduced when the number of progeny from different spawns in the mixture varied. Briefly, over the spawning year 2003, the mean ± SD number of spawns contributing to each released population was 2.84 ± 1.65. The estimate of N_{eR} for the simulated released populations when different spawns were mixed in a release population and when the number of progeny per spawn varied (according to TPWD records) was 5.38 ± 2.32, whereas the estimate of N_{eR} for the simulated released populations when the number of progeny per (different) spawn was equalized was 7.17 ± 4.20. The difference between these two simulation-based estimates indicates that equalizing the number of progeny from different spawns in a released population would generate, on average, a 33% increase in N_{eR}. We also generated N_{eR} estimates when spawns contributing less than 10% of the progeny in a mixture were included as is and the contribution of the remaining spawns in the mixture were equalized. The estimate of N_{eR} for these simulated "pseudoequalized" released populations was 6.53 ± 3.49. The average difference between this estimate of N_{eR} and that when the number of progeny per spawn was equalized ($N_{eR} = 7.17 \pm 4.20$) was approximately 9%.

The purpose of this study was to assess the potential for a Ryman–Laikre effect (a reduction in the effective size of a wild population stemming from the small effective size of a hatchery-released population) in the TPWD red drum stock enhancement program. The empirical data generated demonstrated first, that far fewer dams (16 of 27 [59%]) than sires (16 of 18 [89%]) spawned over the time period studied; second, that the average N_e per spawn (3.02) was approxi-

mately 34% less than the expected maximum N_e of 4.55 had all possible mating combinations occurred at each spawn; and third, that the varying number of progeny generated per mating combination further reduced the average N_e per spawn by about 9% (to 2.59). Overall, the average N_e per spawn was approximately 43% less than the maximum possible, with nearly 80% of the reduction being due to the number of mating combinations that actually occurred. Because the latter represent the number of mating (dam × sire) combinations irrespective of sex, the observed reduction in N_e per spawn appears to be due primarily to nonspawning dams. Finally, using simulation analysis and TPWD spawning and release records in 2003, the average number of spawns from different spawning tanks mixed in a released population was estimated to be 2.84.

Spawning and release records at the TPWD MDC indicated that in 2003 a total of 62 release populations, roughly equivalent in size, were stocked into different localities in each of four different bays or estuaries. The number of released populations per bay or estuary ranged from 7 (Aransas Bay) to 27 (upper Laguna Madre) and included offspring from 11 (Aransas Bay) to 18 (upper Laguna Madre) different spawning tanks. Considering the average maximum N_e of 4.55 for each spawn, the estimated maximum average N_{eR} of all released fish per bay of estuary potentially would range from about 50.1 (Aransas Bay) to about 81.9 (upper Laguna Madre). These estimates assume that the contribution of individual spawning tanks and survival probabilities per released population were equivalent. Considering the average N_e per spawning tank of 2.59 when accounting for variation in the number of progeny generated per observed mating combination, the average N_{eR} per bay or estuary would be reduced to approximately 28.5 (Aransas Bay) and 46.6 (upper Laguna Madre). These values of N_{eR} would be underestimates if the contribution of progeny of individual dams, sires, or both tended to equalize over time; the values would be overestimates if the contribution of different spawning tanks and survival probabilities per released population varied. Based on present data, the latter seems more likely than the former.

Based on a coalescent approach, Turner et al. (2002) estimated the long-term genetic effective size (N_{eI}) of wild red drum populations in each of seven bays or estuaries in the northern Gulf of Mexico. Their estimates ranged from 183 to 517 (average, approximately 263 per bay or estuary). We used the data from the study of Turner et al. (2002) and estimated the contemporaneous variance of the genetic effective size (N_{eV}), using the temporal method (Nei and Tajima 1981; Waples 1989) for the same seven bays of estuaries. The estimates of N_{eV} per bay or estuary ranged from 166 to 356 and averaged 272. The range of N_{eR} estimates (from 28.5 to 46.6) per bay or estuary for all fingerlings released from the TPWD MDC in 2003 are smaller, on average, than both the N_{eI} and N_{eV} estimates per bay or estuary. Moreover, the estimates of N_{eR} are very likely inflated given that the assumption of equivalent survival probability per released population is probably not met. Briefly, Karlsson et al. (2008) genotyped yearling or older red drum from two "stock-enhanced" bays or estuaries along the Texas coast and unequivocally identified 30 of 321 fish (9.3%) sampled from Aransas Bay as being of hatchery origin. The contribution of brood dams, sires, and dam × sire combinations to the hatchery-assigned fish, however, was nonrandom, as was the distribution of hatchery-assigned and wild fish with respect to sampling localities within each bay. Karlsson et al. (2008) interpreted these results as indicating variation in survival probability among releases, which clearly would result in decreases in total N_{eR} over all releases. The above considerations suggest that a Ryman–Laikre effect could occur in Texas bays or estuaries supplemented with hatchery-reared fish.

There are three approaches that might be employed to increase the N_{eR} of TPWD-released fish and decrease the probability of a Ryman–Laikre effect: (1) increase the number of mating combinations per spawn, (2) equalize the number of progeny generated per mating combination, and (3) increase the number of spawns from different spawning tanks in each released population. The first could be accomplished by using two dams and three sires in each spawning tank, given that the proportion of spawning sires appears greater than the proportion of spawning dams. This approach might be problematic, however, as total egg output per spawning tank could be compromised significantly, particularly as far fewer dams than sires appear to participate in individual spawns. The observed spawning activity of dams and sires also raises the question as to whether over a spawning season there are dams (or sires) that contribute few or no progeny to any released population. We are currently studying this issue and, to date, it appears that there are dams (but not sires) in TPWD spawning tanks that contribute negligibly, if at all, over a spawning season. Monitoring and replacing non- or low-contributing dams might be a strategy to at least marginally increase the number of mating combinations per spawn, although this might prove difficult given the need for a "conditioning" period prior to spawning activity. The second approach, equalizing the number of progeny generated per mating combination within a spawning tank, would seem in

practice difficult to impossible to achieve. It also would likely be unproductive given that the observed reduction in N_e per spawn due to variation in progeny produced per mating combination was relatively small (about 9%).

The third approach, increasing the number of spawns from different spawning tanks in a released population, would seem to be the optimal strategy, as the N_{eR} of a released population would on average be the product of the average N_e per spawn (estimated here as 2.59) and the number of spawns from different spawning tanks included in the mixture. For example, if progeny from five different spawning tanks were combined into a single release, the average N_{eR} would be 12.95, a 2.4-fold increase in N_{eR}. A further 33% increase in N_{eR} (to 17.2) could be achieved by equalizing the number of progeny from different spawning tanks mixed in a released population. Even considering a pseudoequalized mixture as simulated above, N_{eR} would still be increased to 16.1. Given the range (7–27) in the number of release populations stocked from the MDC in 2003, the maximum average N_{eR} under this scenario (i.e., using a pseudoequalized mixture) could range from more than 110 to about 260, a substantial increase relative to that estimated under the present-day program and closer to the average contemporaneous (N_{eV}) and long-term (N_{el}) effective size estimates of Saillant and Gold (unpublished) and Turner et al. (2002), respectively.

Acknowledgments

We gratefully acknowledge the help and assistance provided by personnel, in particular R. Chavez and R. Gamez of the Texas Parks and Wildlife Department CCA/CPL Marine Development Center in Flour Bluff. We also thank C. Bradfield and M. Renshaw for help in the laboratory, D. Gatlin III and W. Neill for help in obtaining tissues, and T. Turner for helpful comments on an early draft of the manuscript. Work was supported by the Texas Sea Grant College Program (award NA16RG1078), the Coastal Fisheries Division of the Texas Parks and Wildlife Department, the Coastal Conservation Association–Texas, and the Texas Agricultural Experiment Station (project H-6703). This paper is number 60 in the series "Genetics Studies in Marine Fishes" and contribution 160 of the Center for Biosystematics and Biodiversity at Texas A&M University.

References

Colura, R. L. 1987. Saltwater pond fertilization. Pages (III) 48–50 *in* G. Chamberlain, R. J. Miget, and M. G. Haby, editors. Manual of red drum aquaculture. Texas A&M University, Texas Agricultural Extension Service and Sea Grant College Program, College Station.

Crow, J. F., and M. Kimura. 1973. An introduction to population genetics theory. Harper and Row, New York.

Danzmann, R. G. 1997. PROBMAX: a computer program for assigning unknown parentage in pedigree analysis from known genotypic pools of parents and progeny. Journal of Heredity 88:333.

Efron, B. 1979. 1977 Rietz Lecture: bootstrap methods—another look at the jackknife. Annals of Statistics 7:1–26.

Frankham, R. 1995. Effective population-size adult population size ratios in wildlife: a review. Genetical Research 66:95–107.

Gold, J. R., and L. R. Richardson. 1991. Genetic studies in marine fishes, IV. An analysis of population structure in the red drum (*Sciaenops ocellatus*) using mitochondrial DNA. Fisheries Research 12:213–241.

Henderson-Arzapalo, A. 1987. Red drum egg and larval incubation. Pages (II) 40–42 *in* G. Chamberlain, R. J. Miget, and M. G. Haby, editors. Manual of red drum aquaculture. Texas A&M University, Texas Agricultural Extension Service and Sea Grant College Program, College Station.

Higgins, K., and M. Lynch. 2001. Metapopulation extinction caused by mutation accumulation. Proceedings of the National Academy of Sciences of the USA 98:2928–2933.

Karlsson, S., E. Saillant, B. W. Bumguardner, R. R. Vega, and J. K. Gold. 2008. Genetic identification of hatchery-released red drum (*Sciaenops ocellatus*) in Texas bays and estuaries. North American Journal of Fisheries Management 28:1294–1304.

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

McCarty, C. E. 1987. Design and operation of a photoperiod–temperature spawning system for red drum. Pages (II) 27–31 *in* G. Chamberlain, R. J. Miget, and M. G. Haby, editors. Manual of red drum aquaculture. Texas A&M University, Texas Agricultural Extension Service and Sea Grant College Program, College Station.

McEachron, L. W., C. E. McCarty, and R. R. Vega. 1995. Beneficial uses of marine fish hatcheries: enhancement of red drum in Texas coastal waters. Pages 161–166 *in* H. L. Schramm and R. G. Piper, editors. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Nei, M., and F. Tajima. 1981. Genetic drift and the estimation of effective population size. Genetics 98:625–640.

Ryman, N., and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. Conservation Biology 5:325–329.

Saillant, E., A. Fostier, P. Haffray, B. Menu, J. Thimonier, S. Laureau, and B. Chatain. 2002. Temperature effects and genotype temperature interactions on sex determination in the European sea bass (*Dicentrarchus labrax* L.). Journal of Experimental Zoology 292:494–505.

Saillant, E., K. Cizdziel, K. G. O'Malley, T. F. Turner, C. L. Pruett, and J. R. Gold. 2004. Microsatellite markers for red drum, *Sciaenops ocellatus*. Gulf of Mexico Science 1:101–107.

Turner, T. F., J. P. Wares, and J. R. Gold. 2002. Genetic effective size is three orders of magnitude smaller than

adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162:1329–1339.

Tringali, M. D., and T. M. Bert. 1998. Risk to genetic effective population size should be an important consideration in fish stock enhancement programs. Bulletin of Marine Science 62:641–659.

Vega, R. R., C. Chavez, C. J. Stolte, and D. Abrego. 2003. Marine fish distribution report, 1991–1999. Texas Parks and Wildlife, Data Management Series 212, Austin.

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379–391.