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[Article]

Genetic Identification of Hatchery-Released Red Drum in Texas Bays and Estuaries

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Abstract.—The stock enhancement program for red drum *Sciaenops ocellatus* in Texas annually releases from 25 to 30 million fingerlings into Texas bays and estuaries and represents one of the largest such programs for marine fishes worldwide. We used 16 nuclear-encoded microsatellites and a 370-base-pair fragment of the mitochondrial DNA (mtDNA) D-loop to assign red drum sampled from two bays along the Texas coast to either hatchery or wild origin. A total of 30 hatchery-released fish were identified among 321 red drum belonging to three year-classes sampled from Galveston Bay, while a total of 11 hatchery-released fish were identified among 970 red drum belonging to four year-classes sampled from Aransas Bay. Allelic richness (microsatellites) was significantly lower among hatchery-released fish than among hatchery broodfish and wild fish. Similarly, the expected number of mtDNA haplotypes in hatchery-released fish (based on simulation analysis) was significantly lower than that expected in a random sample of both brood and wild fish. The contribution of brood dams, sires, and dam × sire combinations to the hatchery-released fish was nonrandom, as was the distribution of hatchery-released and wild fish with respect to sampling stations (localities) within each bay. The possibility of a Ryman–Laikre effect is discussed.

Red drum *Sciaenops ocellatus* arguably represent the most important recreational marine fishery in Texas waters, contributing a substantial portion of the approximately US$1.3 billion in annual revenue to coastal communities from marine recreational fishing (U.S. Fish and Wildlife Service 2001). Because of declines in red drum abundance in the northern Gulf of Mexico (Goodyear 1991), in the 1980s the Texas Parks and Wildlife Department (TPWD) began a vigorous program of hatchery-based supplementation (stock enhancement) of red drum stocks (McEachron et al. 1993, 1995). Today, the program releases from 25 to 30 million hatchery-produced, red drum fingerlings (25–40 mm total length [TL]) annually into various Texas bays and estuaries (Vega et al. 2003).

Assessment of the success of the TPWD stock enhancement program in terms of the long-term survival of released fingerlings has primarily consisted of (1) length frequency analysis of red drum captured with gill nets in bays where off-season (i.e., spring, which is well after the fall spawning season of wild fish) releases occurred a year earlier and (2) release–recovery of fingerlings marked with oxytetracycline–HCl (OTC). Comparisons of length frequency distributions of fish sampled a year after off-season releases indicated that released fish may survive in large numbers through the first year of life and led to the suggestion that the releases could have increased the relative abundance of age-1 and age-2 red drum by as much as 21% in some Texas bays and estuaries (McEachron et al. 1998). Scharf (2000), however, did not detect any effect of increased release rates of hatchery-raised fingerlings on the abundance of age-0 and age-1 red drum in nine bays and estuaries along the Texas coast sampled over a 20-year period. The OTC-marking experiments, alternatively, have been informative relative to longer-term survival in that 2 of 15 recovered OTC-marked fish were at large for over 12 months (McEachron et al. 1998). Other approaches,

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FIGURE 1.—Map of the northwestern Gulf of Mexico showing the locations of Galveston and Aransas bays, where hatchery juvenile red drum were released and recovered.

including use of coded wire microtags (Gibbard and Colura 1980) and release of genetically marked fish (Ward et al. 2004) have been uninformative: the former because released fingerlings were too small for tags to be retained, and the latter because of difficulties in resolving statistically significant increases in marker allele frequency. Long-term survival of hatchery-raised red drum following release into the wild has been documented in both Florida and South Carolina (Willis et al. 1995; Jenkins et al. 2004; Tringali et al. 2008).

The paucity of data on the long-term survival of TPWD hatchery releases impedes assessment of whether there are differences in enhancement effectiveness among various stocking methods, whether there are genetically based differences in survival among released fish, and whether genetic diversity among hatchery-released fish differs from that among wild fish. This limits a rigorous and much-needed evaluation of the program's overall success and cost-effectiveness, thereby constraining a risk-averse, cost-effective approach to marine stock enhancement (Blankenship and Leber 1995). In this study, we employed genetic markers (nuclear-encoded microsatellites and a fragment of mitochondrial DNA [mtDNA]) to discriminate age-1 and older hatchery-released red drum from wild conspecifics in two bays and estuaries along the Texas coast. Genetic markers have advantages over other types of markers in that no treatment prior to release is required (e.g., OTC exposure and physical tagging); unlike physical tags, genetic marks are permanent; and fish need not be sacrificed to detect the marker (as with OTC marks). There also is a growing concern about the genetic impact of hatchery-released fish on the wild population (Hindar et al. 1991; Blankenship and Leber 1995; Hansen et al. 2000), making genetic markers even more useful as a means to detect potential genetic differences between hatchery-released and wild fish. The objectives of our study were twofold: (1) to document the long-term survival of hatchery-released fish in Texas bays and estuaries and (2) to compare genetic variability between hatchery-released and wild fish.

Methods

Sample procurement.—The offspring released in the TPWD stock enhancement program are generated in two hatcheries: the Marine Development Center (MDC) in Flour Bluff and Sea Center Texas (SCT) in Lake Jackson. The broodfish at each of the two TPWD hatcheries generally comprise around 60 dams and 40 sires, although the number varies from year to year. At both hatcheries, 25% of the broodfish (both sexes), on average, are replaced each year by adult fish randomly sampled, by angling, from the wild red drum population at offshore localities proximal to each hatchery (McEachron et al. 1995). Broodfish are maintained in brood tanks containing three dams and two sires per tank, and no broodfish are kept in the program for more than 4 years; in addition, both dams and sires are alternated among brood tanks across years, although the same dam and sire often are placed in the same brood tank in subsequent years (McEachron et al. 1995). Temperature and photoperiod are manipulated following a 150-d maturation cycle in order to achieve spontaneous spawning (McCarty 1987). Depending on the contribution of individual dams and sires present in a brood tank, individual spawning events could give rise to up to six dam × sire combinations. Following each spawning event, fertilized (buoyant) eggs are collected at the effluent of each brood tank and incubated for around 72 h under conditions described in Henderson-Arzapalo (1987). Newly hatched larvae are transferred to separate, 0.4- or 0.8-hectare prefertilized ponds where they are grown until they reach a release size of approximately 30 mm (Colura 1987).

Fin clips from all broodfish (dams and sires) maintained in each of the two TPWD red drum hatcheries between 2002 and 2004 were sampled prior to spawning activity and stored in 95% ethanol. The total number of broodfish sampled at each hatchery was 145 (84 dams, 61 sires) at MDC and 80 (48 dams, 32 sires) at SCT. Data on mating design (which dams and sires occurred in the same brood tank in each year) and the location of release and number of fingerlings released from each hatchery (MDC and SCT) into Galveston and Aransas bays (Figure 1) between 2002 and 2004 were obtained from records maintained at each hatchery.

Fin clips were sampled from red drum caught in gill nets set at 90 stations (each) in both Galveston and Aransas bays by TPWD personnel during the fall of

2005 and spring of 2006. Fin clips were removed only from relatively pristine (i.e., not degraded) fish and only from fish less than 500-mm TL. The latter precaution was to ensure that a minimal number of age-1 fish would be excluded from the sample; the 500-mm size restriction was based on examination of age–length keys from previous years. A total of 352 fish (219 in fall 2005 and 133 in spring 2006) were sampled from 62 stations in Galveston Bay, while 985 fish (477 in fall 2005 and 508 in spring 2006) were sampled from 83 stations in Aransas Bay. Otoliths were removed from 155 of the fish sampled from Galveston Bay and 690 of the fish sampled from Aransas Bay, and used to age individual fish following methods in VanderKooy and Guindon-Tisdel (2003). For the remaining fish, age was inferred from length data based on the relationship between known age, based on otoliths, and length for fish from the same bay and sampling season. Data on localities and number of fish sampled at each locality were obtained from records maintained at the TPWD Perry R. Bass Marine Fisheries Research Station.

Genetic assays.—DNA was extracted from ethanol-preserved fin clips using a standard phenol–chloroform procedure (Sambrook et al. 1989) when mitochondrial mtDNA was to be amplified for direct sequencing or using an alkaline-lysis protocol (Saillant et al. 2002) when microsatellite alleles were to be amplified for genotyping. Sequences of a 370-base-pair (bp) fragment of the mtDNA D-loop and genotypes at 16 nuclear-encoded microsatellites were acquired for all brood dams at both hatcheries; genotypes at the 16 microsatellites were acquired for all brood sires. A database containing mtDNA haplotypes (dams) and microsatellites genotypes (dams and sires) of all broodfish in both hatcheries is available at http://wfsc.tamu.edu/doc (under the file name Appendix table for Karlsson et al.–NAJFM).

Sequences of the mtDNA D-loop fragment were acquired from all fish sampled from both bays in fall 2005. Fish with mtDNA sequences that matched those of a brood dam at either hatchery, or fish whose mtDNA sequence differed by only a single base from those of a brood dam, were then genotyped at the 16 microsatellites to confirm or reject whether a sampled fish was of hatchery origin. All fish (both bays) sampled in spring 2006 were genotyped for the 16 microsatellites and tentatively assigned to either of hatchery or wild origin. The 370-bp mtDNA fragment was then acquired from putative hatchery-released fish to confirm a match to a specific brood dam. The different approach in identifying hatchery-released fish in the 2006 sample was largely a function of cost and time efficiency. Both approaches worked equally well in terms of discriminating hatchery-released from wild fish.

The 370-bp fragment of the mtDNA D-loop was amplified in 50-μL reactions as described in Renshaw et al. (2006b). Approximately 50–100 ng of purified polymerase chain reaction product was sequenced using the Big Dye Terminator Kit version 3.1 (Applied Biosystems, Foster City, California) following instructions from the manufacturer. Electrophoresis and base calling were performed on an Applied Biosystems Genetic Analyzer 3100 (Applied Biosystems). Sequences were edited using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, Michigan). Amplification reactions for the 16 microsatellites were carried out using the multiplex protocols described in Renshaw et al. (2006a) for panel 1 (*Soc412*, *Soc416*, *Soc417*, *Soc423*, *Soc428*), panel 3 (*Soc19*, *Soc85*, *Soc138*, *Soc156*, *Soc206*, *Soc410*), and panel 4 (*Soc11*, *Soc83*, *Soc99*, *Soc407*, *Soc424*). Details, including primer sequences, for each of the 16 microsatellites may be found in Saillant et al. (2004). Amplification products were separated and visualized on an ABI Prism 377 DNA sequencer (Applied Biosystems). All gels were analyzed using Genescan Analysis 3.1.2 (Applied Biosystems); allele calling was performed with Genotyper version 2.5 (Applied Biosystems).

Data analysis.—Assignment of the fish sampled from both bays to either hatchery or wild origin proceeded as follows: The program DnaSP 4.0 (Rozas et al. 2003) was used to compare mtDNA sequences of fish sampled in 2005 with mtDNA sequences of brood dams. Sampled fish whose mtDNA haplotype matched any of the brood dams from either hatchery (or having a mtDNA haplotype that differed by one bp from that of a brood dam) were tentatively assigned to hatchery-released fish and subsequently assayed for genotypes at the 16 microsatellites to confirm hatchery origin; sampled fish with mtDNA sequences that differed by more than a single base from those of any brood dam were regarded as of wild origin. Fish sampled (both bays) in spring 2006 were assayed first for genotypes at the 16 microsatellites; the 370-bp mtDNA fragment was then acquired from all fish tentatively assigned to hatchery origin and compared (using DnaSP 4.0) with mtDNA sequences of all brood dams to confirm a match to a specific brood dam.

Assignment of sampled fish to hatchery origin based on microsatellite genotypes followed the recommendations in Renshaw et al. (2006b) and began with a genotype exclusion approach. Assignment analyses were conducted considering the broodfish mating groups in both hatcheries during 2002, 2003, and 2004. Genotype comparisons involved testing the composite microsatellite genotype (over all 16 micro-

satellites) of each sampled fish (both bays) against all possible dam × sire combinations (both hatcheries combined) in each year. We used this approach because fingerlings raised at MDC were released into both Galveston and Aransas bays. Exclusion of sampled individuals from either of the two bays as being of hatchery origin followed Mendelian principles (i.e., a hatchery-released fish must have one allele derived from a specific dam and one allele from a specific sire). Probmax-3 (Danzmann 1997) was used to perform exclusions. Each exclusion analysis was run with from zero to six mismatches allowed between parents and offspring in order to account for scoring errors, mutational events, null alleles, or both. For all mismatches, chromatograms were rechecked for all sampled individuals (and for their putative parents), the individuals and their parents were regenotyped, or both. Ultimately, all but two sampled fish tentatively assigned to hatchery origin matched perfectly a given parental pair. These two offspring appeared to be the consequence of null alleles, at *Soc407* in one instance and at *Soc206* in the other.

To assess possible sexing errors among the broodfish, crossing errors (dams or sires incorrectly assigned to a given spawning tank), or both, assignment tests accounting for all parental-pair combinations within a given hatchery (regardless of sex and spawning tank) were carried out. For this analysis, one large input file that included genotypes of all broodfish at both hatcheries (in 2002, 2003, and 2004), genotypes of all potential offspring, and allele frequencies at the 16 microsatellite loci as estimated from the entire data set (broodfish and fish sampled from both bays), was generated. Assignment was based on the logarithm of odds (LOD) score approach in Famoz (Gerber et al. 2003) where the LOD score is the logarithm of the ratio of the likelihood that a specific dam and sire are the parents of a given sampled fish versus the likelihood that they are not. The distribution of LOD scores of true offspring from hatchery broodfish and offspring from wild parents was determined by simulation in Famoz. Two populations of 200,000 individuals were simulated: one based on Mendelian principles and the known genotypes of broodfish in both hatcheries (representing true offspring from hatchery broodfish), and one based on allele frequencies at each microsatellite in the sampled populations (representing wild offspring unrelated to the hatchery broodfish). The simulation error rate, representing possible mutational events, was set to 0.0005 and was based on consideration of mutation rates in microsatellites (Jarne and Lagoda 1996). The multilocus error rate in LOD score estimation (representing scoring errors, null alleles, or both) was set to 0.05. Logarithm of odds scores for the best parental pair for true offspring from hatchery broodfish varied between 20.6 and 38.2, while LOD scores for the best parental pair of wild offspring (unrelated to hatchery broodfish) varied between 0.3 and 13.2. The strong bimodal distribution, with virtually no overlap between the two sets of LOD scores, indicates that robust assignment of fish assigned to hatchery releases or wild fish was achieved with the 16 microsatellites assayed in this study.

Once sampled fish were assigned to either hatchery or wild origin, genetic variability measures (including the number of alleles observed, allelic richness, and gene diversity [for microsatellites] and the number of haplotypes observed, haplotype diversity, and nucleotide diversity [for mtDNA sequences]) were generated for broodfish, wild fish, and hatchery-released fish using *F*-stat version 2.9.3.2 (Goudet 1995; available at www2.unil.ch/popgen/softwares/fstat.htm) for microsatellites and Arlequin version 3.11 (Schneider et al. 2000; available at cmpg.unibe.ch/software/arlequin3) for mtDNA sequences. Wilcoxon signed rank tests were used to test for homogeneity in allelic richness and gene diversity among hatchery-released fish, broodfish, and wild fish. Homogeneity in the number of mtDNA haplotypes among hatchery-released fish, broodfish, and wild fish was tested via a bootstrap (random) resampling approach (after Dowling et al. 1996) wherein the probability that the number of different haplotypes or the haplotype diversity observed in the hatchery-released fish would be observed in a random sample of the same size taken from the broodfish or the wild fish samples was estimated. We used Pop Tools (a free add-in software for Excel, available at www.cse.csiro.au/poptools/index.htm) to randomly sample the number of fish of hatchery origin (41 total) from the samples of broodfish and wild fish. Random sampling was performed 10,000 times, and the average number of observed haplotypes and average haplotype diversity and their upper (0.975) and lower (0.025) percentiles were recorded.

The same bootstrap resampling approach was used to assess whether the contribution to the hatchery-released fish of all dams, all sires, and all dam × sire combinations in each hatchery was random. As the majority (30 in all) of hatchery-released fish were from the 2004 year-class, a total of 30 fish were randomly sampled 10,000 times from all possible dams, sires, and dam × sire combinations that spawned in 2004 at both hatcheries, and the average number of different dams, sires, and dam × sire combinations and their upper (0.975) and lower (0.025) percentiles were recorded. Finally, Fisher's exact test, using software in Zaykin and Pudovkin (1993), was used to assess whether hatchery-released and wild fish were distrib-

TABLE 1.—Hatchery-released and wild red drum sampled from Galveston Bay during fall 2005 and spring 2006. The spatial array of sampling stations is shown in Figure 2.

	2004 year-class		2003 year-class		2002 year-class		Total	
Sampling station	Wild	Hatchery	Wild	Hatchery	Wild	Hatchery	Wild	Hatchery
1	16	0	1	0	0	0	17	0
2	1	0	0	0	0	0	1	0
3	9	5	1	0	0	0	10	5
4	7	0	0	0	0	0	7	0
5	15	3	6	0	0	0	21	3
6	2	4	0	0	0	0	2	4
7	7	1	0	0	0	0	7	1
8	3	0	0	0	0	0	3	0
9	3	3	0	0	0	0	3	3
10	3	0	4	0	1	0	8	0
11	1	0	0	0	0	0	1	0
12	7	0	0	0	0	0	7	0
13	4	0	0	0	0	0	4	0
14	1	0	0	0	0	0	1	0
15	5	1	1	0	0	0	6	1
16	3	0	0	0	0	0	3	0
17	10	3	8	0	2	0	20	3
18	15	1	4	0	1	0	20	1
19	1	0	1	0	0	0	2	0
20	1	0	1	0	0	0	2	0
21	16	5	6	0	0	0	22	5
22	10	0	2	0	0	0	12	0
23	4	0	1	0	0	0	5	0
24	1	0	0	0	0	0	1	0
25	3	0	1	1	0	0	4	1
26	1	0	1	1	0	0	2	1
27	6	0	0	0	0	0	6	0
28	1	0	0	0	0	0	1	0
29	5	0	0	0	0	0	5	0
30	8	0	2	0	0	0	10	0
31	7	0	4	0	0	0	11	0
32	10	0	4	0	0	0	14	0
33	2	0	0	0	0	0	2	0
34	15	1	3	0	0	0	18	1
35	13	0	2	0	0	0	15	0
36	3	0	1	0	0	0	4	0
37	1	0	0	0	0	0	1	0
38	3	0	1	0	0	0	4	0
39	1	0	0	0	0	0	1	0
40	3	0	0	0	0	0	3	0
41	12	0	3	0	0	0	15	0
42	8	1	0	0	0	0	8	1
43	5	0	0	0	0	0	5	0
44	5	0	1	0	0	0	6	0
45	1	0	0	0	0	0	1	0
All stations	258	28	59	2	4	0	321	30

uted randomly with respect to the localities (field stations) surveyed in either bay. The exact probability (P) value was estimated using a Monte Carlo approach (1,000 replications).

Results

Identification of Hatchery-Released Fish

Genetic data were acquired from a total of 225 broodfish (131 dams, 94 sires) from the two hatcheries and from 351 (of 352) fish sampled from Galveston Bay and 981 (of 985) fish sampled from Aransas Bay. The microsatellite genotypes and/or mtDNA sequences from all fish may be found at wfsc.tamu.edu/doc. The results of the assignment analysis (i.e., identification as hatchery-released or wild fish) of red drum sampled from Galveston and Aransas bays are given in Tables 1 and 2, respectively.

A total of 30 hatchery-released fish (9.35%) were identified among the 321 fish sampled from Galveston Bay (Table 1). Twelve of the hatchery-released fish were assigned to a specific dam × sire combination with no mismatches at any of the 16 microsatellites or at mtDNA, while 17 of the fish matched the mtDNA sequence of a hatchery dam but had mismatches at from one to four of the microsatellites (one mismatch, seven fish; two mismatches, five fish; three mismatch-

TABLE 2.—Hatchery-released and wild red drum sampled from Aransas Bay during fall 2005 and spring 2006.

	2004 year-class		2003 year-class		2002 year-class		2001 year-class		Total	
Sampling station	Wild	Hatchery	Wild	Hatchery	Wild	Hatchery	Wild	Hatchery	Wild	Hatchery
1	0	0	15	0	0	0	0	0	15	0
2	8	0	41	0	0	0	0	0	49	0
3	1	0	0	0	0	0	0	0	1	0
4	4	0	5	2	3	0	1	0	13	2
5	19	1	22	0	3	3	1	0	45	4
6	1	0	4	0	0	0	0	0	5	0
7	11	0	1	0	0	0	0	0	12	0
8	18	0	2	0	0	0	0	0	20	0
9	10	0	1	0	0	0	0	0	11	0
10	13	0	8	0	0	0	0	0	21	0
11	11	0	3	1	2	0	1	0	17	1
12	1	0	1	0	0	0	0	0	2	0
13	10	0	28	1	4	0	1	0	43	1
14	6	0	3	0	0	0	0	0	9	0
15	33	0	18	0	0	0	0	0	51	0
16	9	0	12	0	3	0	0	0	24	0
17	18	0	13	1	0	0	0	0	31	1
18	2	0	20	0	5	0	0	0	27	0
19	5	0	3	0	0	0	0	0	8	0
20	5	0	4	0	0	0	0	0	9	0
21	15	0	14	0	1	0	0	0	30	0
22	3	0	0	0	0	0	0	0	3	0
23	23	0	20	1	1	0	0	0	44	1
24	9	1	14	0	0	0	0	0	23	1
25	48	0	13	0	0	0	0	0	61	0
26	31	0	9	0	0	0	0	0	40	0
27	9	0	4	0	0	0	0	0	13	0
28	3	0	51	0	0	0	0	0	54	0
29	22	0	46	0	2	0	0	0	70	0
30	0	0	1	0	0	0	0	0	1	0
31	25	0	12	0	0	0	0	0	37	0
32	17	0	51	0	3	0	1	0	72	0
33	16	0	37	0	0	0	0	0	53	0
34	3	0	52	0	1	0	0	0	56	0
All stations	409	2	528	6	28	3	5	0	970	11

es, four fish; and four mismatches, one fish). With one exception, reexamination of chromatograms, regenotyping of the 17 fish, or both revealed genotyping errors in either broodfish (dam, sire, or both) or sampled fish which, when corrected, removed all mismatches. The one exception was a mismatch at locus *Soc407*, for which the dam appeared to be homozygous for allele *140*, the sire was heterozygous for alleles *142* and *148*, and the sampled fish appeared to be homozygous for allele *142*. This mismatch is best explained by either occurrence of a null allele in the dam or a newly arisen null allele in the sampled fish. The LOD scores of the 30 hatchery-released fish recovered in Galveston Bay were all above 22.4, while the remaining sampled (wild) fish had LOD scores below 16.4. These results indicate unambiguously that the 30 fish are offspring of hatchery broodfish, while the remaining 291 fish are not.

Altogether, 28 of the 30 hatchery-released fish recovered in Galveston Bay were assigned to a dam and sire at MDC, while 2 of the fish were assigned to a dam and sire at SCT. Of the fish assigned to MDC, 14 could be assigned unequivocally to the 2004 spawning-year cohort based on both occurrence of the mating pair (dam and sire) in the same brood tank only in 2004 and on the length–age key. For the remaining 16 fish, the assigned parental pair contributed to multiple spawns in different years; four of these fish were assigned to an age-class based on otoliths, while 12 were assigned to an age-class based on the age–length key.

Eleven hatchery-released fish (1.13%) were identified among the 970 fish sampled from Aransas Bay (Table 2). Eight of these fish were assigned with no mismatches at any of the 16 microsatellites or at mtDNA, while three of the fish matched the mtDNA sequence of a hatchery dam but had mismatches at from one to four of the microsatellites (one mismatch, one fish; two mismatches, one fish; and four mismatches, one fish). Reexamination of chromatograms, regenotyping of the three fish, or both revealed scoring errors in either broodfish (dam, sire, or both) or sampled fish which, when corrected, removed the mismatches for two of the fish. The third fish appeared

TABLE 3.—Genetic variability in hatchery-released fish, broodfish, and wild fish.

Fish type	Microsatellites			mtDNA		
	Average number of alleles	Average allelic richness (range)	Average gene diversity (range)	Number of haplotypes	Haplotype diversity	Nucleotide diversity
Hatchery-released fish	22	11.6 (4.0–21.8)	0.805 (0.531–0.944)	18	0.923	0.022
Broodfish	35	13.7 (3.8–26.0)	0.813 (0.508–0.948)	83	0.987	0.028
Wild fish	38	13.3 (4.1–25.0)	0.813 (0.485–0.948)	262	0.986	0.032

to be homozygous for allele *253* at *Soc206*. The putative dam was heterozygous for alleles *249* and *257*, while the putative sire appeared to be homozygous for allele *253*. This mismatch could be due to occurrence of a newly arisen null allele in the sampled fish or differential amplification of allele *253* in both the putative sire and the sampled fish. Logarithm of odds scores for the 11 fish identified as hatchery releases were above 24.1, while LOD scores for the remaining fish were below 13.7, indicating unambiguous assignment of the former as hatchery releases and of the latter to wild fish. All 11 hatchery-released fish were assigned to a specific dam and sire at MDC. In each case, the assigned parental pair contributed to multiple spawns in different years; five of the fish were assigned to an age-class based on otoliths, while six were assigned to an age-class based on the age–length key.

Genetic Variation in Hatchery-Released Fish, Broodfish, and Wild Fish

Genetic variability measured as number of alleles, allelic richness, and gene diversity (microsatellites) and number of haplotypes, haplotype diversity, and nucleotide diversity (for mtDNA) among hatchery-released fish, broodfish, and wild fish is presented in Table 3. Pairwise Wilcoxon signed rank tests revealed significantly lower allelic richness in hatchery-released fish than in both broodfish ($P = 0.002$) and wild fish ($P = 0.005$); pairwise tests for homogeneity in gene diversity, however, were nonsignificant ($P = 0.256$ versus broodfish, and $P = 0.393$ versus wild fish). The number of mtDNA haplotypes, haplotype diversity, and nucleotide diversity were lower in the hatchery-released fish than in both broodfish and wild fish (Table 3). The simulated, random resampling (with replacement) of 41 fish (the number of hatchery-released fish) from the sample of broodfish and wild fish indicated that the average number of mtDNA haplotypes expected among the broodfish would be 29.15 (with lower and upper percentiles of 24 and 34, respectively), while the average number of mtDNA haplotypes expected among the wild fish would be 32.52 (with lower and upper percentiles of 28 and 37, respectively). This analysis indicates that the 18 different mtDNA haplotypes found among the hatchery-released fish is significantly less than that which would be expected in a random sample from the broodfish or from the wild fish.

Contribution of Broodfish to Hatchery-Released Fish

The 30 hatchery-released fish from spawns in 2004 represented offspring from 13 dams, 10 sires, and 13 dam × sire combinations. The total number of possible hatchery dams, sires, and dam × sire combinations (both hatcheries) in 2004 was 108, 73, and 214, respectively. The simulated, random resampling (with replacement) analysis indicated that the average number of hatchery dams and sires expected to contribute to a sample of 30 fish would be 26.3 (with upper and lower percentiles of 29 and 23, respectively) and 24.7 (with upper and lower percentiles of 28 and 21). Similarly, the average number of hatchery dam × sire combinations expected would be 28.6 (with upper and lower percentiles of 30 and 25). This analysis indicates that the number of hatchery dams, sires, and dam × sire combinations contributing to the 30 hatchery-assigned fish is significantly less than that which would be expected if each had an equal probability of contributing to the 30 hatchery-released fish.

Spatial Distribution of Hatchery-Released and Wild Fish

The distribution of hatchery-released and wild fish with respect to stations (localities) within each bay appeared to be nonrandom. In Galveston Bay, many of the hatchery-assigned fish were recovered in the same general area (Figure 2). A homogeneity test using the 28 hatchery-released fish from spawns in 2004 at the MDC revealed that the distribution of hatchery-released fish and wild fish across sampling stations (localities) was nonrandom ($P = 0.022$). As examples, four fish of hatchery origin and two fish of wild origin were sampled at one locality, whereas no fish of hatchery origin were found among the 13 fish sampled at a second locality. The same appeared to be the case for hatchery-released fish sampled from Aransas Bay where two fish of hatchery origin and five wild fish

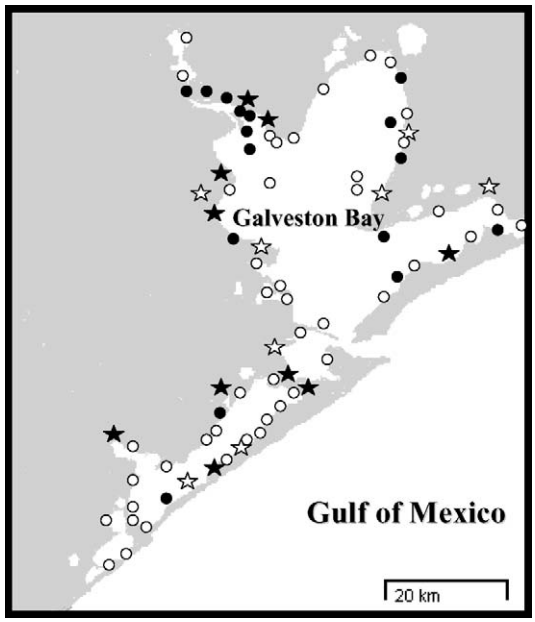

FIGURE 2.—Map of Galveston Bay showing the locations of the release sites (stars) and sampling stations (circles) for red drum. Filled stars indicate releases from the Marine Development Center, open stars releases from Sea Center–Texas. Filled circles indicate stations at which hatchery-reared fish were recovered.

were sampled at one locality, while no fish of hatchery origin were among the 52 fish sampled at a second locality.

Discussion

The genetic markers and analytical approaches employed in this study proved highly effective in discriminating hatchery-released from wild red drum. This was not unexpected, as a prior study in our laboratory (Renshaw et al. 2006b) had demonstrated that (1) both genotype exclusion and likelihood ratio tests could be used to identify hatchery-released and wild red drum within requisite error bounds and within the context of complete parental sampling and (2) using a minimum of 15 microsatellites and the mtDNA fragment assayed generated a cumulative match probability (the chance that a wild fish could be incorrectly identified as a hatchery-reared fish) of less than 10^{-19}. Using mtDNA further permitted unambiguous correction of all but two mismatches at the microsatellite loci. The two mismatches not attributable to misscoring probably represent null alleles but could, in one case, represent differential amplification of alleles. For interested readers, a slightly different but equally effective approach to discriminate hatchery-reared from wild red drum was developed by Tringali (2006).

The percentage of hatchery-released red drum in our study ranged from 1.13% in Aransas Bay to 9.35% in Galveston Bay. Similar percentages have been documented for the red drum stock enhancement program in Florida. Willis et al. (1995) used coded wire or internal anchor tags and identified a total eight hatchery-reared fish (~2%) among a sample of 395 fish trammel-netted from Mosquito Lagoon (on the eastern coast of Florida), while Tringali et al. (2008) used coded wire tags and genetic markers to identify 282 hatchery-reared fish (~2.8%) among nearly 10,000 fish sampled throughout Tampa Bay and neighboring waters (on the western coast of Florida). In both of the Florida studies, the hatchery-released fish recovered were greater than 200-mm TL (up to 586 mm in Willis et al. 1995), indicating survival in the wild for at least 6 months; also, in both studies, the highest recapture probability was observed at sites closest to the release sites.

Considerably higher recovery rates of hatchery-released red drum have been reported in South Carolina. Jenkins et al. (2004) released multiple groups of OTC-marked juveniles into Callawassie Creek (Port Royal Sound estuary); overall, hatchery-reared fish (primarily age 0 but up to age 2) accounted for 19% of 627 fish sampled from the 1995 cohort and 19.4% of fish sampled from the 1996 cohort. Smith et al. (2003) reported that 78% of the 1999 cohort of red drum sampled from the Ashley River (Charleston Harbor estuary) was hatchery reared. The difference in recovery percentages of hatchery-released fish in South Carolina versus those in our study is undoubtedly due to several factors. Notable differences include stocking density, the number of release localities, and the number of sampling or recovery sites. In the study involving Callawassie Creek, approximately 1.57 million hatchery-raised juveniles (22–56 mm) were released between fall 1995 and spring 1997 into a small (535-ha) part of the total of 25,000 ha available in Port Royal Sound estuary (Jenkins et al. 2004), while in the study involving the Ashley River, a total of 617,000 fish (20.9–30.0 mm) were released in 1999 over a 15-km reach of the river into a maximum of 980 ha of potential habitat (Smith et al. 2003). Estimated stocking densities (number of fish/ha) in the Callawassie River were around 3,000/ha if one considers the 535 ha in the release site, or 62.8/ha if one considers the entire Sound; in the Ashley River, the stocking density estimated by Smith et al. (2003) was around 630/ha. In 2004, roughly 3.2 million juvenile red drum (29–40-mm TL) were released at 18 different localities throughout Galveston Bay (Figure 2). The total surface area of Galveston Bay is 141,676 ha

(Matlock and Osborn 1982), yielding a stocking density of approximately 22.6/ha, considerably less than those in the Callawassie and Ashley rivers. Finally, there were only three localities sampled in the Callawassie River study, one of which was adjacent to the release site and in which the majority of hatchery-released fish were recovered (Jenkins et al. 2004). In our study, there were 90 stations sampled randomly throughout Galveston Bay; red drum ($\leq$500 mm) were collected at 62 of the stations (Figure 2). The spatial distribution of hatchery-released and wild fish with respect to sampling stations (localities) within each bay, however, were nonrandom, suggesting (as has been observed in other studies) that individuals from the same hatchery-released population often maintain close proximity to one another.

Comparisons of the metrics of genetic variation among hatchery-released fish, broodfish, and wild fish revealed significantly lower allelic richness at nuclear-encoded microsatellites and significantly fewer, different mtDNA haplotypes in hatchery-released fish than in both broodfish and wild fish. Genetic risks of hatchery-released fish on wild populations have been discussed extensively (Blankenship and Leber 1995; Tringali and Bert 1998; Brannon et al. 2004; Tringali et al. 2007) and generally relate to dangers inherent if released fish differ in allele distribution or frequency of alleles affecting life history traits or if released fish stemming from a small number of broodfish contribute disproportionately to the overall juvenile pool. The latter can reduce the genetic effective size (N_e) of the wild population (Ryman and Laikre 1991) and has been termed a Ryman–Laikre effect (Tringali and Bert 1998). However, there were no differences in genetic variation between hatchery broodfish and wild fish, suggesting that the lower genetic variation observed in the hatchery-released fish is due to nonrandom survival among individual releases, a low number of breeders actually contributing to individual releases, or both. While nonrandom survival of individual releases could reflect genetic differences among the broodfish in genes affecting progeny survival, it seems to us more likely that it would reflect other factors that would generate chance variation in survival probability of individual releases. Such factors include variable physiological condition of released fish, time or season of release, variable water temperature and salinity at a release site, differences in social behavior and/or energy efficiency, and variable presence of predators, all of which are either known or hypothesized to affect the probability of survival of hatchery-released or cultured fish (Niva and Jokela 2000; Weber and Fausch 2003; Fairchild and Howell 2004). Indeed, there was suggestive evidence in our study of an effect of season of release on survival of hatchery-reared fish. Of the 3.2 million red drum fingerlings released into Galveston Bay in 2004, roughly 1.1 million were reared at SCT and released between 3 June and 23 September, while roughly 2.1 million were reared at MDC and released between 28 September and 9 December (Figure 2). All 28 hatchery-released fish recovered from the 2004 cohort were from fingerlings reared at MDC, suggesting increased survival of juveniles released in the fall. However, if SCT fish released in early June could grow to exceed 500-mm TL prior to sampling, they would have been excluded from genetic analysis. Increased recovery of hatchery-reared red drum released in season (i.e., during the fall when wild red drum are spawning) also was observed by Willis et al. (1995).

The reduced genetic variation observed among the hatchery-released fish could also reflect a small number of broodfish giving rise to individual releases. Records at the MDC hatchery for the calendar year 2004 indicate that survival of fingerlings during the prerelease growth period ranged from approximately 7–73% and averaged approximately 34%. If fingerling production is nonrandom with respect to families (dam $\times$ sire combinations) held in grow-out ponds, the number of broodfish contributing to a release potentially would be reduced. In addition, the number of breeders contributing to a release also would be reduced if dams or sires within a spawning tank either did not contribute to a spawn or did not contribute equally. Based on empirical data, Gold et al. (in press) estimated that the maximum, average effective size (N_{eR}) of an actual released population of red drum fingerlings from the MDC hatchery was approximately 2.4 times less than would be expected if all possible dams and sires had contributed equally to the released population. Finally, the notion that relatively few broodfish may contribute to a released population was supported by the observation in this study that the number of hatchery dams, sires, and dam $\times$ sire combinations contributing to the hatchery-reared fish recovered in Galveston Bay was significantly fewer than would be expected had each dam, sire, and dam $\times$ sire combination had an equal probability of contributing to the recovered fish.

The estimates of the average, genetic effective size of a TPWD-released population suggest that a Ryman–Laikre effect on the overall (hatchery-released plus wild) red drum population in waters off the Texas coast is a possibility. Increasing the number of spawns from different spawning tanks in a hatchery-released population would be one strategy to increase the average N_{eR} of a released population and minimize such an effect. Another strategy would be to increase the

survival potential of each hatchery-released population. Pilot studies to assess factors affecting survival potential of TPWD-released population are planned. Results of the present study, however, demonstrate that hatchery-reared red drum released as early juveniles (25–40 mm in length) survive at least 1 year in Texas bays and estuaries, and may comprise a significant fraction of red drum in an individual bay or estuary.

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