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[Article]

Genetic Studies of Hatchery-Supplemented Populations of Red Drum in Four Texas Bays

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Abstract.—Genetic diversity, population structure, average long-term effective population size (N_e), and average long-term genetic migration rate of red drum *Sciaenops ocellatus* in each of four Texas bays were assessed using variation in 13 nuclear-encoded microsatellites among samples from the 2004 and 2005 cohorts. No significant differences in genetic diversity were detected among bays. Levels of gene diversity of red drum in each bay were equal to or greater than estimates reported for microsatellites in red drum sampled previously from two of the four bays and from other bays in the southeastern USA, including some that had not yet been supplemented with hatchery-raised fish. Tests of the homogeneity of allele and genotype distributions (including analysis of molecular variance) among the four bays were nonsignificant. Estimates of the migration rate (m) between bays ranged from 0.08% to 0.15%, with the average long-term number of migrants (calculated as $N_e \times m$) between bays estimated to range from 1.04 to 2.37 fish/generation. Estimates of average long-term N_e in the four bays ranged from 1,302 to 1,581 fish and collectively were well within the range hypothesized to support sustained, long-term persistence. The estimates of N_e also were, on average, five to six times higher than comparable estimates reported for the 1986–1989 red drum cohorts sampled from seven bays across the northern Gulf of Mexico. Adjustment of long-term N_e in each of the four bays relative to bay-specific spatial parameters revealed a positive relationship with red drum abundance as measured by catch-per-unit-effort statistics compiled by the Texas Parks and Wildlife Department between 1982 and 2005. The observed high levels of genetic diversity, estimates of average long-term N_e, and increased N_e over the past 15–20 years are consistent with the hypothesis that the Texas Parks and Wildlife Department's stock enhancement program has not genetically compromised the resident red drum subpopulations in the four bays.

The red drum *Sciaenops ocellatus* is an estuarine-dependent species that historically supported important commercial and recreational fisheries in U.S. waters of the northern Gulf of Mexico (hereafter, Gulf) and western Atlantic Ocean (VanVoorhees et al. 1992; Pattillo et al. 1997). Significant declines in red drum abundance (Goodyear 1991) prompted closure of the commercial fishery (GMFMC 1996; ASMFC 2002), implementation of harvest restrictions, prohibition of the sale of "wild" red drum (Matlock 1990), and initiation of hatchery augmentation (stock enhancement) programs in several southern states, including Texas, Florida, Georgia, and South Carolina (McEachron et al. 1993; Smith et al. 1997; Woodward 2000; Tringali et al. 2008). The red drum stock enhancement program in Texas was initiated by the Texas Parks and Wildlife Department (TPWD) in the 1980s (McEachron et al. 1993). Presently, the TPWD program releases 25–30 million hatchery-produced red drum fingerlings annually into various Texas bays and estuaries (Vega et al. 2003), and there is both indirect (McEachron et al. 1995) and direct (Karlsson et al. 2008) evidence that hatchery-released fish contribute to the red drum fishery in Texas bays and estuaries.

Bert et al. (2007) reviewed several aspects of genetic management of hatchery-based stock enhancement, including the need for rigorous baseline population genetics information on the populations receiving hatchery supplementation. Such information typically includes identification of distinct stocks, should they

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exist; levels of genetic diversity; and estimates of genetic effective population size (N_e). Knowledge of stock structure is important in that different populations (stocks) within a region may possess distinct genetic resources that promote differences in phenotypic and life history traits, such as growth rates, fecundity, abundance, and disease resistance (Stepien 1995). Inadvertent mixing of different stocks via supplementation with hatchery-derived offspring potentially could lead to erosion of these resources (Ryman et al. 1995; Tringali and Bert 1998). Knowledge of genetic diversity is critical because of the strong correlation between genetic diversity and overall fitness (Reed and Frankham 2003; Vandewoestijne et al. 2008), and estimates of N_e are of importance for predicting potential losses in population fitness due to reductions in genetic diversity (Frankham 1995; Higgins and Lynch 2001).

In this study, genetic diversity, population structure, average long-term migration, and average long-term N_e of red drum in four hatchery-supplemented Texas bays (Galveston, San Antonio, and Aransas bays and Upper Laguna Madre) were assessed using variation in 13 nuclear-encoded microsatellites. The four bays have been supplemented annually since 1990 with an average (±SE) of approximately 5.3 ± 0.7 million (Galveston Bay), 1.8 ± 0.4 million (San Antonio Bay), 2.4 ± 0.6 million (Aransas Bay), and 3.4 ± 0.9 million (Upper Laguna Madre) fingerling red drum from TPWD hatcheries (R. Vega, TPWD Coastal Fisheries Division, personal communication). Details regarding TPWD hatchery practices and recent estimates of the percentage of released age-0 fish that were recovered in two Texas bays are described by Karlsson et al. (2008).

Methods

A total of 1,493 red drum from the 2004 and 2005 cohorts were sampled from the four Texas bays (Figure 1) during fall 2005, spring 2006, and fall 2006 by TPWD personnel. Sample sizes at each locality were 144 fish (2004 cohort) and 303 fish (2005 cohort) from Galveston Bay, 317 and 77 fish from San Antonio Bay, 257 and 285 fish from Aransas Bay, and 83 and 27 fish from the Upper Laguna Madre. Fish were obtained by gillnetting, and small pieces (4–5 mm^3) of caudal fin were removed and fixed in 95% ethanol; fin clips were removed only from relatively pristine (i.e., not degraded) fish. All fish sampled were less than 500–550 mm in total length to ensure that virtually all fish were age 0. The size restriction was based on age–length relationships for each bay determined empirically via otolith increment analysis by TPWD personnel at the Perry R. Bass Marine Fisheries Research Station, Palacios.

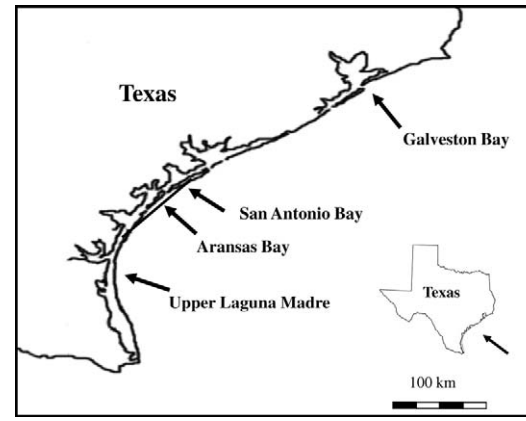

FIGURE 1.—Locations of four bays along the Texas coast, where samples of red drum were obtained.

The DNA was extracted from fin clips by using a standard phenol–chloroform protocol (Sambrook et al. 1989). Sixteen microsatellites were surveyed using the multiplex protocols of Renshaw et al. (2006) for panel 1 (*Soc412*, *Soc416*, *Soc417*, *Soc423*, and *Soc428*), panel 3 (*Soc19*, *Soc85*, *Soc138*, *Soc156*, *Soc206*, and *Soc410*), and panel 4 (*Soc11*, *Soc83*, *Soc99*, *Soc407*, and *Soc424*). Polymerase chain reaction primer sequences and other details for the 16 microsatellites are detailed by Saillant et al. (2004). An ABI Prism 377 DNA sequencer (Applied Biosystems, Inc., Foster City, California) was used to separate and visualize amplification products. Gel analysis was performed using GeneScan version 3.1.2 (Applied Biosystems), with allele calling performed using Genotyper software version 2.5 (Applied Biosystems). Genotypes at the 16 microsatellites assayed and organized by cohorts within bays are available from Texas A&M University (2009).

Initial statistical analysis included the removal of 17 fish from the data set (identified unequivocally as hatchery-released fish based on analytical methods outlined by Karlsson et al. 2008), followed by (1) tests of departure of genotypic proportions from Hardy–Weinberg equilibrium (HWE) expectations for each microsatellite in each cohort, (2) tests of departure from genotypic equilibrium for pairs of microsatellites in each cohort, and (3) tests of homogeneity in allele (genic) and genotype distributions between cohorts within each bay. Tests of conformance to HWE and genotypic equilibrium expectations were carried out using exact probability tests as implemented in GENEPOP version 3.4 (Raymond and Rousset 1995). The exact probability in each test was estimated using a

Markov-chain approach (Guo and Thompson 1992) that employed 5,000 dememorizations, 500 batches, and 5,000 iterations/batch. Sequential Bonferroni correction (Rice 1989) was applied for all multiple tests performed simultaneously. Genotypic proportions at 3 of the 16 microsatellites (*Soc410*, *Soc412*, and *Soc416*) differed significantly from HWE expectations (after Bonferroni correction) in three to five of the eight samples. Analysis using MicroChecker (van Oosterhout et al. 2004) indicated widespread occurrence of null alleles at *Soc410* and *Soc416*. Inferred genotypes at *Soc412* included a number of 1-base-pair shifts that often made scoring at this microsatellite problematic. All three microsatellites were thus omitted from all further analyses. Significant genotypic disequilibrium (after Bonferroni correction) was found only for one pair of microsatellites (*Soc11* and *Soc424*) in the 2005 cohort from Galveston Bay and for two pairs of microsatellites (*Soc19–Soc85*; *Soc19–Soc428*) in the 2004 cohort from San Antonio Bay. Homogeneity of allele (genic) and genotype distributions between cohorts within each bay was tested for each of the remaining 13 microsatellites by using exact tests as implemented in GENEPOP; tests were combined to determine probability values across all 13 loci according to Fisher's method. Sequential Bonferroni correction was employed to account for multiple tests carried out simultaneously and to determine significance of probability values. No significant differences in allele or genotype distributions between cohorts in any of the four bays were found after Bonferroni correction (corrected $\alpha = 0.003$; *P*-values ranged from 0.006 to 0.953 for allele distributions and from 0.025 to 0.954 for genotypic distributions); cohorts within each bay were then pooled for all subsequent statistical analyses. We treated each bay as an independent unit of analysis, in part because the number of hatchery-released fish within and between bays varies annually and in part because a number of hydrographic, hydrological, and water quality parameters also varies annually both within and between bays (http://www.twdb.state.tx.us/data/bays_estuaries/bays_estuary_toc.asp) and could affect varying survival of hatchery-released fish.

Summary statistics, including number of alleles, allelic richness (a measure of the number of alleles independent of sample size), unbiased gene diversity (expected heterozygosity), and the inbreeding coefficient (F_{IS}; measured as the *f* of Weir and Cockerham 1984), were obtained for each microsatellite in red drum from each bay (cohorts pooled) using FSTAT version 2.9.3.2 (Goudet 1995). Tests of departure of genotypic proportions from HWE expectations for each microsatellite and tests of departure from genotypic equilibrium for pairs of microsatellites within each of the four bays were carried out as described above for cohorts within each bay. Homogeneity in allelic richness and gene diversity among samples from the four bays was tested using Friedman rank tests as implemented in the Statistical Package for the Social Sciences (version 11.0.1). Homogeneity of allele and genotype distributions across all four bays was tested via exact tests as implemented in GENEPOP; exact probabilities were estimated using a Markov-chain method and employing the same parameters used in tests of HWE and genotypic equilibrium (see above). The null hypothesis of genetic homogeneity among red drum from the four bays also was tested using analysis of molecular variance (AMOVA) as implemented in Arlequin version 3.11 (Schneider et al. 2000).

Maximum likelihood estimates of the average long-term (mutation-scaled) migration rate (*M*) between adjacent bays and average long-term N_e in each bay were generated using the coalescent-based program MIGRATE version 2.4 (Beerli and Felsenstein 2001). MIGRATE uses Markov-chain Monte Carlo simulations of gene-tree branch lengths in each subpopulation (bays, in this case) and provides bidirectional estimates of *M* between each bay and estimates of theta (Θ, a mutation-scaled approximation of effective population size) for each bay. The value *M* is equal to the migration rate per generation (*m*) divided by the average, per-gene mutation rate (μ); Θ is equal to $4N_e\mu$, where N_e is the average long-term size. Due to computational limitations, estimates of these parameters were based on a random sample of 25 individuals from the 2004 cohorts in each of the four bays (100 individuals total). A preliminary analysis was undertaken to establish initial estimates for both *m* and Θ for use as starting values in a final run, which consisted of three replicates. In the final Markov-chain Monte Carlo simulation, 30 short chains (10^4 gene trees sampled) and two long chains (2.5×10^6 gene trees sampled) were specified. The first 10^4 steps were disregarded as burn-in to ensure parameter stability.

Results

Summary statistics for red drum in each bay, including number of alleles, allelic richness, unbiased gene diversity, results of HWE tests, and F_{IS} for each of the 16 microsatellites, are given in Table 1. As expected, based on initial tests of HWE carried out for each microsatellite in each cohort, significant deviations from HWE were observed at *Soc410*, *Soc412*, and *Soc416* in samples from two or more bays. As before, analysis with MicroChecker indicated occurrence of null alleles at *Soc410* and *Soc416*; this was corroborated by relatively high, positive F_{IS} values (indicating

TABLE 1.—Summary statistics at 16 nuclear-encoded microsatellite loci for the 2004 and 2005 (pooled) cohorts of red drum sampled from four bays along the Texas coast (N = sample size; #A = number of alleles; A_R = allelic richness; H_E = gene diversity, measured as expected heterozygosity; P_{HW} = probability of conforming to expected Hardy–Weinberg genotypic proportions; F_{IS} = inbreeding coefficient, measured as the f of Weir and Cockerham (1984).

Microsatellite and statistic	Galveston Bay	San Antonio Bay	Aransas Bay	Upper Laguna Madre
Soc11				
N	536	446	376	102
#A	12	13	12	11
A_R	9.61	10.13	10.57	10.78
H_E	0.705	0.699	0.735	0.718
P_{HW}	0.003	0.476	0.028	0.938
F_{IS}	−0.024	−0.017	0.012	−0.024
Soc19				
N	536	442	385	102
#A	18	17	17	15
A_R	15.66	15.44	15.74	14.86
H_E	0.903	0.903	0.909	0.903
P_{HW}	0.211	0.896	0.050	0.832
F_{IS}	0	−0.012	0.017	0.012
Soc83				
N	537	442	379	97
#A	18	16	17	16
A_R	15.53	15.05	15.27	15.92
H_E	0.859	0.867	0.863	0.838
P_{HW}	0.159	0.008	0.695	0.030
F_{IS}	0.046	0.042	0.009	0.029
Soc85				
N	537	445	388	104
#A	17	18	17	15
A_R	15.15	15.14	15.09	14.91
H_E	0.863	0.870	0.866	0.867
P_{HW}	0.217	0.756	0.064	0.868
F_{IS}	0.024	0.008	0.044	0.013
Soc99				
N	537	445	384	100
#A	25	29	28	22
A_R	22.36	23.87	23.76	21.85
H_E	0.928	0.937	0.934	0.929
P_{HW}	0.075	0.183	0.000	0.388
F_{IS}	0.002	0.031	0.002	−0.045
Soc138				
N	537	445	389	103
#A	26	25	21	13
A_R	15.43	14.97	13.04	12.53
H_E	0.815	0.804	0.808	0.796
P_{HW}	0.012	0.882	0.010	0.693
F_{IS}	0.024	0.01	0.052	0.049
Soc156				
N	537	446	389	104
#A	6	7	5	5
A_R	4.76	5.14	4.29	4.99
H_E	0.549	0.584	0.542	0.558
P_{HW}	0.636	0.044	0.968	0.085
F_{IS}	−0.025	−0.017	−0.01	−0.018
Soc206				
N	537	446	386	103
#A	5	5	5	5
A_R	4.73	4.83	4.89	4.99
H_E	0.491	0.491	0.510	0.511
P_{HW}	0.989	0.703	0.080	0.906
F_{IS}	−0.032	0.055	0.01	−0.045
Soc407				
N	537	442	385	103
#A	10	12	11	10
A_R	9.55	10.24	9.92	9.98
H_E	0.823	0.824	0.827	0.812
P_{HW}	0.101	0.375	0.042	0.747
F_{IS}	0.011	0.042	0.014	−0.005
Soc410				
N	537	435	366	100
#A	28	20	19	16
A_R	18.06	16.15	15.20	15.84
H_E	0.824	0.795	0.838	0.822
P_{HW}	0.000	0.000	0.000	0.000
F_{IS}	0.126	0.187	0.234	0.197
Soc412				
N	536	440	366	104
#A	33	35	34	26
A_R	26.90	27.94	28.23	25.64
H_E	0.918	0.914	0.923	0.925
P_{HW}	0.000	0.000	0.110	0.014
F_{IS}	0.096	0.157	0.056	0.064
Soc416				
N	537	446	301	102
#A	20	19	20	17
A_R	16.99	17.30	16.57	16.85
H_E	0.810	0.836	0.774	0.835
P_{HW}	0.000	0.000	0.000	0.000
F_{IS}	0.126	0.112	0.111	0.307
Soc417				
N	537	445	385	104
#A	17	17	14	11
A_R	13.24	11.89	11.79	10.83
H_E	0.753	0.760	0.770	0.739
P_{HW}	0.067	0.088	0.752	0.083
F_{IS}	−0.023	0.042	−0.009	0.089
Soc423				
N	537	446	381	104
#A	22	23	21	18
A_R	17.13	17.93	17.93	17.74
H_E	0.894	0.897	0.896	0.897
P_{HW}	0.137	0.666	0.749	0.812
F_{IS}	0.029	0.023	−0.004	−0.029
Soc424				
N	537	418	352	95
#A	28	24	24	20
A_R	21.12	20.50	19.81	20.00
H_E	0.857	0.851	0.843	0.874
P_{HW}	0.605	0.004	0.135	0.740
F_{IS}	−0.008	0.039	0.029	0.036
Soc428				
N	537	435	384	103
#A	34	34	34	27
A_R	30.26	31.17	31.26	26.84
H_E	0.945	0.949	0.953	0.949
P_{HW}	0.487	0.003	0.364	0.093
F_{IS}	0.013	0.048	0.008	0.069

a deficit of heterozygotes) at both microsatellites (Table 1). With the exception of *Soc99* in the sample from Aransas Bay, no significant departures from HWE (after Bonferroni correction) were observed. For the remaining 13 microsatellites, the number of alleles per microsatellite ranged among bays between 5 and 34 and averaged 17.1. Allelic richness averaged 14.81 (range = 4.29–31.26), and unbiased gene diversity averaged 0.801 (range = 0.491–0.953). Only 5 of 312 pairwise tests of genotypic disequilibrium were

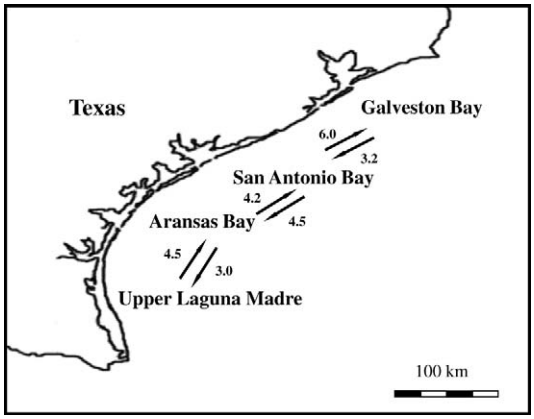

FIGURE 2.—Bidirectional values of the average long-term migration rate (M; mutation scaled) of red drum between adjacent bays in Texas.

significant after Bonferroni correction. No significant differences in either allelic richness ($P = 0.595$) or gene diversity ($P = 0.152$) among bays were revealed by Friedman rank tests. Exact tests of homogeneity of both allele and genotype distributions among bays were nonsignificant after Bonferroni correction, and the among-bays component of molecular variance as estimated by AMOVA did not differ significantly from zero (genetic differentiation index $F_{ST} = -0.00001$, $P = 1.000$).

Results of the analysis (using MIGRATE) of average long-term, mutation-scaled M and average long-term N_e are shown in Figure 2 and Table 2, respectively. Only 10 of the microsatellites could be used in the analysis. *Soc138* (a tetranucleotide repeat) and *Soc156* (an imperfect trinucleotide repeat) deviated from the stepwise mutation model (Kimura and Ohta 1978) assumed in the analysis in that both possessed alleles that differed from the sizes expected based on additions or deletions of their repeat motifs. *Soc99* was also omitted because of an infrequent allele that was well outside the size range of the remaining alleles and that confounded the analysis. Estimates of M ranged from 3.0 (from Aransas Bay to the Upper Laguna Madre) to 6.0 (from San Antonio Bay to Galveston Bay). Bidirectional estimates of M between Aransas and San Antonio bays were fairly symmetric, whereas estimates of M from the Upper Laguna Madre to Aransas Bay and from San Antonio Bay to Galveston Bay were 1.50 and 1.87 times greater, respectively, than those in the reverse direction (Figure 2). A range of estimates of m was derived from the M-values using an average per-gene (modal) μ of 2.53×10^{-4}. This value of μ was obtained using the 10-microsatellite data set and the Bayesian coalescent approach of Beaumont (1999). Specific details regarding the latter can be obtained from the first author.

Estimates of the long-term, average m between bays (with $\mu = 2.53 \times 10^{-4}$) ranged from 0.08% to 0.15%. When coupled with the estimates of average long-term N_e (see below), the average, long-term effective number of migrants ($N_e m$) between bays was estimated to be 1.04–2.37 fish/generation. Estimates of average long-term N_e for each bay (Table 2) were derived from Θ values generated in MIGRATE ($N_e = \Theta/4\mu$), with μ equal to the modal rate of 2.53×10^{-4} derived from the Bayesian coalescent approach of Beaumont (1999). The N_e estimates ranged from 1,302 (Aransas Bay) to 1,581 (Galveston Bay) red drum; the estimates for Galveston Bay, San Antonio Bay, and the Upper Laguna Madre did not differ from one another based on the confidence intervals, whereas the N_e for Aransas Bay differed from estimates for the other three bays (Table 2).

Alternatively, the four bays differ markedly in physical size, with both surface area and shoreline distance in Galveston Bay being considerably greater than those of both San Antonio and Aransas bays. We adjusted the estimates of N_e to reflect these differences by dividing each estimate by the estimated surface area (in km^2) and shoreline distance (in km) of each bay. Surface area estimates for Galveston, San Antonio, and Aransas bays were obtained from GulfBase (2009). The lower estimate for the Upper Laguna Madre was based on data provided by TPWD personnel and

TABLE 2.—Estimates of average long-term effective population size (N_e) and 95% confidence limits (CLs) for red drum from each of four Texas bays, and estimates of average N_e per square kilometer of surface area and N_e per kilometer of shoreline distance. See Results for description of surface area and shoreline distance calculations used for Upper Laguna Madre (upper and lower estimates of N_e/km^2 and N_e/km).

Bay system	Long-term N_e	Lower 95% CL	Upper 95% CL	Surface area (km^2)	N_e/km^2	Shoreline distance (km)	N_e/km
Galveston Bay	1,581	1,453	1,724	1,399	1.13	661.7	2.39
San Antonio Bay	1,545	1,439	1,661	531	2.91	362.4	4.26
Aransas Bay	1,302	1,211	1,403	539	2.42	424.1	3.07
Upper Laguna Madre	1,510	1,408	1,623	410–816	1.85–3.68	357.7–633.4	2.38–4.22

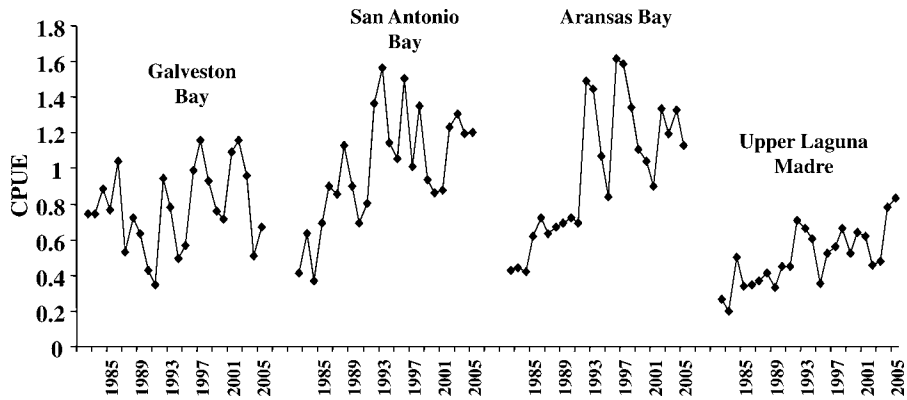

FIGURE 3.—Estimates of red drum catch per unit effort (CPUE; fish/h) with 45 gill nets set overnight twice annually between 1982 and 2005 in four Texas bays (M. Fisher, Texas Parks and Wildlife Department, unpublished data).

includes only the Upper Laguna Madre proper, while the upper estimate includes the estimate from GulfBase (2009; which includes the contiguous Corpus Christi Bay) along with estimates for several small bays (Alazan Bay, Cayo del Grullo, Laguna de los Olmos, and Cayo del Infiernillo) that also are contiguous with the Upper Laguna Madre and collectively total approximately 100 km^2 in surface area (McKee 2008). Shoreline distances (km) for each bay were obtained from Matlock and Osborn (1982). Adjusting for the differences in surface area and shoreline distance indicated that the N_e of red drum per square kilometer (surface area) and the N_e per kilometer (shoreline distance) in both San Antonio and Aransas bays were greater than those in Galveston Bay and the lower estimate (based on total potentially available surface area) in the Upper Laguna Madre system. Interestingly, these estimates of red drum N_e per square kilometer appear to correlate reasonably well with long-term estimates of catch per unit effort (CPUE; Figure 3) based on catch per hour in 45 gill nets set overnight twice annually between 1982 and 2005 (Martinez-Andrade et al. 2005; M. Fisher, TPWD Coastal Fisheries Division, unpublished data).

Discussion

Levels of genetic variability in red drum from the four bays, measured as number of alleles, allelic richness, and expected heterozygosity (gene diversity) at 13 microsatellites, were generally greater than analogous variability estimates reported for microsatellites in other vertebrates, including fish (DeWoody and Avise 2000; Neff and Gross 2001), and were equal to or greater than microsatellite variability reported in prior studies of red drum. With regard to the latter, Chapman et al. (2002) reported that gene diversity values per microsatellite ranged from 0.560 to 0.903 (average = 0.658) for seven cohorts (1990–1996) sampled from major estuaries in South Carolina, while M. D. Tringali (Fish and Wildlife Research Institute, Tampa, Florida, personal communication) found that gene diversity values per microsatellite ranged from 0.52 to 0.92 (average = 0.79) for two cohorts (1999–2000) sampled from bays along the west (Gulf) coast of Florida. The fish surveyed by Tringali were taken from bays prior to stock enhancement and ostensibly represent "natural" levels of genetic variability in "wild" red drum in Florida. Finally, Gold and Turner (2002) surveyed red drum from four cohorts (1986–1989) sampled from seven bays in the northern Gulf and reported gene diversity values ranging from 0.560 to 0.903 (average = 0.658). Gene diversity values for the four bays surveyed in our study ranged from 0.501 to 0.949 (average = 0.810), with values greater than 0.800 for 12 of the 16 microsatellites. Although no data on genetic variability prior to stock enhancement are available for Texas bays and estuaries, the comparatively high gene diversity values for the four bays and the strong correlation between genetic diversity and overall fitness (Reed and Frankham 2003; Vandewoestijne et al. 2008) would certainly appear to mitigate concerns (Busack and Currens 1995) about the loss of genetic variability and fitness in populations undergoing stock enhancement, at least for red drum in these four bays.

The AMOVA and the tests of homogeneity in microsatellite allele and genotype distributions among the four bays were nonsignificant. Previously, Gold and Turner (2002) reported a significant isolation-by-distance effect among red drum in the northern Gulf, with a genetic neighborhood size (the area around an individual's birthplace that also encompasses the parents' birthplace: Fox et al. 2001) of approximately 700–900 km. The geographic distance (approximately

350 km) between Galveston Bay to the northeast and the Upper Laguna Madre is well within the estimated neighborhood size, potentially alleviating concerns by TPWD (R. Vega, personal communication) about supplementing bays along the upper Texas coast (e.g., Galveston Bay) with red drum fingerlings from broodstock taken along the lower coast (e.g., Aransas Bay or the Upper Laguna Madre). These considerations are based on neutral genetic variation and gene flow assessed using microsatellites and do not rule out the possibility of local genetic adaptation(s) in bays along the upper coast versus bays along the lower coast or even in individual bays.

The estimates of average long-term N_e in the four bays ranged from 1,302 (Aransas Bay) to 1,581 (Galveston Bay) red drum. When coupled with the estimates of average long-term m, the average long-term N_em per generation between bays was estimated to range from 1.04 to 2.37 fish. This level of N_em is, in theory, sufficient to homogenize populations at selectively neutral loci under conditions of migration–drift equilibrium and an island model of population structure (Wright 1931; Whitlock and McCauley 1999). Neither condition, particularly the latter, was likely to have been met here. In addition, the range of long-term m (0.08–0.15%) among the four bays is sufficiently low to allow independent response to demographic perturbation (Hauser and Carvalho 2008).

The estimates of average long-term N_e in the four bays, when summed together, are well within the range of long-term N_e suggested to be compatible with sustained, long-term persistence and resistance to extinction from environmental stochasticity, genetic factors, or both (Whitlock 2000; Reed 2005). Moreover, the estimates for the four bays are, on average, five to six times higher than those reported by Turner et al. (2002) in their study of red drum from the 1986–1989 cohorts sampled from seven bays or estuaries in the northern Gulf. The estimates of Turner et al. (2002) ranged from 184 to 517 (average = 265) and included two of the bays sampled in this study (Galveston Bay, referred to as West Bay in their study, and the Laguna Madre). Long-term N_e is a backward-looking statistic (Crandall et al. 1999) that refers to the number of parents in a reference population in the past (Templeton 2006). Smaller long-term N_e thus essentially reflects fewer parents and a higher probability of identity by descent under random mating (Templeton 2006). The significant increase in long-term N_e over the past 15–20 years suggests that the red drum sampled in our study descended from a significantly larger number of parents than those sampled by Turner et al. (2002). It would be difficult to discern whether this increase is due to management measures implemented in the mid- to late 1980s (Matlock 1990), stock enhancement, or both.

Interestingly, adjusting the estimated long-term N_e in each of the four bays relative to the estimated surface area or the estimated shoreline distance in each bay indicated a positive relationship between N_e per square kilometer (surface area) or N_e per kilometer (shoreline distance) and red drum abundance as measured by CPUE. Many studies (e.g., Shrimpton and Heath 2003) have found little agreement between census population size and N_e estimated using MIGRATE, yet none to our knowledge have taken into account the size of potentially available habitat and adjusted estimates of N_e accordingly. A caveat to the observed correlation is that surface area and shoreline distance per se do not necessarily correspond to the available amount of suitable red drum habitat. Nonetheless, the relationship is minimally of heuristic interest and certainly warrants further investigation as a possible fishery-independent approach to estimating relative abundance.

The original purpose of the study was to obtain baseline population genetics data on red drum populations receiving hatchery supplementation. We reported previously (Gold et al. 2008) that the average effective size of hatchery-released populations (N_{eR}) in each of four Texas bays or estuaries in 2003 ranged from approximately 28.5 to 46.6 fish and that these values of N_{eR} indicated a reasonable potential for a Ryman–Laikre effect (Tringali and Bert 1998), where inbreeding and accumulation of deleterious genotypes could lead to a reduction in population fitness. The observed high levels of genetic diversity, the estimates of average long-term N_e, and the increase in N_e over the past 15–20 years are consistent with the hypothesis that the TPWD stock enhancement program has neither genetically compromised the resident red drum subpopulations in the four bays nor led to an obvious Ryman–Laikre effect.

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