



Spatial and temporal variation in recovery of hatchery-released red drum (*Sciaenops ocellatus*) in stock-enhancement of Texas bays and estuaries

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ABSTRACT

Spatial and temporal variation in recovery of hatchery-released red drum (*Sciaenops ocellatus*) was assessed in nine bays and estuaries along the Texas coast. Sixteen nuclear-encoded microsatellites were used to identify a total of 167 hatchery-released fish among 4325 red drum sampled between the fall of 2006 and the spring of 2009; these were added to 41 recovered hatchery-released fish identified in a prior study of 1332 red drum sampled from Galveston and Aransas bays in the fall of 2005 and spring of 2006. Recovery of hatchery releases across bays and years ranged from 0.28% to 17.65%. Of the 208 recovered hatchery-released fish, 158 were age 0–1, 46 were age 1–2, and four fish were age 2–3. The distribution of recovered hatchery-released fish and wild fish differed significantly across sampling periods within and among bays or estuaries. Allelic richness in recovered hatchery-released fish was significantly lower than in both hatchery brood fish and wild fish. In addition, recovered hatchery-released fish were produced by fewer dams, sires, and dam × sire combinations in 2005 and 2006 and by fewer dams and sires in 2007 than would be expected if reproductive success was distributed randomly among brood fish. The spatial distributions of recovered hatchery-released and wild fish deviated from random expectations for samples from the Upper Laguna Madre in the fall of 2006 and spring of 2007 and from West Matagorda Bay in the spring and fall of 2008. Correlation and principal components analysis identified a significant, positive relationship between annual average CPUE and relative annual mortality. No relationship was observed between the number of fish released per hectare and the percentage of recovered hatchery-released fish. Comparisons to other studies of red drum stock enhancement are discussed.

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1. Introduction

Red drum (*Sciaenops ocellatus*) comprises one of the most important recreational marine fisheries in bays and estuaries along the Texas coast, contributing approximately \$350 million annually to coastal communities in the state (Vega et al., 2011). Because of declines in red drum abundance across the northern Gulf of Mexico in the 1970s and 1980s (Goodyear, 1991), restoration

(stock-enhancement) programs to supplement the red drum fishery were developed in several Gulf Coast states. The largest of these is in Texas where 20–30 million red drum fingerlings (25–40 mm, total length) are released annually by the Texas Parks and Wildlife Department (TPWD) into nine Texas bays and estuaries (Vega et al., 2003, 2011). Until recently, assessment of the TPWD restoration program in terms of survival of released fish included (i) short-term trawl recovery of fingerlings released during the ‘off’ season (i.e., spring) when wild red drum were not spawning, (ii) release/recovery of oxytetracycline-HCl marked fingerlings, and (iii) length-frequency analysis of fish captured with gill nets in bays where ‘off’ season releases occurred a year earlier (McEachron et al., 1998). Use of coded-wire microtags has been precluded largely because of the small size of released fish. Comparisons of length-frequency distributions of fish sampled a year after ‘off’ season releases indicated that released fish survive in large numbers through the first year, with estimates of the contribution of hatchery-produced red drum to the fishery ranging as

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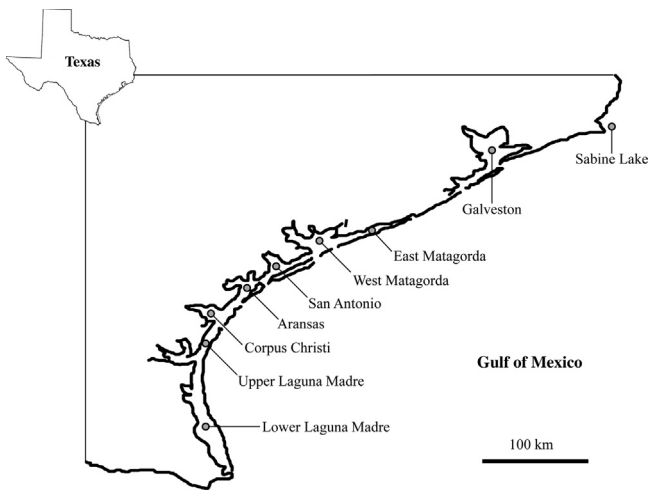


Fig. 1. Location of the nine bays and estuaries sampled in this study.

high as 30% (McEachron et al., 1995, 1998; Scharf, 2000). Karlsson et al. (2008) used a genetic approach to assign red drum sampled from two bays along the Texas coast as either of hatchery or wild origin. They identified 30 hatchery-released fish among 351 red drum sampled from Galveston Bay and 11 hatchery-released fish among 981 red drum sampled from Aransas Bay. They also found that allelic richness, a measure of genetic variation, was significantly reduced in recovered hatchery-released fish as compared to hatchery brood fish and wild fish, and that the contribution of brood dams, sires, and dam \times sire combinations to the recovered hatchery-released fish was non-random.

In this study, we used the 16 nuclear-encoded microsatellites employed by Karlsson et al. (2008) to genotype additional red drum from the two bays (Galveston Bay and Aransas Bay) assessed previously and from the seven other Texas bays or estuaries stocked annually by TPWD: Sabine Lake, East and West Matagorda bays, San Antonio Bay, Corpus Christi Bay, and the Upper and Lower Laguna Madre (Fig. 1). Our objectives were to expand the assessment of survival of hatchery-released red drum to include all of the bays and estuaries along the Texas coast where red drum are stocked and to assess variation in survival across years within the same bay or estuary. Quantifying spatial and temporal rates of recovery of hatchery-released marine species is important for long-term restoration programs that span large geographic scales (Hamasaki and Kitada, 2008) as efficient future management may rely on locality-specific restoration. Additional objectives were to generate base-line data for continued monitoring to assess possible occurrence of adverse genetic effects (Laikre et al., 2010) and to assess possible relationships between recovery of hatchery-released fish and other variables, including abundance and mortality of similar-sized wild fish and available shallow-water habitat.

2. Materials and methods

Details regarding production of hatchery-raised red drum at TPWD hatcheries (Marine Development Center [MDC] in Corpus Christi and Sea Center Texas [SCT] in Lake Jackson) and release of hatchery fish into Texas bays and estuaries are described in Karlsson et al. (2008). Releases from both hatcheries generally start in mid-May and continue through late November. Fin clips from all brood dams and sires at both TPWD hatcheries have been sampled since the 2002 production year and stored in 95% non-denatured ethanol (ETOH). Data on mating design (which dams and sires occurred in the same brood tank in each year) and on location and number of fingerlings released from each hatchery into various

bays and estuaries were obtained from records maintained at each hatchery.

Fin clips from 4325 red drum (total), caught in gill nets set at various sampling stations in nine Texas bays or estuaries between the fall of 2006 and the spring of 2009, were obtained by TPWD personnel and stored in 95% ETOH. Total length was recorded for each fish and fin clips removed only from non-degraded fish less than 500 mm total length to insure that a minimal number of yearling (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. Otoliths were removed from roughly two-thirds of the fish sampled and used to age individual fish, following methods in VanderKooy and Guindon-Tisdell (2003). Age was inferred for the remaining fish from length data based on the relationship between known age, based on otoliths, and length of fish from the same bay collected during the same sampling season. Data from the 1332 red drum sampled in the fall of 2005 and spring of 2006 and reported in Karlsson et al. (2008) were included in data analysis in this study.

DNA was extracted using the phenol-chloroform protocol of Sambrook et al. (1989). Polymerase-chain-reaction (PCR) amplification reactions for the 16 microsatellites used by Karlsson et al. (2008) were carried out using the multiplex protocols for Panels 1, 3, and 4 described in Renshaw et al. (2006). Amplification products were separated and visualized on an ABI Prism 377 DNA sequencer (Applied Biosystems) and analyzed using GENESCAN v. 3.1.2 (Applied Biosystems); allele calling was performed with GENOTYPER v. 2.5 (Applied Biosystems). The microsatellite genotypes of all brood fish (dams and sires) in both hatcheries and of all fish sampled from Texas bays and estuaries to date are available at <http://agrillife.org/gold/doc/> in the folder entitled 'Carson et al., 2013 - Recovery of hatchery-released red drum.'

Assignment of individual fish to origin (hatchery-released or wild) followed Karlsson et al. (2008) and began with a genotype exclusion analysis as implemented in PROBMAX-3 (Danzmann, 1997). Wild, in this case, refers to a fish that was not identified as having been produced by a specific dam \times sire combination from one of the two TPWD hatcheries. From zero to six mismatches were allowed and all mismatches were crosschecked on GENOTYPER files and corresponding gel-image files to rule out scoring error. The logarithm of odds (LOD) approach in FAMoZ (Gerber et al., 2003) was then used to assign individual fish as either hatchery released or wild, as described in Saillant et al. (2009). Hierarchical log-linear analysis was used to compare the distribution of hatchery and wild fish recovered in samples from different localities and in different year classes. The significance of partial associations between the variables origin (hatchery versus wild) and locality or cohort was tested using the Statistical Package for the Social Sciences (SPSS) v. 18.0.0 (IBM SPSS Statistics, Armonk, NY), with reference to the saturated model as described in Bishop et al. (1975). Standard genetic variability measurements (allelic richness and gene diversity) were generated for brood fish, recovered hatchery-released fish, and wild fish, using F stat v. 2.9.3.2 (Goudet, 1995). Homogeneity of allelic richness and gene diversity among brood fish, recovered hatchery-released fish, and wild fish was tested using Friedman's rank test; pair-wise comparisons employed Wilcoxon signed-rank tests, using SPSS. The bootstrap re-sampling method of Dowling et al. (1996) was used to test whether the contribution of all dams, all sires, and all dam \times sire combinations to recovered hatchery-released fish was random; re-sampling was conducted using PopTools (Hood, 2010). The Monte Carlo simulation approach used by Karlsson et al. (2008) and implemented using a program developed by Zaykin and Pudovkin (1993) was used to determine whether recovered hatchery-released and wild fish were distributed randomly according to sampling station within a bay or estuary.

Possible relationships between the percentage of recovered hatchery-released fish and a number of variables were assessed via pairwise, Spearman's rank-order correlations between variables and by principal component analysis (PCA). Variables examined included: (i) number of hatchery fish stocked per hectare; (ii) annual average catch-per-unit-effort (CPUE), relative annual mortality, and shallow-water surface area. Annual average CPUE was based on the gill-net samples taken by TPWD personnel during the spring and fall seasons (45/season/bay except for 20/season in East Matagorda Bay); CPUE was estimated by dividing the number of red drum (>500 mm) caught by the soak time of the net. Relative annual mortality was based on bag-seine samples (20/month/bay except 10/month in East Matagorda Bay) taken by TPWD personnel. Relative annual mortality was estimated from the slope of the right-hand side of the length–frequency curve (i.e., those length classes fully recruited into the gear), with CPUE for each length class log-transformed for linearity. A steep slope represented higher relative annual mortality, and a flat slope represented lower relative annual mortality. CPUE was estimated by dividing the number of fish per hectare caught by the area sampled. Shallow-water surface area for all sampled bays and estuaries was taken from Matlock and Osborn (1982). Because estimates of annual CPUE (gill-net surveys) and annual relative mortality (bag-seine surveys) were based on fish up to age 1, the percentages of recovered hatchery-released fish used in correlation analysis and PCA were based on hatchery fish released in the same year. Spearman's pairwise correlation (ρ) between variables was computed in SPSS; the probability that $\rho=0$ was estimated by assuming that the quantity t , where $t = \rho\sqrt{(n-2)/(1-\rho^2)}$, is distributed approximately as a Student's t distribution with $n-2$ degrees of freedom under the null hypothesis. PCA was used to summarize the variability of the dataset in a lower number of dimensions than the original observations, using uncorrelated linear combinations of the original variables that extracted the highest proportion of the variance present in the dataset (Legendre and Legendre, 1998). PCA was performed on the covariance matrix of the data normalized to a mean of zero and unit variance. Computations were performed in SPSS; input data may be found at <http://agrillife.org/gold/doc/>.

3. Results

A total of 167 hatchery-released fish initially were identified among the 4325 fish sampled in the six periods from fall of 2006 through spring of 2009, with an overall total of 208 hatchery-released fish among 5657 fish over the eight sampling periods for which red drum have been collected (Table 1). Thirty-nine of the initially identified, hatchery-released fish were assigned immediately to a specific dam and sire with no mismatches at any of the 16 microsatellites. The remaining 128 fish had mismatches at one to five microsatellites: one mismatch, 76 fish; two mismatches, 41 fish; three mismatches, seven fish; four mismatches, three fish; and five mismatches, one fish. Re-examination of chromatograms or re-genotyping the 128 fish revealed that all mismatches resulted from incorrect scoring of brood fish (dams, sires, or both) and/or of sampled fish, with the exception of: (i) two individuals in the fall 2007 sample from Lower Laguna Madre – one that possessed a double motif shift at Soc424 and one that possessed a null allele at Soc412; and (ii) one individual in the spring 2008 sample from West Matagorda Bay that possessed a single motif shift at Soc428. These three fish were considered hatchery-released fish. LOD scores of the 167 recovered hatchery-released fish ranged from a high of 24.99 to a low of 13.77, with two exceptions. One fish in the spring 2007 sample in San Antonio Bay had an LOD score of 13.27, while one fish in the fall 2006 sample from the Upper Laguna Madre had a LOD score of 13.4. No mismatches were found in either fish, but three and five microsatellites, respectively, could not be scored satisfactorily. These two fish were considered hatchery-released fish. LOD scores of all presumed wild fish were 13.72 or lower and had five or more confirmed mismatches.

The number (percentage) of recovered hatchery-released fish in each bay or estuary in each sampling period (Table 1) ranged from 0.28% (Galveston Bay, fall 2006) to 17.65% (West Matagorda Bay, spring 2008), with an overall average (\pm SE) of $4.56\% \pm 1.11$. The distribution of recovered hatchery-released fish across sampling periods and bays or estuaries was highly skewed to the right ($G_1 \pm SE = 1.56 \pm 0.55$), with a median value between 1.97% and 2.46%. Exceptionally high percentages (17.65% and 15.48% in the

Table 1

Hatchery-released and wild red drum collected between 2005 and 2009 across nine bays and estuaries along the Texas coast. Percent recovery is total number of hatchery-released fish/total number of 'wild' plus hatchery-released fish. Asterisks identify collections from Karlsson et al. (2008).

System	Sampling period	Hatchery fish	Wild fish	Total	Percent recovery
Sabine Lake	Fall 2007	1	284	285	0.35
	Spring 2008	2	183	185	1.08
Galveston Bay	Fall 2005*	17	203	219	7.76
	Spring 2006*	13	118	132	9.85
	Fall 2006	1	359	360	0.28
	Spring 2007	3	610	613	0.49
East Matagorda Bay	Spring 2009	15	288	303	4.95
West Matagorda Bay	Spring 2008	51	238	289	17.65
	Fall 2008	39	213	252	15.48
San Antonio Bay	Fall 2006	5	396	401	1.25
	Spring 2007	11	547	558	1.97
Aransas Bay	Fall 2005*	5	460	465	1.08
	Spring 2006*	6	510	516	1.12
	Fall 2006	6	298	304	1.97
Corpus Christi Bay	Spring 2009	5	198	203	2.46
Upper Laguna Madre	Fall 2006	6	107	113	5.31
	Spring 2007	8	89	97	8.25
Lower Laguna Madre	Fall 2007	10	198	208	4.81
	Spring 2008	3	75	78	3.85
	Fall 2008	1	75	76	1.32

Table 2

Genetic variability of recovered hatchery-released and wild red drum sampled between 2006 and 2009 and of brood fish from both TPWD hatcheries that gave rise to hatchery cohorts between 2004 and 2007. 95% confidence intervals are in parentheses. A_R , allelic richness, and H_E , gene diversity. A_R was significantly lower (Wilcoxon signed-rank tests) in hatchery-released fish than in both brood fish and wild fish (cf. text).

Origin	Average A_R	Average H_E
Recovered hatchery-released fish	15.8 (4.0–29.7)	0.80 (0.50–0.94)
Wild fish	17.4 (5.1–32.1)	0.81 (0.49–0.95)
Brood fish	17.6 (5.1–33.3)	0.81 (0.51–0.95)

spring and fall of 2008, respectively) were found in West Matagorda Bay. The distribution of recovered hatchery-released and wild fish by bay, cohort, and gill-net station for each sampling period in this study may be found in Appendix Tables A–F (available at <http://agrillife.org/gold/doc/>). Of the 208 hatchery-released fish identified in this study and by Karlsson et al. (2008), 158 were age 0–1, 46 (22.1%) were age 1–2, and four fish (1.9%) were age 2–3.

Hierarchical log-linear analysis of the distribution of hatchery and wild fish among samples revealed a highly significant association between origin and locality ($X^2_{[8]}=96.7$, $P<0.0001$) and between origin and year class ($X^2_{[5]}=50.2$, $P<0.0001$). Genetic variability, measured as allelic richness and gene diversity, among recovered hatchery-released fish, brood fish, and wild fish is presented in Table 2. Average allelic richness ranged from 15.8 (recovered hatchery-released fish) to 17.6 (brood fish); average gene diversity was essentially the same among all three groups. Friedman's rank test indicated significant differences in allelic richness ($X^2_{[2]}=17.20$, $P<0.0001$) but not gene diversity ($X^2_{[2]}=2.95$, $P=0.229$) among recovered hatchery-released fish, brood fish, and wild fish. Wilcoxon signed-rank tests revealed significant pair-wise differences (before and after Bonferroni correction) in allelic richness between wild and recovered hatchery-released fish ($Z=-3.067$, $P=0.002$) and brood fish and recovered hatchery-released fish ($Z=-3.181$, $P=0.001$); the pairwise difference between wild fish and brood fish was non-significant ($Z=-1.363$, $P=0.256$).

Table 3

Hatchery-released red drum (2004–2007 spawning years) recovered between 2006 and 2009, possible number of contributing dams, sires, and dam × sire combinations (both hatcheries) for each spawning year, and observed and expected dams, sires, and dam × sire combinations contributing to the recovered hatchery-released fish. Asterisks indicate values significantly lower than expected, based on the estimated lower (0.025) percentile.

	Hatchery-releases			
	2004	2005	2006	2007
Number recovered	24	17	100	26
	Possible brood stock			
	2004	2005	2006	2007
Dams	107	91	81	84
Sires	74	64	60	56
Dam × sire combinations	216	174	124	117
	Observed contribution			
	2004	2005	2006	2007
Dams	19	10*	33*	20*
Sires	19	9*	24*	18*
Dam × sire combinations	22	11*	37*	22
	Expected contribution			
	2004	2005	2006	2007
Dams	21.6 (19–24)	15.6 (14–17)	50.8 (46–55)	24.0 (21–27)
Sires	20.6 (18–23)	15.0 (13–17)	38.1 (34–42)	22.2 (19–25)
Dam × sire combinations	22.8 (21–24)	16.2 (14–17)	68.8 (63–74)	25.0 (22–28)

The number of recovered hatchery-released fish from the 2004–2007 year classes is presented in Table 3, along with (i) the total number of possible dams, sires, and dam × sire combinations (both hatcheries combined) for each year class, (ii) the observed number of dams, sires, and dam × sire combinations that contributed to the 167 recovered hatchery-released fish, and (iii) the average number of hatchery dams, sires, and dam × sire combinations that would be expected to contribute at random to a sample equal to the number of recovered hatchery-released fish for each cohort. Based on the estimated upper (0.975) and lower (0.025) percentiles, the contribution of dams, sires, and dam × sire combinations to recovered hatchery-released fish released in 2004 did not differ significantly from that expected under randomly distributed reproductive success. The contribution of dams, sires, and dam × sire combinations to recovered hatchery-released fish released in 2005 and 2006, however, was non-random, with fewer of each contributing relative to that expected; in 2007, fewer dams and sires contributed to the 2007 cohort than was expected.

The spatial distributions of recovered hatchery-released and wild fish with respect to stations (Table 4) deviated from random expectations for Upper Laguna Madre in the fall 2006 ($P=0.018$) and spring 2007 ($P=0.010$) samples and for West Matagorda Bay in the spring 2008 ($P=0.0001$) and fall 2008 ($P=0.044$) samples. As examples, during the spring 2008 sampling in West Matagorda Bay, four fish of hatchery origin and five fish of wild origin were sampled at Station 85; whereas zero fish of hatchery origin and 33 fish of wild origin were sampled at Station 498. Similarly, two fish of hatchery origin and three fish of wild origin were sampled at Station 202; whereas zero fish of hatchery origin and 20 fish of wild origin were sampled at Station 496.

Estimates of ρ (rho, Spearman's rank-order correlation) and the probability that $\rho=0$ for pairwise comparisons of the variables percent recovery of recovered hatchery-released fish, the number of fish released per hectare, annual CPUE, annual relative mortality, and shallow-water surface area are given in Table 5. A significant, positive correlation was found between annual CPUE and annual relative mortality ($\rho=0.606$, $P=0.004$). No significant relationship was found between the number of fish released per hectare and the percent of recovery of hatchery releases ($\rho=0.063$, $P=0.786$).

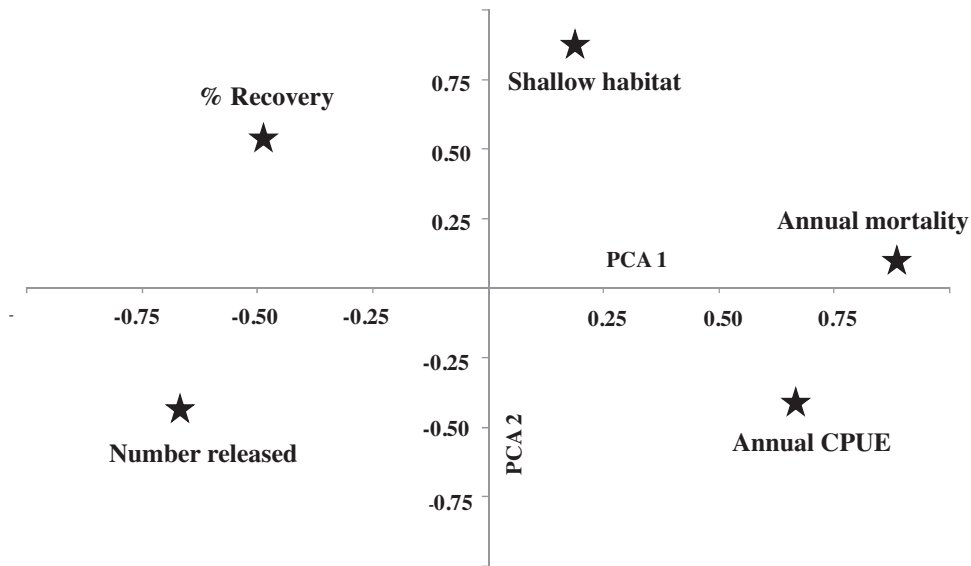


Fig. 2. Projection of studied variables (percent recovery of recovered hatchery-released fish, number of fish released per hectare, annual CPUE, annual relative mortality, and shallow-water surface area) on principal component 1s and 2 (PCA 1 and 2). PCA 1 and 2 are orthogonal linear functions of the original variables and extract 39 and 29%, respectively, of the total variation present in the dataset.

Table 4
Spatial distribution of recovered hatchery-released and wild fish in relation to collection stations within each bay. Significant values (in bold) indicate a non-random distribution of hatchery-released and wild red drum.

System	Sampling period	P-value
Sabine Lake	Fall 2007	1.000
	Spring 2008	0.653
Galveston Bay	Fall 2006	1.000
	Spring 2007	0.384
East Matagorda Bay	Spring 2009	0.162
West Matagorda Bay	Spring 2008	<0.001
	Fall 2008	0.044
San Antonio Bay	Fall 2006	0.194
	Spring 2007	0.247
Aransas Bay	Fall 2006	0.260
Corpus Christi Bay	Spring 2009	0.0528
Upper Laguna Madre	Fall 2006	0.018
	Spring 2007	0.010
Lower Laguna Madre	Fall 2007	0.660
	Spring 2008	0.091
	Fall 2008	0.789

Table 5
Estimates of Spearman’s rank-order correlation rho (ρ) between pairs of variables. P is the probability that $\rho=0$ (two-tailed test).

	# Released hatchery fish	Annual CPUE	Annual mortality	Shallow-water surface area
% recovered hatchery fish	$\rho=0.063$ $P=0.786$	$\rho=-0.148$ $P=0.522$	$\rho=-0.180$ $P=0.435$	$\rho=0.248$ $P=0.278$
# released hatchery fish		$\rho=-0.154$ $P=0.505$	$\rho=-0.416$ $P=0.061$	$\rho=-0.291$ $P=0.200$
Annual CPUE			$\rho=0.606$ $P=0.004$	$\rho=-0.186$ $P=0.420$
Annual mortality				$\rho=-0.031$ $P=0.893$

Interestingly, the correlation of available shallow-water surface area, reflecting availability of more optimal habitat for small, juvenile fish (Stunz et al., 2002), was positive in the comparison with the percent of recovered hatchery-released fish but negative in

comparisons with the number of released hatchery fish, annual CPUE, and annual relative mortality. Results from PCA (Fig. 2) essentially paralleled those from correlation analysis. The eigenvalue and percentage of variance explained by each of five principal components extracted during principal component analysis and the loading of each original variable on the five principal components may be found at <http://agriflife.org/gold/doc/>. The first two principal axes of the PCA accounted for 68% of the total variance of the dataset. Axis 1 (39% of the overall variance) opposed annual CPUE and relative annual mortality to the % recovery and the number released, while the second axis (29% of the total variance) opposed shallow-water surface area and % recovery to the number released and annual CPUE.

4. Discussion

Release of hatchery-raised juveniles to augment the wild population and optimize harvests of commercially or recreationally exploited marine species has increased substantially over the past decade as one of the approaches whereby depleted stocks of fisheries worldwide can be restored and the global demand for marine-fishery products can be met (Bell et al., 2008). Studies on large-scale, stock-enhancement of marine fish over multiple generations, however, are exceedingly sparse and limited primarily to studies of red sea bream (*Pagrus major*) and Pacific herring (*Clupea pallasii*) in Japanese waters (Kitada et al., 2009) where recovery percentages (hatchery-raised/wild fish) over 10–15 year periods ranged from $2.5 \pm 1.8\%$ to $41.2 \pm 26.8\%$ (red sea bream) and from 17.1 ± 12.2 to $28.9 \pm 26.8\%$ (Pacific herring). The large variance in recovery percentages indicates both spatial and temporal heterogeneity in recovery of hatchery-raised fish, consistent with findings in stock enhancement of non-fisheries (Hamasaki and Kitada, 2008).

Results from our studies of red drum (Karlsson et al., 2008; this study) revealed significant differences in the recovery percentage of recovered hatchery-released red drum among different bays or estuaries as well as among sampling periods within the same bay or estuary. The majority (158 of 208 total) of recovered hatchery-released fish were age 0–1; the remainder (50 fish) were either age 1–2 or age 2–3. The frequency of recovered hatchery-released red drum beyond age 0–1 in Texas bays and estuaries may well

be greater as sampling was restricted to fish 500 mm (TL) or less in part to maximize recovery of age 0–1 fish. That recovered hatchery-released red drum survive for at least two or more years raises the issue of whether recovered hatchery-released fish are spawning successfully, given that red drum in Texas waters reach sexual maturity between the third and fourth year (<http://www.tpwd.state.tx.us/huntwild/wild/species/reddrum/>). Finally, as noted in [Karlsson et al. \(2008\)](#), if fish released in late spring grew larger than 500 mm (TL) before sampling in the fall, these individuals would have been excluded from our analysis. Both TPWD hatcheries begin to release fish in mid- to late-May, and simulations of growth rate based on a physiological model (W. Neill and R. Vega, unpublished) do suggest that some of these fish could have exceeded 500 mm (TL) by late fall.

Variable recovery rates of hatchery-released fish are hypothesized to reflect a number of factors: (i) physiological condition of released fish, (ii) time and season of release, (iii) size and/or density at release, (iv) tolerance to handling and transport stress, (v) variable water temperature and salinity at release sites, (vi) differences among released fish in social behavior and/or energy efficiency, (vii) variable presence of predators, and (viii) suitability of particular bay or estuarine systems to successful enhancement ([Leber and Arce, 1996](#); [Munro and Bell, 1997](#); [Kellison et al., 2003](#); [Kitada and Kishino, 2006](#); [Loneragan et al., 2006](#)). Seasonal and annual variation in temperature, salinity, dissolved oxygen, and turbidity are documented among Texas bays and estuaries (<http://www.twdb.texas.gov/surfacewater/conditions/index.asp>) and would be expected to impact survival of hatchery releases. There was suggestive evidence in the study by [Karlsson et al. \(2008\)](#) that red drum released in the fall exhibited greater survival. The evidence was based on a rare instance when fingerlings from both TPWD hatcheries were released into the same bay or estuary. Barring this type of situation, experimentally assessing rigorously possible factors impacting survival of hatchery-released red drum in large-scale, ongoing programs such as carried out in Texas will be problematic, if only for logistical reasons.

In this study and in the prior study by [Karlsson et al. \(2008\)](#), allelic richness was significantly less in recovered hatchery-released fish than in either brood fish or wild fish. No significant difference in this parameters between hatchery brood fish and wild fish was found in either study, suggesting that the lower genetic variation observed in recovered hatchery-released fish could stem from non-random survival prior to or following release, a low number of breeders actually contributing to individual releases, or both. [Kitada et al. \(2009\)](#) suggested that the loss of rare alleles and reduced allelic variation observed in recovered hatchery-released versus wild red sea bream stemmed from the random genetic drift expected when a small number of hatchery brood fish are used repeatedly over successive generations. Red drum brood fish at each of the two TPWD hatcheries generally comprise around 60 dams and 40 sires, and roughly 25% of the brood fish (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](#)). However, because of the large size of adult red drum, each spawning tank contains only five-six fish (generally three dams and two-three sires), and studies ([Gold et al., 2008, 2010](#)) on the spawning efficiency of red drum brood fish at TPWD hatcheries have shown that not all fish in a spawning tank participate in a spawning event and, moreover, that there are considerable differences in the productivity of individual brood fish in different spawning tanks. In this study, the number of dams, sires, and dam \times sire combinations that contributed to hatchery releases in 2005 and 2006, and the number of dams and sires that contributed to hatchery releases in 2007, were significantly less than expected if reproductive success was randomly distributed among brood fish, consistent with the effects

of random genetic drift due to the limited number of spawning brood fish in the hatcheries as compared to the wild. A small study is now being carried out by TPWD to assess whether non-random survival occurs during grow-out prior to release. Finally, [Karlsson et al. \(2008\)](#) found that the spatial distribution of recovered hatchery-released and wild red drum with respect to sampling stations (localities) within the two bays they sampled were non-random. The spatial distributions of recovered hatchery-released and wild fish in this study deviated significantly from random expectations in the spring and fall 2008 samples from West Matagorda Bay and in the fall 2006 and spring 2007 samples from the Upper Laguna Madre. We interpret these results as indicating that individuals from the same hatchery-released population may often maintain close proximity to one another following release.

We used correlation analysis and principal components analysis to examine possible relationships between recovered hatchery-released fish, the number of hatchery fish released, annual average CPUE (based on gill-net surveys), relative annual mortality (based on bag-seine surveys), and shallow-water surface area. All input data were from individual years; for recovered hatchery-released fish, we used individual cohorts (year classes) regardless of the year caught. A significant, positive correlation was found only between annual average CPUE and relative annual mortality, suggesting that high recruitment is accompanied by high mortality, a situation known as density-dependent mortality or density dependence ([Hazlerigg et al., 2012](#)) and which has been observed in other fish species ([Anderson, 2001](#); [Hixon and Jones, 2005](#)). No relationship was observed between the number of fish released per hectare and the percentage of recovered hatchery-released fish. There were negative correlations between available shallow-water surface area, reflecting suitable habitat for small, juvenile fish ([Stunz et al., 2002](#)), and number of fish released per hectare, annual CPUE, and annual relative mortality. We interpret these to simply represent greater density of juvenile fish in patches of more optimal habitat. Principal components analysis essentially paralleled correlation analysis.

Studies of red drum stock enhancement in Florida have documented similar percentages of recovered hatchery-released fish. [Willis et al. \(1995\)](#) identified eight recovered hatchery-released fish (~2%) among 395 fish sampled from Mosquito Lagoon on the east coast of Florida, while [Tringali et al. \(2008\)](#) identified 282 recovered hatchery-released fish (~2.8%) among nearly 10,000 fish sampled in Tampa Bay and neighboring waters on the west coast of Florida. Considerably higher recovery percentages have been reported from long-term studies carried out in South Carolina. [Jenkins et al. \(2004\)](#) reported recovery rates of recovered hatchery-released fish of 19 and 19.4% from two cohorts released into Callawassie Creek (Port Royal Sound estuary), while [Denson et al. \(2012\)](#) reported a range of 28.4–88.9% from three hatchery cohorts (1999–2001) released into the Ashley River, a tributary that empties into Charleston Harbor. Lower recovery rates (0.0–14.6%) in the same study were found for hatchery cohorts released into the Wando River, another tributary that empties into Charleston Harbor. Significantly, the annual hatchery contribution to Charleston Harbor in 1 year (1999) was as high as 51.2%. [Denson et al. \(2012\)](#) did note that larger fingerlings (61–64 mm total length [TL]) released earlier were recovered at rates almost 10 times as high as smaller fingerlings (22–27 mm TL) released later in the season. This supported previous studies ([Leber, 1995](#); [Willis et al., 1995](#); [Hervas et al., 2010](#)) that documented higher survival of larger hatchery-released fish. However, as pointed out by [Denson et al. \(2012\)](#) it was not possible to determine which fish from a wide size distribution actually survived, and moreover that recovery rates in 1999 of smaller-sized releases still ranged from 10% to 82.4%.

Because of the number of variables hypothesized to affect recovery rates of hatchery-released fish (see above), it is difficult to assess

why recovery rates of hatchery-released red drum are so high in South Carolina compared to those documented in Texas (Karlsson et al., 2008; this study) and Florida (Willis et al., 1995; Tringali et al., 2008). Denson et al. (2012) found that the highest percentage of recovered hatchery releases were in years where there was lower natural recruitment, while lower recovery rates occurred in years with higher natural recruitment. Such a relationship was not found in our study. One possibility to account at least in part for the difference in recovery rates might be differences in the total area surrounding the stocking sites. The sites in South Carolina (in the Ashley and Wando rivers) were described as small creeks adjacent to high-marsh, mud flats, and oyster reefs (Denson et al., 2012); releases of red drum in Texas, alternatively, generally occur directly into bays or estuaries either from shore or from stocking boats (R. Vega, pers. comm.). The range in size across the nine bays or estuaries in Texas supplemented with red drum is considerable, from ~153 km² (East Matagorda Bay) to ~1600 km² (Galveston Bay). Even considering just available, shallow-water habitat, the habitable fingerling area in Galveston Bay (~418 km²) would still be considerably greater than the total available area (based on data in Denson et al., 2012) in either the Ashley River (~9.8 km²) or the Wando River (~28 km²). There also is a possibility that differences in the number or proximity of stocking sites may impact recovery rates. The number of stocking sites in each of the two rivers in the study by Denson et al. (2012) was not indicated but may have been few; given the total area of the two rivers, different stocking sites also may not have been far from one another. In Texas, roughly 11.5 sites are stocked annually in each of the nine bays or estuaries, with individual stocking sites spread variously about each bay or estuary (e.g., Fig. 2 in Karlsson et al., 2008). The number and proximity of stocking sites could inversely impact recovery rates in that the studies in South Carolina (Jenkins et al., 2004; Denson et al., 2012) found high concentrations of recovered hatchery-released fish close to release sites. In our studies (Karlsson et al., 2008; this study) we have not necessarily found higher concentrations of recovered hatchery-released fish near stocking sites. Karlsson et al. (2008, Fig. 2), however, did find a relatively large number of recovered hatchery-released fish near two stocking sites in Galveston Bay that were located in the small, northwest arm of the bay where a relatively small inlet allows access into a succession of small bays. These observations may indicate that higher recovery rates are associated with release sites located in relatively confined areas. Possibly related to this may be the differences in the number of fish sampled from a given area. Denson et al. (2012) sampled an average of 69 and 62 fish each year over a three-year period from the Ashley and Wando rivers, respectively; whereas in our two studies, an average of ~283 fish were sampled during each sampling period (spring and fall) in each bay or estuary. If recovered hatchery-released fish tend to remain close to release sites and only a few fish are sampled from few sites, the proportion of hatchery releases recovered could be biased upward. Finally, there do appear to be large differences in stocking density between releases in South Carolina and those in Texas. Average stocking densities over the three-year period in the Ashley and Wando rivers were 661 ha⁻¹ and 180 ha⁻¹, respectively (Denson et al., 2012). In Texas, and just considering available, shallow-water habitat, the average stocking density per bay or estuary over the period of our two studies was 110 ha⁻¹. Comparing the results reported in the studies from South Carolina with those from Texas, there does appear to be a coarse correlation between stocking density and recovery of hatchery-released fish and a coarse, inverse correlation between recovery percentage and the size of the area where fish are released.

Implications of our studies relative to stock enhancement of other marine species are difficult to assess, in part because red drum is a warm-water, estuarine-dependent fish with a unique life history, in part because the fish is stocked in large

numbers into comparatively large bays and estuaries, and in part because the TPWD stock-enhancement program has been ongoing for decades and spawning, larviculture, and release procedures are well established (Vega et al., 2011). The only comparable, long-term stock-enhancement programs that have been studied are those in Japan on red sea bream and Pacific herring (Kitada et al., 2009). Based on the work in Japan and on red drum in Texas, the take-home message appears to be that stocked fish often survive in relatively large numbers and there is a large variance in survival across stocking sites and time. On the surface this suggests that minimal needs for successful stock enhancement include a stable, financial support system, reliable background information of the species to be restored, firmly established spawning, rearing, and release strategies, and a highly competent staff to carry out the work. Clearly, many variables impact survival of stocked fish, but assessing these experimentally in a large-scale, ongoing stock-enhancement program will be problematic. Additional studies of stock enhancement involving large, open areas such as found along the Texas coast are needed as are studies to ask whether stocked fish contribute to spawning populations.

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