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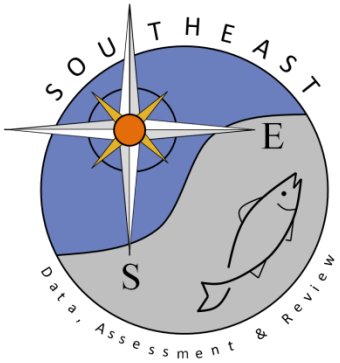
Eric Saillant, S. Coleen Bradfield, and John R. Gold

SEDAR74-RD65

March 2021



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Article *in* ICES Journal of Marine Science · August 2010

DOI: 10.1093/icesjms/fsq011

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Saillant, E., Bradfield, S. C., and Gold, J. R. 2010. Genetic variation and spatial autocorrelation among young-of-the-year red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. – ICES Journal of Marine Science, 67: 1240–1250.

Temporal and spatial genetic variations at 18 nuclear-encoded microsatellites were assayed among age-0 Gulf red snapper (*Lutjanus campechanus*), sampled from the 2004 and 2005 cohorts in each of five regions in the northern Gulf of Mexico (Gulf) and from a mixed-age group sampled off northwest Florida. Hierarchical analysis of molecular variance revealed genetic heterogeneity among habitat patches within regions, but not among regions. A significant, positive spatial autocorrelation of microsatellite genotypes among fish sampled within the geographic range 50–100 km was detected. Bayesian coalescent analysis of historical demography indicated a decline of nearly an order of magnitude in the effective population size for red snapper across the area surveyed. The highest posterior probability for the current effective population size was 2163, approximately four orders of magnitude smaller than the estimates of red snapper census size. The results of the study demonstrate that spatial genetic structuring among young-of-the-year red snapper in the Gulf occurs at small geographic scales and is consistent with a metapopulation stock-structure model of partially connected populations. This accentuates the importance of maintaining healthy local spawning populations of red snapper in all regions across the northern Gulf.

Keywords: effective population size, population structure, red snapper, spatial autocorrelation.

Received 16 June 2009; accepted 30 January 2010; advance access publication 4 March 2010.

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Introduction

The Gulf red snapper (*Lutjanus campechanus*) is a reef fish of considerable economic importance in the southern United States. Annual landings of the US commercial fishery between 2003 and 2007 averaged ~4.1 million pounds, an estimated dockside value of more than US $11 million (http://www.st.nmfs.noaa.gov/st1/commercial/landings/annual_landings.html); annual landings of the US recreational fishery over the same period averaged ~3.7 million pounds (http://www.st.nmfs.noaa.gov/st1/recreational/queries/catch/time_series.html). Fishing for red snapper also contributes significantly to the ~$1.8 billion in annual expenditure and >56 000 fulltime jobs in coastal communities associated with recreational fishing in US waters of the Gulf of Mexico (Adams *et al.*, 2004). The abundance of red snapper in the northern Gulf of Mexico (hereafter Gulf), however, decreased by an estimated 90% between the 1970s and the early 1990s (Goodyear and Phares, 1990), at which time the fishery was supported primarily by fish aged 1–3 years (SEDAR 7, 2004). Principle factors thought to be involved in the decline in red snapper include overfishing by directed fisheries, habitat alteration and degradation, and mortality of juveniles taken as bycatch during trawling for shrimps (Christman, 1997; Schirripa and Legault, 1997; Gallaway *et al.*, 1998).

Despite intensive management since the early 1990s, red snapper remain overfished and undergoing overfishing (SEDAR 7, 2004). The assessment of population structure and demographic dynamics is a central issue for developing rebuilding plans and for the management of fishery resources. Knowledge of population structure is essential, because life history, demographic, and genetic characteristics may differ among constituent subpopulations; failure to recognize underlying population structure within a fishery may lead to overexploitation and depletion of localized subpopulations and to loss of unique genetic resources inherent in those subpopulations (Carvalho and Hauser, 1994; Begg *et al.*, 1999; Hilborn *et al.*, 2003). The loss of such genetic resources potentially compromises long-term sustainability (Hilborn *et al.*, 2003). In addition, for fisheries undergoing rebuilding programmes, differential rebuilding of non-identified subpopulations can lead to an inability to anticipate future recruitment in those non-identified units (Ruzzante *et al.*, 1999).

The possible presence of multiple stocks of red snapper in US waters has been examined extensively during the past two decades through studies of life history, demography, and genetic variation. Genetic studies (Gold *et al.*, 1997, 2001; Pruett *et al.*, 2005; Saillant and Gold, 2006) generally have indicated homogeneity in the distribution of both nuclear and mitochondrial genetic

variants across the northern Gulf. Alternatively, studies of life history (Woods *et al.*, 2003; Fischer *et al.*, 2004) and of genetic effective population size or N_e (Saillant and Gold, 2006) have indicated significant differences among geographic regions. These findings, along with the occurrence of both geological and habitat differences (Rezak *et al.*, 1985; Gallaway *et al.*, 1998) and differences in landings and fishing effort across the northern Gulf (SEDAR 7, 2004), have led to the definition of eastern and western "stocks" on either side of the Mississippi River (SEDAR 7, 2004). Further subdivision of the western stock has been suggested based on the differences in growth parameters (Fischer *et al.*, 2004) and estimates of N_e (Saillant and Gold, 2006) between red snapper sampled off the coasts of Louisiana and Texas.

Recent studies based on tagging and otolith microchemistry indicate that the magnitude and spatial scale of red snapper (larval) dispersal and (adult) movement remain unclear (Patterson *et al.*, 2001, 2008). Relative site fidelity of adult red snapper has been demonstrated in several studies (Fable, 1980; Szedlmayer and Shipp, 1994; Szedlmayer, 1997), and studies by Workman and Watson (1995), Szedlmayer and Conti (1999), and Workman *et al.* (2002) have shown that juvenile red snapper exhibit both attraction and homing to structure following settlement. Based on the observations of relative site fidelity, Saillant and Gold (2006) pointed out that gene flow in red snapper across the continental shelf should be more-or-less linear regardless of the life-history stage at which movement occurs and, moreover, should follow a pattern of isolation by distance where fish from proximal localities are more similar genetically than fish from more distal localities. To date, however, no such correlation has been documented (Gold *et al.*, 1997, 2001; Saillant and Gold, 2006). We hypothesize that gene flow might be restricted, yet red snapper populations might not be in equilibrium, leading to the absence of genetic divergence among regions within the northern Gulf. If that is the case, temporary stable demographic assemblages might exist, and genetic structure on a small spatial scale might be detected at the level of a single cohort.

Here, we used nuclear-encoded microsatellites to examine temporal and spatial genetic variation among young-of-the-year (age-0) red snapper at five regions across the northern Gulf. We sampled multiple habitat patches, using a randomized design within each region to assess spatial genetic variation at both micro- (within region) and macro- (across regions) geographic scales. Sampling at the micro-geographic scale allowed us to assess restricted gene flow within cohorts of red snapper at smaller spatial scales than previously studied. Another question of interest for the management and conservation of the red snapper is whether 30 years of overfishing have led to significant erosion of genetic diversity. A recent study based on sequences of a mitochondrial (mt) DNA-coding gene revealed an increase in N_e of Gulf red snapper following glacial retreat (Pruett *et al.*, 2005). The data, however, were uninformative relative to the recent reduction in population size considering the star-like phylogeny of haplotypes revealed by the mitochondrial marker. Microsatellites markers, on the other hand, are characterized by higher mutation rates and therefore are more appropriate for studying recent demographic history. We employed a Bayesian coalescent approach to assess recent changes in N_e of red snapper across the northern Gulf region based on the microsatellite dataset.

Material and methods

Young-of-the-year red snapper were sampled in 2004 and 2005 during "fall" (autumn) groundfish surveys by the National Marine Fisheries Service (NMFS). Sampling was conducted by trawling on benthic habitats offshore from Brownsville (Texas), Port Aransas (Texas), Freeport (Texas), along the west coast of Louisiana, and along the coastline of the Mississippi–Alabama border (Figure 1). Only red snapper <125 mm total length were selected for genetic analysis to ensure that fish were young-of-the-year (age 0; Patterson *et al.*, 2005; Wells *et al.*, 2008). We restricted sampling to fish aged 0 to examine temporal stability of spatial genetic structure. Sample sizes for the two cohorts (2004/2005) in each region were 110/100 (Brownsville), 105/103 (Port Aransas), 103/103 (Freeport), 103/102 (Louisiana), and 104/110 (Mississippi–Alabama). Samples were obtained from multiple tows in each region. The average length ($\pm$s.e.) of a tow was $\sim 3.27 \pm 0.06$ km, and the numbers of tows per region varied between 2 and 22 (average 10.2). The average distance between tows within regions was $\sim$52 km, and the number of red snapper sampled per tow averaged 10.2 (range 1–53). A sample of 85 juvenile red snapper was obtained as bycatch from a shrimp trawler offshore from northwest (NW) Florida in summer 2004. The fish in the shrimp trawl were of mixed age, so that sample was used only in inferences on spatial genetic variation among regions (see below).

Fin clips and small pieces of muscle tissue were removed from each fish sampled during the groundfish surveys and fixed immediately in 95% ethanol. Fish obtained as bycatch from shrimp trawling were kept frozen after capture until small pieces of muscle tissue could be removed; the tissues were then fixed in 95% ethanol. All tissue samples ultimately were transported to College Station and maintained at ambient room temperature until DNA extraction. All fish were assayed for allelic variation at 18 nuclear-encoded microsatellites. Details regarding DNA extraction, multiplex PCR amplification and electrophoresis, sizing of fragments, and allele calling may be found in Renshaw *et al.* (2006). Descriptions of the PCR primers may be found in Bagley and Geller (1998) and Gold *et al.* (2001).

Summary statistics, including number of alleles, allelic richness, and unbiased gene diversity (expected heterozygosity), for each microsatellite in each of the 11 samples, i.e. two cohorts in five regions plus the sample from the northwest coast of Florida, were obtained using F-STAT (Goudet, 1995; v. 2.9.3, http://www2.unil.ch/popgen/softwares/fstat.htm). Homogeneity of allelic richness and gene diversity among samples was tested via the Friedman rank tests as implemented in PROC FREQ of SAS® (SAS Institute, Cary, NC, USA). Departure of genotypic proportions from Hardy–Weinberg (HW) equilibrium expectations in each sample was measured as Weir and Cockerham's (1984) f. The probability that f differed significantly from zero (P_{HW}) was estimated using a Markov-chain method (Guo and Thompson, 1992), as implemented in GENEPOP (Raymond and Rousset, 1995; v. 4.0.7, http://kimura.univ-montp2.fr/~rousset/Genepop.htm). Markov-chain parameters employed in estimation were 5000 dememorizations, 500 batches, and 5000 iterations per batch. Genotypic disequilibrium between pairs of microsatellites within samples was tested by exact tests, as implemented in GENEPOP and employing the same Markov-chain parameters as above. Sequential Bonferroni correction (Rice, 1989) was applied for all multiple tests performed simultaneously. Possible occurrence of

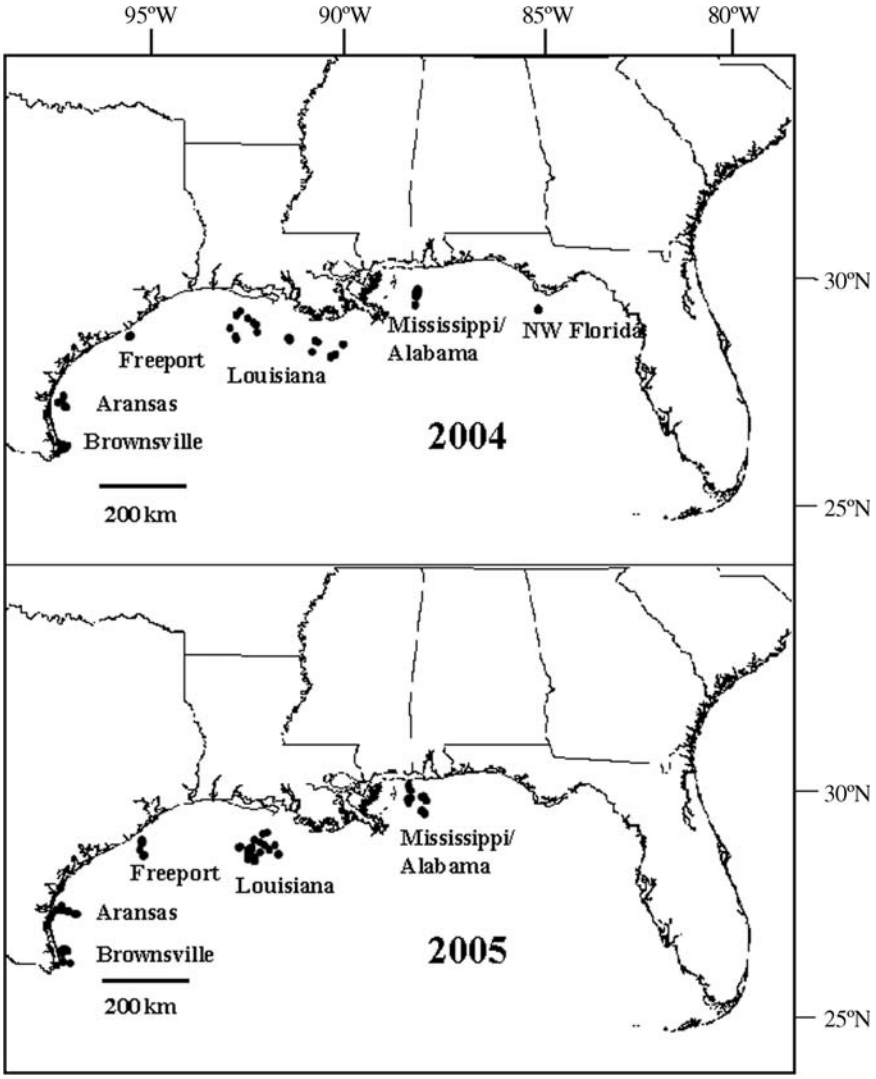

Figure 1. Sample localities for red snappers (*Lutjanus campechanus*) in the northern Gulf of Mexico; dots indicate the locations of individual sampling tows.

stuttering, large allele dropout, and/or null alleles was evaluated for each microsatellite in each sample using MICROCHECKER (van Oosterhout *et al.*, 2004).

The homogeneity of allele and genotype distributions among the 11 samples was tested using exact tests; significance of probability values was assessed by a Markov-chain method, as implemented in GENEPOP and using the same Markov-chain parameters as above. The degree of divergence between pairs of samples was estimated as Weir and Cockerham's (1984) θ, as implemented in F-STAT. Sequential Bonferroni correction (Rice, 1989) was applied for all multiple tests performed simultaneously. The analysis of molecular variance or AMOVA (Excoffier *et al.*, 1992), as implemented in ARLEQUIN (Excoffier *et al.*, 2006; v. 3.11, http://cmpg.unibe.ch/software/arlequin3/), was used to partition molecular variance according to two hierarchical models; Model 1 examined variance components attributable to region and to cohorts within regions, whereas Model 2 examined variance components attributable to region and among tows within regions. The sample of mixed-age fish from NW Florida was included only in the analysis under Model 1. The significance of variance components and their associated fixation indices was assessed with 10 000 permutations, as implemented in ARLEQUIN.

The possible correlation between genetic and geographic distances, i.e. variation according to an isolation-by-distance pattern of population structure, was tested using a Mantel test (Mantel, 1967; Smouse *et al.*, 1986) as implemented in GENEPOP. Initially, Mantel tests were implemented accounting for the five regions surveyed. A second analysis of the dataset was then conducted accounting for individual sampling tows. The quantity $F_{ST}/(1 - F_{ST})$ was used as a genetic distance according to Rousset (1997). Geographic distances between tows were determined from GPS coordinates (longitude and latitude) recorded during trawling. Coordinates were averaged over tows within a region to calculate the geographic distances among regions. In all tests, the significance of the correlation was assessed using 10 000 random permutations.

Spatial genetic variation was assessed further via spatial autocorrelation analysis (Smouse and Peakall, 1999), as implemented in GenAlEx (Peakall and Smouse 2006, v. 6.0, http://www.anu.edu.au/BoZo/GenAlEx/). The spatial autocorrelation coefficient

(r) was computed using the geographic distance between individual trawl tows, determined as above, and the multilocus genetic distance outlined in Smouse and Peakall (1999). Under isolation by distance, estimated values of r differ significantly from zero for geographically proximal samples, and decrease with increasing geographic distance between samples. The distance between samples at which r no longer differs significantly from zero provides an approximation of the distance at which population structure can be detected (Peakall *et al*., 2003). As the estimation of spatial autocorrelation is influenced by the size of the distance class (Peakall *et al*., 2003), r was computed based on a series of increasing distances between sampling tows. Distance-class sizes were determined based on the observation of the distribution of pairwise geographic distances between sampling tows; the distance classes used were 1, 50, 100, 150, 250, 350, 450, 550, 650, and 750 km. The significance of r was determined via random permutations of the genotypes of individuals sampled in single tows. The distribution of r values under the null hypothesis of random spatial distribution of genotypes was used to determine the probability of significance of the observed values of r according to a one-tailed test, i.e. r is significant if it lies beyond the upper 95% bound of the distribution. The significance of r was tested also by generating bootstrap 95% confidence intervals (*CI*s) for r. Bootstrap values were obtained by sampling with replacement pairs of sampling tows within a given distance class. Bootstrap resampling was performed 1000 times, and the significance of r was inferred when the 95% *CI* did not overlap zero. Initial GenAlEx runs by cohort revealed consistent patterns of autocorrelation in each cohort; a combined analysis of the two cohort datasets was then implemented to maximize the power of inference in estimating r at different spatial scales, as described in Peakall *et al*. (2003).

The Bayesian coalescent approach developed by Beaumont (1999) was used to infer the demographic history of red snapper across the area sampled. The model employed assumed a population changing exponentially in size from an initial (historical) effective size to a current (contemporaneous) effective size. The demographic parameters estimated are current (N_0) and historical/initial (N_1) effective sizes, average mutation rate (μ) across loci per generation, and time (t_a) in generations since the beginning of the expansion or decline phase. The ratio of N_0/N_1 is <1 in a declining population and >1 in an expanding population. The posterior distributions of the genealogical (mutational and coalescent events) and demographic (initial and final effective population size, time since expansion/decline) parameters were estimated using a Monte Carlo–Markov-chain (MCMC) approach, as implemented in MSVAR v. 1.3 (http://www.rubic.rdg.ac.uk/~mab/stuff/). Computations were performed initially on three random subsamples of 100 chromosomes from Brownsville, Galveston, and Mississippi, using the program SINF (included in the MSVAR package). For each dataset, computations were replicated three times, using different starting parameters, to assess convergence of the MCMC. Posterior distributions for each run were estimated based on 2.5×10^9 steps; the first 5×10^8 steps in each run were discarded as burn-in. All runs gave consistent posterior distributions for the estimated parameters and were therefore combined to derive final summary statistics of each parameter's posterior distribution. The parameters of the prior distributions for N_0, N_1, and t_a were set as described in Saillant *et al*. (2004). An average generation time of 6.3 years, estimated as described in Jorde and Ryman [1996; their Equation (10)], was considered based on life-history data available for red snapper. Considering the stepwise mutation model assumed in MSVAR, the complex microsatellite *Lca*43 was discarded from the analysis of demographic history.

Results

Genotypes at the 18 microsatellites for each fish assayed in each of 11 samples (two cohorts in each of the five regions and the shrimp-trawl sample from NW Florida) are available at http://wfsc.tamu.edu/doc/ under the file name "Red Snapper 2004–2005 Genotypes". Summary statistics for each microsatellite in each sample are given in Appendix Tables A1 and A2. The number of alleles detected ranged from three at *Lca*20 in the 2004 cohort from Mississippi–Alabama to 21 at *Prs*248 in the 2004 cohort from Aransas and the 2005 cohort from Freeport. Allelic richness ranged from 3.0 at *Lca*20 in the 2004 cohort from Mississippi–Alabama to 19.6 at *Prs*240 in the 2004 cohort from Freeport. Unbiased gene diversity (expected heterozygosity) ranged from 0.112 at *Prs55* in the 2004 cohort from Freeport to 0.920 at *Prs*240 in the 2004 cohort from Louisiana. Neither allelic richness nor gene diversity differed significantly among samples ($Q = 10.6$, d.f. $= 10$, $p = 0.39$; and $Q = 6.87$, d.f. $= 10$, $p = 0.74$, respectively).

Significant departure from HW equilibrium expectations was found in 32 tests before Bonferroni correction. Only four tests (2% of the total number of tests) remained significant following Bonferroni correction: *Prs*221 (2004 cohort from NW Florida and 2005 cohort from Freeport), *Prs*303 (2005 cohort from Freeport), and *Lca*91 (2005 cohort from Mississippi–Alabama). Estimates of F_{IS} among samples ranged from -0.032 (2005 cohort from Mississippi–Alabama) to 0.045 (2004 cohort from Brownsville). F_{IS} estimates for the 2005 cohort from Freeport, the 2004 cohort from NW Florida, and the 2004 cohort from Louisiana were 0.021, 0.024, and 0.028, respectively, and differed significantly from zero after Bonferroni correction. All remaining F_{IS} estimates per sample did not differ significantly from zero after Bonferroni correction. Analysis using MICROCHECKER indicated possible occurrence of null alleles only at *Prs*221 and *Prs*303 in the 2005 cohort from Freeport. There was no indication of stuttering or large allele dropout affecting scoring at these two microsatellites in other samples. Only one of 1683 tests of linkage disequilibrium (*Prs*137 and *Prs*240 in the 2004 cohort from Mississippi–Alabama) was significant following Bonferroni correction. Based on these results, all 18 microsatellites were used in subsequent analyses.

Exact tests over all microsatellites revealed significant heterogeneity among the 11 samples in both allele and genotype distributions ($p < 0.0001$, allele distributions; $p = 0.0004$, genotype distributions). Of 55 exact tests over all microsatellites between pairs of samples, 13 were significant before Bonferroni correction, and none were significant following correction. To examine further the temporal/spatial distribution of genetic variation, we used two hierarchical models of AMOVA. Under Model 1, neither spatial (among regions) nor temporal (between cohorts within regions) heterogeneity was detected, although the temporal component of molecular variance (variation between cohorts within regions) was 20 times greater than the spatial component, and the probability that it was greater than zero was 0.062 (Table 1). Model 2 considered molecular variation within each cohort attributable to region and sampling (trawl) tows within regions. In both cohorts, the component of molecular variance allocated to "sampling tows within regions" was close to an

Table 1. Results of analysis of molecular variance, where d.f. is degrees of freedom, and p the probability of Φ_{CT} or $\Phi_{SC} > 0$.

Hierarchical model	Source of variation	d.f.	% molecular variance	p-value
Model 1	Among regions (Φ_{CT})	5	0.004	0.493
	Between cohorts within regions (Φ_{SC})	5	0.08	0.062
Model 2	2004 cohort			
	Among regions (Φ_{CT})	4	0.045	0.204
	Among sampling tows within regions (Φ_{SC})	36	0.314	0.188
	2005 cohort			
	Among regions (Φ_{CT})	4	−0.018	0.311
	Among sampling tows within regions (Φ_{SC})	56	0.489	0.009

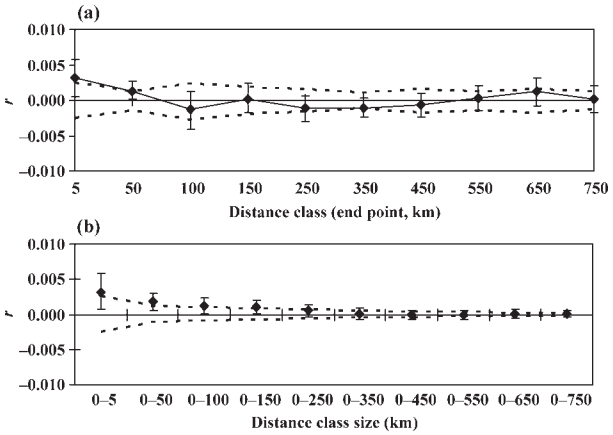

Figure 2. Correlograms illustrating the influence of distance on spatial autocorrelation. Abscissa, distance class; ordinate, spatial autocorrelation (r). 95% confidence error bars for r were estimated by bootstrapping over pairs of samples; dashed lines represent upper and lower bounds of a 95% *CI* for r generated under the null hypothesis of a random geographic distribution of red snapper. (a) Spatial autocorrelation r (black diamonds) as a function of geographic distance classes for the 2004 and 2005 cohorts combined; (b) spatial autocorrelation (r) when the first distance class was increased in increments of 50 km.

order of magnitude greater than the component allocated to "among regions", and the variance "among tows within regions" for the 2005 cohort was significantly >0 (Table 1).

Further analysis of spatial genetic variation utilized Mantel tests of the correlation between genetic and geographic distance and spatial autocorrelation analysis. Mantel tests were non-significant among both regions ($0.94 < p < 0.99$) and sampling tows ($0.58 < p < 0.59$). Significant, positive autocorrelation (r), however, was observed for the distance classes 0–1 and 1–50 km (Figure 2a); the former reflects autocorrelation among fish collected during the same tow, and the latter autocorrelation among fish collected from separate tows up to a distance of 50 km. Spatial autocorrelation among fish collected from separate tows at distances of 100 km or more did not differ significantly from zero. We also examined spatial autocorrelation by estimating r when the first distance class was increased in increments of 50 km (Figure 2b); here, the spatial autocorrelation among individuals collected from proximal localities (tows) was confirmed by high and significant r values obtained when the first distance class was 0–1, 0–50, 0–100, and 0–150 km. Estimates of r did not differ significantly from zero when the size of the first distance class was >250 km. These results were consistent when the two cohorts were analysed separately (data not shown).

Table 2. Summary statistics for posterior distributions of the parameters N_0 (contemporaneous effective size), N_1 (historical effective size), μ (mutation rate), and t_a (time since beginning of expansion/decline) in red snapper (*Lutjanus campechanus*) sampled from five regions in the northern Gulf of Mexico.

Parameter	Mode	0.05 percentile	0.95 percentile
N_0	2 163	202	16 924
N_1	17 766	2 236	149 142
μ	2.71×10^{-4}	4.23×10^{-5}	1.64×10^{-3}
t_a (years)	12 445	465	178 735
$\log_{10}(N_0/N_1)$	−0.92	−1.61	−0.28

Estimates are based on variation at 17 nuclear-encoded microsatellites.

Summary statistics of the posterior distributions, obtained during Bayesian coalescent analysis of population growth, are listed in Table 2. The mode of the posterior distribution of current effective population size (N_0) was 2163 (90% highest posterior density interval, HPD: 202–16 924), whereas the mode of the posterior distribution of the ancestral effective population size (N_1) was 17 766 (90% HPD: 2236–149 142). Log_{10} value of the ratio N_0/N_1 was −0.92 (−1.61 to −0.28), indicating that red snapper in the northern Gulf have experienced a decline in N_e of nearly an order of magnitude. The mode of the posterior distribution of the average mutation rate over the 17 microsatellites was 2.71×10^{-4} (90% HPD: 4.23×10^{-5}–1.63×10^{-3}), whereas the mode of the posterior distribution for the time since decline was 12 445 years (90% HPD: 465–178 735 years).

Discussion

Spatial genetic variation

Exact tests of homogeneity over all loci among all 11 samples examined revealed significant heterogeneity in both allele and genotype distributions. Pairwise exact tests between pairs of samples, however, were non-significant following Bonferroni correction. Results from two models of hierarchical analysis of molecular variance indicated that the component of molecular variance attributable to "among sampling tows within regions" was significant ($p = 0.009$) for the 2005 cohort and approximately one order of magnitude greater than the "among region" component in both cohorts. The component of molecular variance attributable to "between cohorts within regions" also was greater than the component "among regions". In both models, the component of variance from "among regions" was non-significant, underscoring that the spatial genetic variance detected among "sampling tows within regions" reflects genetic heterogeneity of red snapper at small geographic scale. The occurrence of significant spatial heterogeneity within regions is particularly noteworthy, because this result provides the first evidence to date of a non-random spatial distribution of red snapper genotypes in the northern Gulf of Mexico.

Significant spatial genetic heterogeneity was demonstrated by the significant, positive autocorrelation (r) of microsatellite genotypes in both cohorts among fish sampled within a geographic range of ~50–100 km, even when different distance-class sizes were used to alleviate concerns (Peakall *et al.*, 2003) that the distribution of r values depends in part on the size of the distance classes employed. No autocorrelation was found when the size of the distance class was >250 km. However, in part because we were interested more in micro-geographic (within region) variation, and in part because of logistic constraints, distances of 100–250 km were not well sampled. Further study using a more continuous sampling design, particularly at distances between 50 and 250 km, is clearly warranted, first to identify more precisely the geographic distance at which autocorrelation occurs, and second to determine whether there are different patterns of spatial autocorrelation among regions.

Spatial autocorrelation generally reflects isolation by distance (Sokal and Waretenberg, 1983) and potentially stems from limited dispersal within a continuous distribution, differences in population density (Rousset, 1997; Hardy and Vekemans, 1999), or a combination of both factors. Consequently, the spatial autocorrelation observed could reflect the dispersal process of red snapper larvae from spawning events in nearby habitat patches. Larval drift of red snapper has been hypothesized to last up to 3–4 weeks (SEDAR 7, 2004), during which time larvae could be transported over large geographic distances. However, Cowen *et al.* (2006), based on the studies of larval transport in the Caribbean region, found that larval dispersal of ecologically relevant magnitudes for a variety of reef fish species were on the scale of only 10–100 of kilometres or so. The scale of dispersal distances found by Cowen *et al.* (2006) is consistent with the spatial scale (distance) of autocorrelation found in the current study and with mean dispersion rates estimated from tag-and-recapture studies (Strelcheck *et al.*, 2007). Interestingly, the significant, spatial autocorrelation pattern detected did not translate into significant isolation by distance as measured by the correlation between genetic and geographic distance. Detection of a relationship between genetic and geographic distance using a Mantel test requires that the signal be strong across the entire dataset, i.e. across the entire geographic range sampled. Indeed, the pattern of population structure we detected appears not to be a linear increase in genetic distance as a function of geographic distance, but rather a positive correlation between proximal samples that disappears when samples are separated by >100 km. The spatial scale of 100 km or less is much smaller than that across the entire geographic range sampled (~1050 km), effectively precluding detection of a correlation between genetic and geographic distance across the sampling surface. This demonstrates that autocorrelation analyses, which explore genetic correlation at multiple distance classes, are typically more powerful than Mantel tests for uncovering genetic structure (Peakall *et al.*, 2003). Additionally, over the past few years and especially in 2005, there was an unusually large number of hurricanes in the northern Gulf. Hurricanes have been associated with significant movement of adult red snapper in the northern Gulf (Patterson, 2007), and potentially could result periodically in more extensive dispersal of red snapper at a variety of life-history stages. This would tend to diminish what historically might have been a greater distance over which spatial genetic heterogeneity and isolation-by-distance occur. A final point is that spatial autocorrelation may be influenced by different size or density of local breeding populations (Rousset, 1997; Hardy and Vekemans, 1999), a likely situation for red snapper given observed spatial differences in fishing intensity and mortality (SEDAR 7, 2004) and differences in effective size (Saillant and Gold, 2006).

The results obtained to date are consistent with the metapopulation stock-structure model proposed by Pruett *et al.* (2005) and Saillant and Gold (2006) for red snapper in the northern Gulf. The model follows closely that proposed by Kritzer and Sale (2004) where the metapopulation consists of a series of partially closed subpopulations that can impact each other's demographics via periodic gene flow. Evidence for this model in red snapper was based on geographic asynchrony in local population dynamics (Woods *et al.*, 2003; Fischer *et al.*, 2004), different temporal episodes of both range expansion and restricted gene flow with isolation by distance (Pruett *et al.*, 2005), and spatial differences in genetic effective size (Saillant and Gold, 2006). The finding here of significant genetic heterogeneity among habitat patches and of significant spatial autocorrelation at 50–100 km demonstrates independent demographic assemblages of red snapper at relatively small spatial scales. The notion that population subdivision can occur in a marine fish with buoyant (pelagic) eggs and larvae and where adults are capable of long-distance movement is not new and has been documented on geographic scales ranging from tens to a few hundreds of kilometres (Buonaccorsi *et al.*, 2004, 2005; Cunningham *et al.*, 2009). As pointed out by Hauser and Carvalho (2008), the notion that marine fish with Type III survivorship (large brood sizes, high death rate early in life, little to no parental care) and major population sizes have extensive gene flow with no population subdivision is no longer exclusively tenable.

Two final comments are needed regarding, first, the occurrence of detectable spatial autocorrelation in red snapper at small spatial scales but the absence of a significant correlation between genetic and geographic distance across the entire sampling surface, and second, the implications of our findings relative to the management of red snapper in the northern Gulf.

A critical assumption in many population-genetic inferences is that populations are in equilibrium between genetic drift and migration. However, in non- (drift-migration) equilibrium populations, equilibrium is achieved sooner at shorter distances, generating a significant isolation-by-distance effect at short but not long geographic distances (Slatkin, 1993). Disruption of the equilibrium can arise from a variety of causes that generate spatial/temporal differences in effective size, gene flow, or a combination of these two factors. In red snapper, this could include population expansion/decline, recruitment differences, differential fishing intensity and mortality, other pressures such as habitat deterioration and shrimp trawling, and environmental perturbation from periodic climatic events such as hurricanes, all of which have been documented variously as influencing red snapper in the northern Gulf (SEDAR 7, 2004). Following these disturbances, isolation by distance is more likely to be established between neighbouring populations than between more geographically distant ones (Crow and Aoki, 1984). The inference here is that red snapper on a range-wide scale are not in equilibrium with respect to migration and genetic drift, a possibility not inconsistent with findings for other marine species (Johnson and Black, 1998; Hellberg *et al.*, 2002; Buonaccorsi *et al.*, 2004).

With regard to the implications of our findings relative to the management of red snapper in the northern Gulf, both theory (Armsworth, 2002) and simulated modelling (James *et al.*, 2002) indicate that in structure-associated reef fish with pelagic–

benthic life histories, only a few local populations can be expected to replenish themselves consistently and to sustain the stock across a broader region. If this is the case for red snapper, identifying the critical but potentially few source populations will be a challenge. Isolation by distance *per se* does not provide discrete boundaries, but it could be used to assess geographic sectors that may respond differently (and independently) to exploitation and other environmental pressures (Cunningham *et al.*, 2009). As noted above, further study of red snapper using a more-continuous sampling design is clearly warranted to assess whether there are different patterns of spatial autocorrelation across the northern Gulf.

Effective population size and demographic history

Bayesian coalescent analysis indicated an approximately eightfold decline in N_e of red snapper. The mode of the timing of the beginning of the decline phase was roughly 12 500 years ago, corresponding to the Holocene era. Minor glacial advances that lasted until roughly 2000 years ago (Roberts, 1989) may have contributed to the inferred decline in addition to overfishing, habitat deterioration, and shrimp trawling. The last three are factors hypothesized (Christman, 1997; Schirripa and Legault, 1997; Gallaway *et al.*, 1998) to have contributed to the sharp reduction in red snapper abundance beginning in the 1970s (Goodyear and Phares, 1990). This result contrasts with the increase in N_e following the Pleistocene epoch inferred from sequences of a mitochondrial DNA coding gene (Pruett *et al.*, 2005). Observation of a signal of demographic expansion, based on mtDNA, in currently declining populations is not uncommon (Lessios *et al.*, 2001; Saillant *et al.*, 2004) and is likely related to the occurrence of a star-like phylogeny of mtDNA haplotypes in a population that was expanding (Slatkin and Hudson, 1991) before the decline. Indeed, Rogers (1997) showed by simulation that populations may conserve a star-like phylogeny of mtDNA haplotypes, and the signature of demographic expansion, during a demographic bottleneck. The phylogeny of red snapper mtDNA haplotypes analysed by Pruett *et al.* (2005) was star-like and hence probably unsuited to reveal the reduction in effective size inferred from microsatellite genotypes. A cautionary note, however, is that bias in estimating population growth rate may arise if the population sampled is receiving migrants from a divergent source, because this could mimic the signal of a demographic bottleneck (Storz and Beaumont, 2002). Although this possibility cannot be ruled out here, there is no evidence for such migrants because all populations of red snapper surveyed to date have shown homogeneous allele and genotype distributions.

The highest posterior probability for current/recent N_e was 2163, potentially reflecting the effective size of the metapopulation of red snapper under study. Censuses (N) of red snapper in the northern Gulf range from 7.8 to 11.7 million (J. Cowan, Louisiana State University, pers. comm.), indicating that the effective size/census size (N_e/N) ratio for red snapper in the northern Gulf is in the range $1.85–2.77 \times 10^{-4}$. Effective size/census size ratios reported for a number of marine fish species (reviewed in Hauser and Carvalho, 2008) range between two and four orders of magnitude smaller than estimated census sizes. Potential causes for such low N_e/N ratios have been discussed by a number of authors (Turner *et al.*, 2002; Hoarau *et al.*, 2005; Hauser and Carvalho, 2008). The most likely factors in red snapper, as in many other marine fish, would seem to be high variance in individual reproductive success, high variance in productivity among habitat patches (Saillant and Gold, 2006), or a combination of both factors. Nevertheless, the short-distance genetic heterogeneity and spatial autocorrelation pattern observed in red snapper across the northern Gulf is consistent with the hypothesis that recruitment is essentially local and within a 50–100-km range. This underscores the importance of maintaining healthy local spawning populations of red snapper in all regions across the Gulf. Assessing potential spatial variation in neighbourhood size (dispersal distance) across the northern Gulf and evaluating regional differences in density are the next challenges relative to developing efficient regional management of genetic resources of the species.

Acknowledgements

We thank the National Marine Fisheries Service Mississippi Laboratory personnel for assistance during the groundfish surveys, M. Renshaw for assistance both during sampling and in the laboratory, W. Patterson and B. Barnett for providing the sample of red snapper from northwest Florida, and W. Patterson and D. Portnoy for helpful comments on an early draft of the manuscript. We also thank B. Pellegrin for providing the data on the average distance of a sample tow. The work was supported in part by the MARFIN Program of the US Department of Commerce (Grant NA04-NMF-4330074), and in part by Texas AgriLIFE Research (Project H–6703). The views expressed in this paper are those of the authors and do not necessarily reflect those of the sponsors. This article is number 69 in the series "Genetic Studies in Marine Fishes", and Contribution Number 172 of the Center for Biosystematics and Biodiversity at Texas A&M University.

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Appendix

Table A1. Summary statistics for 18 nuclear-encoded microsatellites for the 2004 cohort of age-0 red snapper, *Lutjanus campechanus*, sampled from five regions in the northern Gulf of Mexico and from a mixed-age-group red snapper sample from NW Florida (Figure 1).

Locus	BR	PA	FR	LA	MA	NWF	Locus	BR	PA	FR	LA	MA	NWF
			*Lca*20							*Prs*240			
N	110	105	103	103	104	85	*N*	109	103	100	103	104	85
#A	4	5	5	4	5	4	#A	19	19	20	19	19	19
A_R	3.71	4.44	4.53	3.94	4.65	3.93	A_R	18.51	18.19	19.63	18.69	18.76	18.84
H_E	0.169	0.192	0.139	0.138	0.129	0.124	H_E	0.876	0.897	0.907	0.920	0.898	0.879
P_{HW}	0.046	0.347	1.000	0.007	1.000	1.000	P_{HW}	0.633	0.044	0.029	0.637	0.032	0.147
F_{IS}	0.142	−0.042	−0.049	0.229	−0.047	−0.043	F_{IS}	0.026	0.048	−0.025	−0.045	0.004	−0.057
			*Lca*22							*Prs*248			
N	110	104	103	103	104	85	*N*	110	104	103	102	104	84
#A	12	10	13	11	13	10	#A	17	21	19	20	19	15
A_R	11.08	9.52	12.21	10.30	12.39	9.86	A_R	15.43	19.48	17.89	15.52	18.21	14.87
H_E	0.734	0.710	0.731	0.694	0.751	0.700	H_E	0.874	0.854	0.880	0.874	0.881	0.861
P_{HW}	0.774	0.181	0.362	0.258	0.230	0.563	P_{HW}	0.963	0.147	0.171	0.710	0.482	0.004
F_{IS}	−0.064	0.107	−0.049	0.063	0.026	−0.059	F_{IS}	−0.020	0.009	−0.004	0.002	0.007	0.143
			*Lca*43							*Prs*260			
N	110	105	102	103	104	83	*N*	110	105	103	103	104	84
#A	8	6	7	8	7	9	#A	4	5	3	6	4	4
A_R	7.64	6.00	6.76	7.88	7.00	8.90	A_R	3.92	4.69	3.00	5.30	4.00	4.00
H_E	0.451	0.472	0.552	0.562	0.522	0.572	H_E	0.409	0.301	0.393	0.423	0.431	0.393
P_{HW}	0.038	0.195	0.748	0.637	0.262	0.589	P_{HW}	0.041	0.464	0.636	0.515	0.693	0.775
F_{IS}	0.074	0.052	−0.083	0.051	−0.032	−0.054	F_{IS}	0.134	−0.043	−0.037	0.081	0.018	−0.001

Continued

Table A1. *Continued*

Locus	BR	PA	FR	LA	MA	NWF	Locus	BR	PA	FR	LA	MA	NWF
			*Lca*64							*Prs*275			
N	109	104	103	103	104	82	*N*	110	105	103	103	104	82
#A	10	10	9	9	11	9	#A	6	8	6	8	6	7
A_R	8.90	9.49	8.70	8.66	10.34	8.96	A_R	5.64	7.48	5.95	7.07	5.75	7.00
H_E	0.782	0.770	0.773	0.770	0.784	0.770	H_E	0.571	0.618	0.632	0.564	0.616	0.573
P_{HW}	0.063	0.704	0.121	0.256	0.553	0.829	P_{HW}	0.933	0.058	0.117	0.131	0.249	0.237
F_{IS}	0.097	0.051	−0.017	−0.047	0.007	−0.030	F_{IS}	0.061	−0.016	0.201	0.157	0.032	0.107
			*Lca*91							*Prs*282			
N	109	103	102	102	104	84	*N*	110	105	103	103	104	82
#A	6	6	6	4	5	7	#A	12	12	12	13	11	10
A_R	5.44	5.93	5.72	3.95	4.70	6.82	A_R	11.38	11.55	11.44	12.26	10.68	9.96
H_E	0.559	0.601	0.555	0.57	0.569	0.554	H_E	0.670	0.661	0.648	0.684	0.625	0.611
P_{HW}	0.303	0.601	0.522	0.887	0.005	0.167	P_{HW}	0.226	0.755	0.642	0.019	0.453	0.757
F_{IS}	0.098	−0.065	0.134	0.050	0.122	−0.010	F_{IS}	−0.005	0.006	−0.019	0.048	−0.000	0.082
			*Lca*107							*Prs*303			
N	110	104	103	102	103	84	*N*	110	105	103	101	104	84
#A	10	9	10	10	10	10	#A	8	10	10	8	11	9
A_R	9.90	8.94	9.52	9.76	9.76	9.88	A_R	7.61	9.18	9.25	7.34	9.54	8.82
H_E	0.798	0.796	0.797	0.820	0.781	0.798	H_E	0.438	0.477	0.452	0.446	0.359	0.475
P_{HW}		0.729	0.792	0.079	0.336	0.667	P_{HW}	0.389	0.670	0.269	0.173	0.335	0.190
F_{IS}	0.032	0.046	−0.048	0.055	0.006	−0.074	F_{IS}	0.107	0.022	−0.053	0.112	0.037	−0.053
			*Prs*55							*Prs*328			
N	109	104	103	102	104	84	*N*	110	105	103	103	104	81
#A	5	6	4	6	4	5	#A	5	5	5	5	3	4
A_R	4.71	5.52	3.76	5.32	3.94	4.88	A_R	4.70	4.88	5.00	4.76	3.00	3.98
H_E	0.285	0.268	0.112	0.200	0.171	0.244	H_E	0.546	0.560	0.574	0.581	0.555	0.558
P_{HW}	0.519	0.744	0.003	0.155	1.000	0.783	P_{HW}	0.047	0.786	0.427	0.899	0.441	0.351
F_{IS}	−0.030	−0.006	0.220	−0.031	−0.070	−0.120	F_{IS}	0.051	−0.038	0.086	−0.069	0.048	0.072
			*Prs*137							*Prs*333			
N	110	104	103	103	102	79	*N*	110	105	103	103	104	85
#A	12	10	13	13	10	10	#A	6	5	7	6	6	5
A_R	10.79	9.21	12.43	11.78	9.73	10.00	A_R	5.90	4.75	6.43	5.52	5.46	5.00
H_E	0.730	0.670	0.672	0.721	0.681	0.695	H_E	0.305	0.393	0.319	0.347	0.333	0.326
P_{HW}	0.024	0.010	0.421	0.064	0.849	0.004	P_{HW}	1.000	0.138	0.353	0.024	0.013	0.908
F_{IS}	0.166	0.168	0.033	0.044	0.007	0.144	F_{IS}	−0.072	0.078	−0.003	−0.007	0.046	−0.046
			*Prs*221							*Ra*6			
N	110	105	103	103	104	82	*N*	110	105	103	102	103	82
#A	15	15	15	11	15	12	#A	6	7	7	8	6	6
A_R	13.97	13.62	14.48	10.47	13.98	11.93	A_R	5.70	6.72	6.52	7.32	6.00	5.96
H_E	0.800	0.759	0.832	0.775	0.791	0.817	H_E	0.438	0.373	0.340	0.424	0.395	0.393
P_{HW}	0.020	0.379	0.523	0.586	0.490	**0.002**	P_{HW}	0.077	0.154	0.259	1.000	0.048	0.005
F_{IS}	0.045	0.072	0.054	0.036	−0.009	0.075	F_{IS}	0.024	0.106	0.142	−0.063	0.163	0.254

Regions are BR (Brownsville), PA (Port Aransas), FR (Freeport), LA (Louisiana), MA (Mississippi – Alabama), and NWF (NW Florida). *N*, sample size; #A, number of alleles; A_R, allelic richness; H_E, gene diversity (expected heterozygosity); P_{HW}, probability of conforming to expected HW genotypic proportions; F_{IS}, inbreeding coefficient measured as Weir and Cockerham's (1984) *f*. Enboldened value indicates significant departures from HW equilibrium following (sequential) Bonferroni correction.

Table A2. Summary statistics for 18 nuclear-encoded microsatellites for the 2005 cohort of age-0 red snapper, *Lutjanus campechanus*, sampled from five regions in the northern Gulf of Mexico (Figure 1).

Locus	BR	PA	FR	LA	MA	Locus	BR	PA	FR	LA	MA
*Lca*20						*Prs*240					
N	100	103	103	102	110	*N*	100	103	103	102	110
#A	4	5	4	4	6	#A	20	20	20	19	18
A_R	3.96	4.53	3.95	4.00	5.35	A_R	13.68	13.72	13.95	12.59	12.76
H_E	0.243	0.214	0.190	0.175	0.171	H_E	0.904	0.894	0.901	0.872	0.901
P_{HW}	0.234	0.779	0.149	0.485	0.479	P_{HW}	0.079	0.414	0.008	0.224	0.197
F_{IS}	0.142	−0.042	−0.049	0.229	−0.047	F_{IS}	0.026	0.048	−0.025	−0.045	0.004
*Lca*22						*Prs*248					
N	100	103	103	102	110	*N*	99	103	103	102	110
#A	12	14	12	16	12	#A	18	17	21	18	21

Continued

Table A2. *Continued*

Locus	BR	PA	FR	LA	MA	Locus	BR	PA	FR	LA	MA
A_R	11.32	13.42	11.96	14.76	11.28	A_R	16.94	15.54	18.97	16.95	19.45
H_E	0.666	0.740	0.706	0.737	0.732	H_E	0.881	0.877	0.877	0.867	0.888
P_{HW}	0.132	0.828	0.259	0.828	0.357	P_{HW}	0.012	0.993	0.022	0.871	0.446
F_{IS}	−0.064	0.107	−0.049	0.063	0.026	F_{IS}	−0.020	0.009	−0.004	0.002	0.007
*Lca*43						*Prs*260					
N	100	103	103	102	110	*N*	100	103	103	102	110
#A	6	8	9	7	7	#A	6	4	6	4	5
A_R	6.00	7.70	8.66	6.95	6.92	A_R	5.37	3.77	5.48	3.99	4.44
H_E	0.551	0.523	0.584	0.516	0.514	H_E	0.416	0.315	0.348	0.371	0.355
P_{HW}	0.597	0.227	0.972	0.395	0.733	P_{HW}	0.533	1.000	1.000	0.145	0.371
F_{IS}	0.074	0.052	−0.083	0.051	−0.032	F_{IS}	0.134	−0.043	−0.037	0.081	0.018
*Lca*64						*Prs*275					
N	100	103	103	102	110	*N*	100	103	103	102	110
#A	7	9	11	10	10	#A	5	7	6	6	7
A_R	6.75	8.52	10.51	9.45	9.67	A_R	4.96	6.65	5.66	5.77	6.08
H_E	0.766	0.759	0.786	0.776	0.786	H_E	0.561	0.605	0.592	0.611	0.545
P_{HW}	0.168	0.729	0.979	0.540	0.754	P_{HW}	0.994	0.688	0.395	0.643	0.508
F_{IS}	0.097	0.051	−0.017	−0.047	0.007	F_{IS}	0.061	−0.016	0.201	0.157	0.032
*Lca*91						*Prs*282					
N	100	103	103	102	110	*N*	100	103	103	102	110
#A	5	7	6	6	7	#A	14	12	11	11	12
A_R	5.00	6.46	5.75	5.76	6.08	A_R	13.22	11.64	10.59	10.66	11.58
H_E	0.589	0.549	0.566	0.592	0.558	H_E	0.595	0.613	0.619	0.627	0.641
P_{HW}	0.010	0.187	0.626	0.555	**0.001**	P_{HW}	0.622	0.165	0.493	0.431	0.787
F_{IS}	0.098	−0.065	0.134	0.050	0.122	F_{IS}	−0.005	0.006	−0.019	0.048	−0.000
*Lca*107						*Prs*303					
N	100	103	103	102	110	*N*	100	103	103	102	110
#A	9	11	10	10	9	#A	9	6	9	7	9
A_R	8.79	10.25	9.71	9.76	8.82	A_R	8.44	5.71	8.23	6.72	8.29
H_E	0.790	0.772	0.805	0.770	0.808	H_E	0.393	0.379	0.306	0.409	0.385
P_{HW}	0.040	0.355	0.103	0.692	0.274	P_{HW}	0.621	0.521	**0.002**	0.600	0.254
F_{IS}	0.032	0.046	−0.048	0.055	0.006	F_{IS}	0.107	0.022	−0.053	0.112	0.037
*Prs*55						*Prs*328					
N	100	103	103	102	109	*N*	100	103	103	102	110
#A	4	5	6	6	5	#A	5	5	5	4	5
A_R	3.91	4.74	5.52	5.62	4.65	A_R	4.75	4.76	4.53	3.77	4.64
H_E	0.216	0.280	0.231	0.158	0.247	H_E	0.581	0.555	0.557	0.573	0.558
P_{HW}	1.000	0.790	0.055	0.029	0.037	P_{HW}	0.471	0.775	0.527	0.213	0.537
F_{IS}	−0.030	−0.006	0.220	−0.031	−0.070	F_{IS}	0.051	−0.038	0.086	−0.069	0.048
*Prs*137						*Prs*333					
N	100	103	103	102	110	*N*	100	103	103	102	110
#A	11	12	10	11	12	#A	5	6	7	6	5
A_R	10.44	11.17	9.92	10.65	11.14	A_R	4.79	5.74	6.48	5.72	4.90
H_E	0.699	0.634	0.706	0.690	0.713	H_E	0.328	0.264	0.398	0.355	0.343
P_{HW}	0.325	0.639	0.009	0.007	0.507	P_{HW}	1.000	0.138	0.353	0.024	0.013
F_{IS}	0.166	0.168	0.033	0.044	0.007	F_{IS}	−0.072	0.078	−0.003	−0.007	0.046
*Prs*221						*Ra*6					
N	100	103	103	102	110	*N*	100	103	103	102	110
#A	15	17	14	13	14	#A	8	9	5	8	6
A_R	14.22	15.46	13.54	12.20	12.41	A_R	7.37	8.24	5.00	7.66	5.97
H_E	0.794	0.793	0.786	0.789	0.780	H_E	0.409	0.405	0.390	0.324	0.243
P_{HW}	0.365	0.525	**0.001**	0.364	0.873	P_{HW}	0.837	1.000	0.259	0.261	0.359
F_{IS}	0.045	0.072	0.054	0.036	−0.009	F_{IS}	0.024	0.106	0.142	−0.063	0.163

Regions are BR (Brownsville), PA (Port Aransas), FR (Freeport), LA (Louisiana), and MA (Mississippi–Alabama). *N*, sample size; #A, number of alleles; A_R, allelic richness; H_E, gene diversity (expected heterozygosity); P_{HW}, probability of conforming to expected HW genotypic proportions; F_{IS}, inbreeding coefficient measured as Weir and Cockerham's (1984) *f*. Emboldened values indicate significant departures from HW equilibrium following (sequential) Bonferroni correction.

doi:10.1093/icesjms/fsq011