Conservation Genetics of Gray Snapper (*Lutjanus griseus*) in U.S. Waters of the Northern Gulf of Mexico and Western Atlantic Ocean

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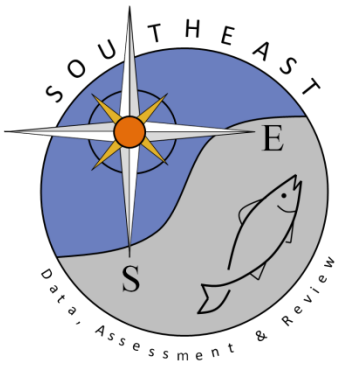
SEDAR51-RD-04

June 2016



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Source: *Copeia*, Vol. 2009, No. 2 (Jun. 12, 2009), pp. 277-286
Published by: American Society of Ichthyologists and Herpetologists (ASIH)
Stable URL: http://www.jstor.org/stable/25512226
Accessed: 15-06-2016 18:52 UTC

REFERENCES

Copeia 2009, No. 2, 277–286

Conservation Genetics of Gray Snapper (*Lutjanus griseus*) in U.S. Waters of the Northern Gulf of Mexico and Western Atlantic Ocean

John R. Gold[1], Eric Saillant[2], N. Danielle Ebelt[1], and Siya Lem[1]

Population structure of Gray Snapper (*Lutjanus griseus*) in U.S. waters was assessed via analysis of allele and genotype distributions at 13 nuclear-encoded microsatellites and mitochondrial (mt)DNA haplotype distribution among samples from five localities in the northern Gulf of Mexico (Gulf) and one locality on the Atlantic coast of Florida. Exact tests of homogeneity over all microsatellites were significant for both allele ($P = 0.004$) and genotype ($P = 0.020$) distributions; homogeneity tests for mtDNA haplotype distributions were not significant ($P = 0.940$). Weak but significant divergence ($\Phi_{CT} = 0.007$, $P = 0.020$) among localities (microsatellites) was indicated by spatial analysis of molecular variance (SAMOVA), where three distinct groups (one from the northwestern Gulf, one from the northcentral/northeastern Gulf, and one from the east coast of Florida) were inferred. Spatial autocorrelation analysis (microsatellites) revealed an isolation-by-distance effect among samples from the northern Gulf. Levels of genetic variation in both microsatellites and mtDNA were low as compared to other lutjanids in U.S. waters, and Bayesian analysis of genetic demography revealed a two to three order-of-magnitude decrease in effective population size of Gray Snapper over the past 5,300 or so years (0.05 quartile of 81 years). The evidence of genetically distinct stocks and the decline in effective population size have implications for management of Gray Snapper resources in U.S. waters.

THE Gray Snapper, *Lutjanus griseus*, is a marine fish found in the western Atlantic Ocean, with its center of distribution being the northern Gulf of Mexico (hereafter Gulf) and the Caribbean Sea (Sumner et al., 1911; Robins et al., 1986). Until recently, Gray Snapper were not heavily targeted by either commercial or recreational fisheries. Increased exploitation and diminishing numbers of other, more popular snapper species such as the Gulf Red Snapper (*Lutjanus campechanus*), however, likely will lead to increased fishing pressure on species such as Gray Snapper (Fischer et al., 2005). Burton (2001), in agreement with an earlier study by Manooch and Matheson (1981), found that Gray Snapper in Atlantic waters of northern Florida achieved a much larger size-at-age than did Gray Snapper in Atlantic waters of southern Florida and suggested that these differences indicated geographic differences in exploitation rates. The latter was supported by estimates of instantaneous rate of fishing mortality (F) that were two to five times higher along the southeastern Florida coast (Manooch and Matheson, 1981; Burton, 2001). Ault et al. (2002, 2005) reported that the spawning potential ratio (SPR) of Gray Snapper in the Florida Keys coral-reef system, including the Dry Tortugas, was below that of a healthy stock, indicating overfishing. Very recent studies of Gray Snapper off the West Florida shelf indicate increasing mortality and decreasing size-at-age from northwest to southwest Florida (R. Allman, pers. comm.).

At present, Gray Snapper in U.S. waters are managed in separate fishery management plans of the South Atlantic and Gulf of Mexico fishery management councils. Wallace et al. (2003) investigated the genetic stock structure of the Gray Snapper in U.S. waters by acquiring sequences of a 351-base-pair (bp) fragment of the mitochondrial (mt)DNA control region from Gray Snapper sampled from four localities along the U.S. Atlantic coast, one locality in the Florida Keys, two localities along the west Florida coast, and one locality in the northwestern Gulf. No differences in mtDNA haplotype frequency or distribution among these eight samples were detected. Their finding of genetic homogeneity is consistent with the hypothesis that gene flow (migration) in Gray Snapper is sufficient to maintain genetic homogeneity across the sampling area. Life-history data of the species, however, are not necessarily consistent with this hypothesis. Adult Gray Snapper, like many reef fishes, are thought to exhibit a sedentary lifestyle in offshore hard-bottom habitats that include reefs, rocky outcroppings, and shipwrecks (Miller and Richards, 1980). Results of tagging studies (Bortone and Williams, 1986) indicated that adult Gray Snapper moved less than nine km over a four-year period. The potential for Gray Snapper dispersal occurs primarily when pelagic eggs and larvae are transported inshore to shallow seagrass and mangrove areas by favorable currents (Burton, 2001). Once established, larvae and juveniles remain on the nursery grounds until they reach a length of about 80 mm (3–4 years of age) when they return to offshore habitats (Starck, 1971; Rutherford et al., 1989; Chester and Thayer, 1990).

In part because of expected increases in exploitation, particularly in waters of south Florida, it was recommended (Reef Fish Stock Assessment Panel, 1999) that Gray Snapper be considered in a future stock assessment. Critical to stock assessment are data on both genetic stock structure and genetic demography (Carvalho and Hauser, 1995). Knowledge of stock structure can ensure that sub-regional exploitation does not unknowingly extirpate unique biological characters (Stepien, 1995), while data on genetic demography may indicate population growth or decline (Beaumont, 1999). In this study, we assessed population structure and genetic demography of Gray Snapper in U.S. waters via analysis of genetic variation at 13 nuclear-encoded microsatellites and in a 590-base-pair fragment of a mitochondrial protein-coding gene. Genetic markers such as microsatellites and mtDNA have been used extensively in studies of stock structure of exploited marine fishes

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Submitted: 22 April 2008. Accepted: 11 November 2008. Associate Editor: D. Buth.
 DOI: 10.1643/CI-08-071

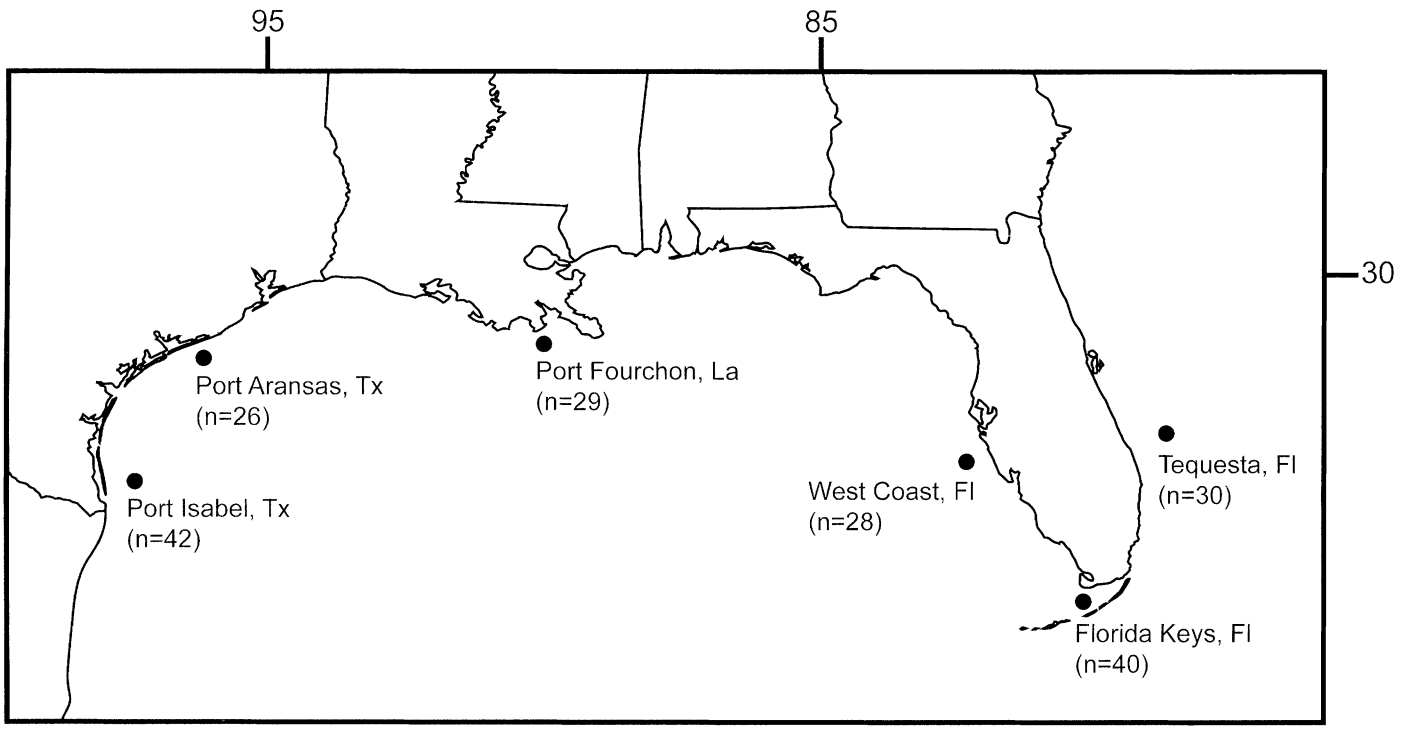

Fig. 1. Sample localities and sample sizes of Gray Snapper (*Lutjanus griseus*) in the northern Gulf of Mexico and the east coast of Florida.

(Carvalho and Hauser, 1995), including several species in the northern Gulf and western Atlantic (Gold et al., 1997, 2001; Gold and Richardson, 1998a; Saillant and Gold, 2006).

MATERIALS AND METHODS

Gray Snapper of varying age and size were sampled from six nearshore localities (Fig. 1) during the spring and summer of 2005: five localities (Port Isabel, TX, Port Aransas, TX, Port Fourchon, LA, Tampa, FL, and the lower Florida Keys) are in the northern Gulf; one locality (Tequesta, FL) is along the east coast of Florida. Individuals from Tampa were obtained by bottom trawling; the remaining samples were obtained by angling. Fin clips or internal tissues (muscle or liver) were taken from each fish at capture. Tissue samples from fish collected at Port Fourchon were frozen in liquid nitrogen and subsequently stored at −80°C; tissue samples from fish collected at the other localities were fixed in 95% ethanol and preserved at ambient temperature. Voucher specimens were collected previously from the Texas coast near Port Isabel (TCWC 575.05, 575.19, 8361.04, 11210.07) and near Port Aransas (TCWC 1584.01, 12352.05), the Louisiana coast near Port Fourchon (TCWC 11198.17; GCRL 90, 91, 1301, 23413; TU 164093, 164439), the west Florida coast near Tampa (TCWC 305.07; GCRL 7061, 7386, 10031, 11393, 11403, 11406, 13353), and the Florida Keys (TCWC 772.07, 795.04, 832.06, 2429.01). Institutional abbreviations are as listed at http://www.asih.org/codons.pdf.

Total genomic DNA was extracted using a phenol/chloroform method (Sambrook et al., 1989), and each fish was assayed for variation at 13 nuclear-encoded microsatellite loci. Polymerase-chain-reaction (PCR) primers used to amplify individual microsatellites were among those developed by Gold et al. (2001) for Gulf Red Snapper (*L. campechanus*) and by Bagley et al. (1999) for Vermilion Snapper (*Rhomboplites aurorubens*). PCR primer sequences and specific annealing temperatures can be found in Gold et al. (2001) and Bagley et al. (1999). Primers were combined into multiplexes for PCR and electrophoresis as described in Renshaw et al. (2007). Microsatellite amplification products were electrophoresed using an ABI 377 automated sequencer (Applied Biosystems Inc., Foster City, CA), following manufacturer instructions. Resulting chromatograms were analyzed in Genescan (ver. 3.1.2, Applied Biosystems); alleles were scored using Genotyper (ver. 2.5, Applied Biosystems).

Ten individuals from each locality were assayed for variation in a 590-base-pair sequence of the mitochondrially encoded ND-4 gene. The primers NAP-2 (Arevalo et al., 1994) and ND4LB (Bielawski and Gold, 2002) were used for amplification and sequencing of PCR products. PCR amplification and sequencing of mtDNA fragments followed Pruett et al. (2005). Amplification products were sequenced using the Big Dye Terminator Kit (ver. 1.1, Applied Biosystems); sequencing reaction products were electrophoresed on an ABI 3100 capillary sequencer (Applied Biosystems), following manufacturer instructions.

Summary statistics for microsatellite data, including number of alleles, allelic richness, and unbiased gene diversity, were obtained for each sample using F-Stat (ver. 2.9.3.2, http://www2.unil.ch/popgen/softwares/fstat.htm). Homogeneity among samples in allelic richness and gene diversity was tested using Friedman rank tests as implemented in SPSS (ver. 11.0.1, http://www.spss.com/statistics/). Departure of genotypic proportions from Hardy-Weinberg expectations within samples was measured as Weir and Cockerham's (1984) f as implemented in F-Stat. Significance of f was evaluated using an exact probability test as implemented in Genepop (ver. 3.4, http://genepop.curtin.edu.au/). The exact probability was estimated using a Markov Chain approach (Guo and Thompson, 1992) that employed 5,000 dememorizations, 500 batches, and 5,000 iterations per

batch. Genotypic disequilibrium between pairs of microsatellite loci also was evaluated using exact tests in Genepop; the exact probability was estimated via a Markov Chain method and using the same parameters as above. Sequential Bonferroni correction (Rice, 1989) was applied for all multiple tests performed simultaneously. Occurrences of null alleles, large allele dropout, or stuttering were evaluated for each microsatellite in each sample, using the software Microchecker (van Oosterhout et al., 2004).

Homogeneity of allele and genotype distributions (microsatellites) among localities was tested using exact tests as implemented in Genepop. The exact probability was estimated using a Markov Chain method and employing the same parameters as above. Population structure was examined using the Spatial Analysis of Molecular Variance (SAMOVA) approach developed by Dupanloup et al. (2002). SAMOVA (ver. 1.0, http://web.unife.it/progetti/genetica/Isabelle/samova.html) employs a simulated annealing algorithm to optimize allocation of N geographic populations into K groups ($2 < K < N$) to maximize the proportion of total genetic variance due to genetic variation among the inferred groups. A total of 100 simulated annealing processes were used to determine optimal allocation of the six geographic samples into two, three, four, or five groups.

Microsatellite data also were used to examine whether there was a relationship between genetic divergence and geographic distance, that is, an isolation-by-distance effect, using multilocus spatial autocorrelation analysis (Smouse and Peakall, 1999; Peakall et al., 2003) as implemented in GenAlEx 6.0 (Peakall and Smouse, 2006). The spatial autocorrelation coefficient (r) was computed using a linear, one-dimensional coastline geographic distance and the multilocus genetic distance outlined in Smouse and Peakall (1999). With isolation by distance, the estimated value of r among proximal localities (small distance classes) will differ significantly from zero and decrease with increasing distance between localities (larger distance classes). The distance class at which r no longer differs significantly from zero provides an approximation of the distance at which genetic divergence (population structure) can be detected (Peakall et al., 2003). Because estimation of spatial autocorrelation is influenced by the size of the distance class, r was computed based on a series of increasing distances between pairs of localities (Peakall et al., 2003). We used multiples of 400 km to construct distance classes so that at least two pairs of localities would be included in the computation of r. Significance of r was determined via random permutations of genotypes among localities. The distribution of r values under the null hypothesis of random spatial distribution of genotypes was used to determine the probability of significance of 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). Significance of r also was tested by generating bootstrap 95% confidence intervals for r. Bootstrap values were obtained by sampling with replacement pairs of localities within a given distance class. Bootstrap resampling was performed 1,000 times and significance of r inferred when the 95% CI did not overlap zero.

Summary statistics for mtDNA, including number of haplotypes, haplotype (nucleon) diversity, and nucleotide diversity, were obtained for each sample, using Arlequin (Schneider et al., 2000). Homogeneity in mtDNA haplotype distribution among localities was tested using an exact test as implemented in Genepop; the exact probability was estimated using a Markov Chain method and employing the same parameters as above for microsatellites. Population structure was examined using both SAMOVA and spatial autocorrelation analysis as described above for microsatellites.

The demographic history of the samples of Gray Snapper was examined using the microsatellite data and a Bayesian coalescent approach (Beaumont, 1999; Storz and Beaumont, 2002). The model implemented considers a population changing in size exponentially from an initial (historical/ancestral) effective size to a current (contemporaneous) effective size. The demographic parameters estimated were current (N_0) and historical/ancestral (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 (r) 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 and time since expansion/decline) parameters were estimated using a Monte Carlo Markov Chain (MCMC) approach as implemented in Msvar (ver. 1.3, http://www.rubic.rdg.ac.uk/cgi-bin/MarkBeaumont/dirlist1.cgi). In order to reduce computation times, 100 chromosomes were sub-sampled at random from the pooled data set. Chromosomes were sub-sampled using the program Sinf (included in the Msvar package) and used in estimation. Computations were performed on three independent subsamples and were replicated three times, using different starting parameters in order to assess convergence of the MCMC. 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 mean of the prior distributions of means N_0, N_1, μ, and t_a were set to 10^5, 10^5, $10^{-3.5}$, and 10^4, respectively; their standard deviations (SD) were set to 10^3, 10^3, $10^{0.5}$, and 10^3, respectively. Priors for N_0, N_1, and t_a provided support for a broad range of values. The prior distribution of μ provided support for values between $10^{-2.5}$ to $10^{-4.5}$ in accordance with published information on microsatellite mutation rates (Storz and Beaumont, 2002; Turner et al., 2002). The standard deviation (SD) of the variance of N_0, N_1, and t_a among microsatellites was set to 0.5 so that expected ratios for pairs of microsatellites would be approximately five-fold under the prior (Storz and Beaumont, 2002). The SD of the variance of mutation rates among microsatellites was set to two so that ratios of mutation rates between individual microsatellites up to 700-fold would be supported under the prior (Storz and Beaumont, 2002). A generation time of seven years was considered based on life-history data available for Gray Snapper (Burton, 2001; Fischer et al., 2005) and assuming a Type II survivorship model (Nunney and Elam, 1994).

RESULTS

Summary statistics, including number of alleles, allelic richness, and unbiased gene diversity for each of the 13 microsatellites, by locality, are given in Appendix 1. Number of alleles per locus ranged among localities between one and 14 and averaged 7.0. Allelic richness and unbiased gene diversity averaged 4.82 (range = 1.96–8.08) and 0.545 (range = 0.066–0.823), respectively. Allelic richness differed significantly among samples ($P = 0.024$), whereas gene diversity

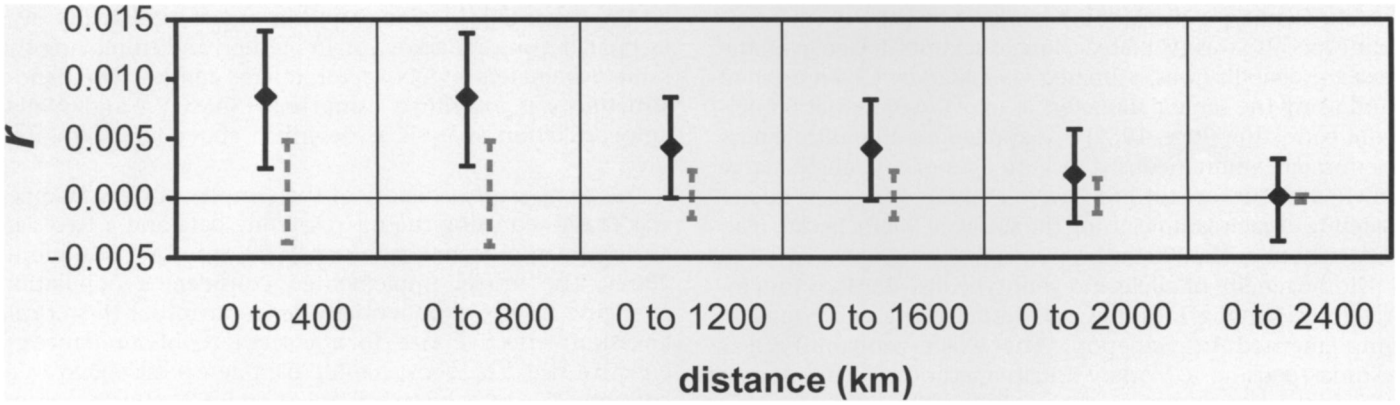

Fig. 2. Correlogram showing spatial autocorrelation (*r*) among Gray Snapper (*Lutjanus griseus*) sampled from five localities in the northern Gulf of Mexico. Estimates of *r* (diamonds) are computed for increasing distance class sizes; 95% confidence error bars for *r* (plain error bars) as determined by bootstrapping over population pairs; upper and lower bounds (dashed error bars) of a 95% CI for *r* generated under the null hypothesis of a random distribution of Gray Snapper among localities.

did not ($P = 0.249$). Significant departures from Hardy-Weinberg equilibrium expectations before, but not after, Bonferroni correction were found at microsatellite *Ra*1 in the sample from Port Isabel (TX), at microsatellites *Lca*43 and *Ra*7 in the sample from Port Fourchon (LA), and at microsatellites *Prs*240 and *Lca*22 in the sample from Tequesta (FL). Significant departure from Hardy-Weinberg equilibrium expectations both before and after Bonferroni correction was found for microsatellite *Prs*275 in the sample from Tequesta, FL ($f = 0.319$, $P = 0.001$). Analysis using Microchecker did not indicate occurrence of null alleles or large-allele dropout at *Prs*275, so the microsatellite was used in subsequent analysis. A total of 28 of 468 pair-wise tests of genotypic disequilibrium were significant before, but not after, Bonferroni correction.

Exact tests of homogeneity of allele and genotype distributions among localities were significant over all microsatellites ($P = 0.004$, allele distribution; $P = 0.020$, genotype distribution). Results from simulated annealing processes in SAMOVA that optimized the partitioning of the set of six sample localities into two, three, four, or five groups revealed that only the partition of localities into three groups led to a significant, among-groups component of molecular variance ($\Phi_{CT} = 0.007$, $P = 0.020$). The optimal inferred partition allocated the samples from the northwestern Gulf (Port Isabel and Port Aransas) into one group, the samples from the northcentral and northeastern Gulf (Port Fourchon, Tampa, and the Florida Keys) into a second group, and the sample from the east coast of Florida (Tequesta) into a third group.

Spatial autocorrelation analysis was conducted initially using only the five sample localities from the northern Gulf of Mexico. The sample from Tequesta (east coast of Florida) was not included based on results of SAMOVA (above), which had indicated that the sample from Tequesta differed significantly from the remainder. Occurrence of a significant barrier to gene flow between Tequesta and the Florida Keys, together with the close proximity between the two localities (approximately 395 km), potentially could compromise estimates of the spatial autocorrelation coefficient (*r*). In initial runs (Tequesta sample excluded), estimates of *r* differed significantly from zero ($0.008 > r > 0.004$) in the first three–four distance classes (Fig. 2), becoming non-significant at distances between localities greater than 1,600 km. Estimates of *r* when the sample from Tequesta was included were lower ($0.005 > r > 0.002$ in the first four distance classes), becoming non-significant at distances between localities greater than 2,000 km. The decrease in spatial autocorrelation when the sample from Tequesta was included further supports results from SAMOVA that Gray Snapper from the Atlantic coast of Florida are a genetically different group from Gray Snapper in the northeastern Gulf.

Summary statistics for mtDNA sequences, including the spatial distribution of haplotypes, haplotype diversity, and nucleotide diversity are presented in Table 1. Only five different haplotypes (GenBank accession numbers EU669440–EU669444) were found among the 60 individuals surveyed; haplotype 2 was the most common, occurring in half or more of the individuals at each locality. Haplotype diversity (h) was low, ranging from 0.200 (Tequesta) to 0.711 (Tampa). Nucleotide diversity (π) ranged from 0.001 (Tequesta) to 0.003 (all other localities). No significant difference in mtDNA haplotype distributions among localities was indicated by exact tests ($P = 0.940$), and no significant partitions of localities were indicated by SAMOVA. A 95% parsimony network of the mtDNA haplotypes (not shown) did not reveal any visual association between individual haplotypes and locality, and estimates of the spatial autocorrelation coefficient (*r*) did not differ significantly from zero in runs with or without the sample from Tequesta included.

The posterior distributions of $\log_{10}(r)$ obtained during replicate runs of Bayesian coalescent analysis of the microsatellite data, using varying starting parameters in replicate random subsamples of the data set, were essentially identical; posterior distributions obtained during the various replicate runs were then combined to derive final summary statistics as per recommendations of M. A. Beaumont (pers. comm.). Summary statistics of obtained posterior distribution are shown in Table 2. The mode of the posterior distribution of current effective population size was 308, while the mode of the posterior distribution of the ancestral effective population size was 16,185 (Table 2). The mode of the posterior distribution of the average mutation rate over all microsatellites was approximately 2.5×10^{-4}, while the mode of the posterior distribution for the time since decline was 5,305 years; the 0.05 quartile was 81 years (Table 2).

Table 1. Summary Statistics for mtDNA (Ten Individuals Surveyed per Locality). h = haplotype diversity; π = nucleotide diversity. Sequences of haplotypes 1–5 are listed under GenBank accession numbers EU669440–EU669444.

Haplotype#	Port Isabel	Port Aransas	Port Fourchon	Tampa	Florida Keys	Tequesta
#1	1	1	1	1	1	–
#2	8	7	6	5	5	9
#3	2	2	3	3	3	1
#4	–	–	–	–	1	–
#5	–	–	–	1	–	–
h	0.511	0.511	0.600	0.711	0.711	0.200
π	0.003	0.003	0.003	0.003	0.003	0.001

DISCUSSION

Genetic population structure.—Exact tests of homogeneity in both microsatellite allele and genotype distributions across all six localities were significant, and results of spatial analysis of molecular variance (SAMOVA) indicated three distinct groupings. The optimal inferred partition identified one group from the northwestern Gulf (samples taken from offshore of Port Isabel and Port Aransas, TX), one group from the northcentral and northeastern Gulf (samples from offshore of Port Fourchon, LA, Tampa, FL, and the Florida Keys), and one group from the east coast of Florida (offshore of Tequesta, FL). Spatial autocorrelation analysis further indicated significant genetic differences among Gray Snapper in the northern Gulf, with genetic divergence occurring at least in part as a function of geographic distance. The autocorrelation correlogram revealed a progressive decline from significant, positive r values observed when localities within 400–1,200 km from one another were compared to no significant autocorrelation when pairwise distances between localities greater than 1,600 km were considered. The results of spatial autocorrelation analysis are not discordant with results from SAMOVA, as the average distance between the two samples from the northwestern Gulf (Port Isabel and Port Aransas) and the three samples from the northcentral and northeastern Gulf (Port Fourchon, Tampa, and the Florida Keys) is approximately 1,680 km. A final point to note is that the distance between the sample locality (Tequesta) on the east coast of Florida and the sample locality in the Florida Keys is only approximately 395 km, well within the 1,600 km range at which positive autocorrelation was found among samples from the northern Gulf. Inclusion of the sample from Tequesta in GenAlEx runs, however, led to a lower spatial autocorrelation (r value), likely reflecting occurrence of a significant barrier to gene flow between Tequesta and the Florida Keys as also inferred in spatial analysis of molecular variance (SAMOVA). This result further supports the hypothesis of significant genetic difference between Gray Snapper along the U.S. Atlantic coast and those in the northern Gulf. Genetic divergence between subpopulations along the U.S. Atlantic coast and the northern Gulf has been documented previously for a number of marine species and has been hypothesized, depending on the species, to stem from a variety of factors including historical processes, absence of suitable habitat in spatially intermediate regions, current patterns, and behavioral characteristics (Avise, 1992; Gold and Richardson, 1998b).

Table 2. Summary Statistics for the Posterior Distributions of Parameters. r (ratio of current/ancestral effective size), N_0 (contemporaneous effective size), N_1 (historical or ancestral effective size), μ (mutation rate), and t_a, (time since beginning of expansion/decline).

All Samples	Mode	0.05 Quartile	0.95 Quartile
$\text{Log}_{10}(r)$	−2.671	−2.072	−4.373
N_0	308	4	5,804
N_1	16,866	1,467	195,209
μ	2.49×10^{-4}	2.8×10^{-5}	2.21×10^{-3}
t_a (years)	5,305	81	142,495

The genetic difference indicated by SAMOVA between Gray Snapper in the northwestern Gulf (samples from the Texas coast) and those in the northcentral and northeastern Gulf (samples from Louisiana, the Florida west coast, and the Florida Keys) suggests limited movement (gene flow) of Gray Snapper between Texas and Louisiana waters. Interestingly, genetic studies, using essentially the same nuclear and mitochondrial markers, of the related congeners *Lutjanus campechanus* (Gulf Red Snapper) and *Lutjanus synagris* (Lane Snapper) have not revealed any genetic differences between samples of those species from Texas and Louisiana (Saillant and Gold, 2006; Karlsson et al., in press). Gray Snapper differ from these two congeners in that larval and juvenile Gray Snapper appear highly dependent on inshore, shallow seagrass-bed and mangrove nursery habitats (Rutherford et al., 1989; Chester and Thayer, 1990); larval and juvenile Lane Snapper and Gulf Red Snapper, alternatively, are found much more often in continental shelf areas further from shore (Gallaway et al., 1999; Patterson et al., 2007; Mikulas and Rooker, pers. comm.). Assuming that east–west movement/dispersal of Gray Snapper would occur at a larval or juvenile life-history stage during colonization of nursery habitats, the genetic hiatus observed between Texas and Louisiana could be due to disruption or patchiness of suitable habitat. Loss of seagrass beds in the northern Gulf is well documented (Handley et al., 2007), particularly in the Galveston Bay system along the northeastern Texas coast where over 95% percent of historic seagrass beds have been lost (Gulf of Mexico Program, 2004). There also is the well-publicized hypoxic zone (Rabalais et al., 1999; Ferber, 2001) that extends westward from the Mississippi Delta to the northeastern Texas coast and that occurs typically from late spring through the summer coinciding with peak abundance of Gray Snapper larvae and juveniles (Tolan and Fisher, pers. comm.).

Wallace et al. (2003), in their study of geographic variation in the Gray Snapper mitochondrial control region,

did not find evidence of genetic heterogeneity among seven samples from Florida (both east and west coasts) and one sample from the northwestern Gulf. MtDNA haplotype diversities in their samples, however, ranged from 0.889 to 1.000 (average of 0.963), indicating that most samples were comprised of unique, singleton mtDNA haplotypes that would constrain effective testing of statistical homogeneity. In our study, only five mtDNA haplotypes were found among 60 individuals surveyed, also precluding effective statistical testing.

The occurrence of different groupings (stocks) of Gray Snapper along the Atlantic coast and in the northern Gulf is consistent with *de facto* current management in that Gray Snapper resources in U.S. waters are under the jurisdiction of separate fishery management councils. The finding that separate groupings of Gray Snapper also may occur in the northern Gulf warrants further investigation, in part because the heterogeneity observed in SAMOVA simulations was not strong ($\Phi_{CT} = 0.007$, $P = 0.02$) and needs to be confirmed by replicate temporal sampling, and in part because, if substantiated, occurrence of distinct groups (stocks) in the northern Gulf may necessitate rethinking of current management of Gray Snapper resources in the region.

Genetic diversity and demographic dynamics.—Gray Snapper in our study exhibited low genetic variation compared to other marine fishes. DeWoody and Avise (2000) reported an average of 20.6 alleles per locus in marine fishes (66 loci averaged over 12 species); Gray Snapper averaged only seven alleles (average) per microsatellite, with a maximum of 14 alleles at a single microsatellite. In addition, recent studies (Saillant and Gold, 2006; Karlsson et al., in press) employing some of the microsatellites used here revealed higher polymorphism in both Gulf Red Snapper (*L. campechanus*—average of 11.67 alleles/microsatellite) and Lane Snapper (*L. synagris*—average of 11.2 alleles/microsatellite). There also were only five mtDNA haplotypes detected among our samples of Gray Snapper, generating a haplotype diversity (h) of 0.495, significantly lower (based on a bootstrap resampling approach as described in Karlsson et al., 2008) than that (h = 0.770–0.797) reported by Pruett et al. (2005) for the same 590 bp fragment of ND-4 in Gulf Red Snapper. Wallace et al. (2003), however, in their study of variation in a 351 bp fragment of the Gray Snapper mtDNA control region, reported haplotype diversities ranging from 0.889 to 1.000 (average of 0.963) among the seven samples from Florida (both coasts) and one sample from the northwestern Gulf (average sample size of approximately 30, with a range of 11–55 individuals/sample) of Gray Snapper. The roughly two-fold difference in mtDNA haplotype diversity between our study and that of Wallace et al. (2003) is likely due to significantly elevated mutation rates in the mtDNA control region. Mutation rates in the mtDNA control region are known to be highly heterogeneous (Excoffier and Yang, 1999), with mutation rates in mtDNA control regions significantly elevated in comparison to mtDNA protein-coding genes (Avise, 2000; Sivasundar et al., 2001). Sturmbauer and Meyer (1992), for example, reported a relative rate of control region/cytochrome *b* evolution in a cichlid from Lake Tanganyika to be approximately 15:1, while McMillan and Palumbi (1997) found that sequence changes at one end of the control region in a Pacific butterfly fish accumulated 33–43 times more rapidly than did changes within the cytochrome *b* gene.

Results from the coalescent approach indicated a decline in effective population size of Gray Snapper from U.S. waters of two to three orders of magnitude, and a recent effective size of only a few hundred individuals. The mode of the posterior distribution of the time since decline was approximately 5,300 years ago, suggesting inception during the Holocene era. In the earlier part of this era, global sea levels rose by about 40 meters due to Pleistocene glacial retreat. From 10,000 years ago until about 5,000 years ago, the global climate experienced an increase in temperature of 0.5–2.0°C, followed by minor glaciation that lasted until about 2,000 years ago (Roberts, 1989). While these geologic factors could have contributed to a decrease in effective population size, their relative role (and possible effects of more recent events, including human impacts) remains unclear. Other marine species have suffered similar declines in the recent past (less than 1,000 years) due to exploitation and habitat destruction (Worm et al., 2006). Although the peopling of the Americas began around 10,000 years ago (Roberts, 1989), human impacts beginning approximately 5,300 years ago are not likely an initial cause of the Gray Snapper decline. However, the lower 0.05 quartile for the time since decline was 81 years, suggesting that human impact (e.g., overfishing, deterioration of shallow seagrass and mangrove habitat favored by larval and juvenile Gray Snapper) could have contributed to the decline in effective size.

The decline in effective population size could account for the lower level of genetic diversity observed among Gray Snapper in our study and may suggest that Gray Snapper are potentially compromised in terms of abundance of genetic resources. The minimum effective size needed to insure long-term maintenance of genetic resources is not well known. Franklin (1980) and Schultz and Lynch (1997) hypothesized that an effective size of a few hundred is needed to maintain the equilibrium between loss of adaptive variation from genetic drift and its replacement by mutation, whereas Lynch and Lande (1998) suggested that an effective size for a conservation/restoration program should be in the range 1,000–5,000. Our estimate of 308 for the current effective population size of Gray Snapper, along with the evidence of genetically distinct stocks within the northern Gulf, suggests that management planning for Gray Snapper resources in U.S. waters may need to be reexamined.

A final comment regards the recent study by Tolan and Fisher (pers. comm.) in which a near exponential increase in Gray Snapper abundance in Texas bays and estuaries over the past 30 years was documented. Tolan and Fisher (pers. comm.) hypothesize that the increased abundance is due largely to changing climate patterns and increasing winter-time surface water temperatures and, moreover, that Gray Snapper should continue to flourish until a series of successive cold winters generates a 'thermal closure' of nursery habitats. Our estimate of genetic effective size in Gray Snapper is not inconsistent with their findings in that episodic fluctuation in abundance is one of the factors that can lead to reduction in effective population size (Vucetich et al., 1997). The caution here is that increases in abundance *per se* may not necessarily indicate that a population or stock is no longer compromised in terms of genetic resources.

ACKNOWLEDGMENTS

We thank the crew of the RV *Tommy Munro* (Gulf Coast Research Laboratory, University of Southern Mississippi), the scientific team of the baitfish survey (Florida Fish and

Wildlife Research Institute), and E. Ault, K. Doncaster, A. Fischer, R. Lehnert, B. Mahmoudi, M. Murphy, M. Renshaw, and J. Tolan for assistance in sampling, H. Bart for providing voucher specimens from the Tulane Museum of Natural History, and W. Patterson for helpful, constructive comments on a draft of the manuscript. Thanks also to C. Bradfield and M. Renshaw for assistance in the laboratory. Work was supported in part by the *Marfin* Program of the U.S. Department of Commerce (Grant NA04-NMF-4330074), and in part by Texas AgriLIFE Research (Project H-6703). Views expressed in this paper are those of the authors and do not necessarily reflect views of the sponsors. This article is number 61 in the series 'Genetic Studies in Marine Fishes' and Contribution Number 163 of the Center for Biosystematics and Biodiversity at Texas A&M University.

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APPENDIX 1

Summary statistics at 13 nuclear-encoded microsatellites in six geographic samples of Gray Snapper (*Lutjanus griseus*). *n* = sample size; #A = number of alleles; AR = allelic richness; H_E = gene diversity; P_{HW} = probability of conformity to Hardy-Weinberg genotypic expectations; and F_{IS} = inbreeding coefficient.

Locality		Port Isabel	Port Aransas	Port Fourchon	Tampa	Florida Keys	Tequesta
Microsatellite							
*Lca*20	*n*	29	25	29	28	40	30
	#A	4	4	4	4	5	4
	AR	4.00	4.00	4.00	4.00	4.60	4.00
	H_E	0.698	0.750	0.731	0.708	0.744	0.736
	P_{HW}	0.097	0.275	0.0518	0.730	0.723	0.265
	F_{IS}	0.001	0.093	−0.132	0.092	−0.008	0.048
*Prs*260	*n*	29	25	29	28	40	30
	#A	7	6	9	9	10	8
	AR	7.00	6.00	8.28	8.71	8.82	7.76
	H_E	0.833	0.826	0.773	0.844	0.814	0.823
	P_{HW}	0.625	0.596	0.080	0.058	0.637	0.594
	F_{IS}	0.040	−0.211	0.242	−0.185	0.078	−0.053
*Ra*1	*n*	29	25	29	28	40	30
	#A	5	8	4	7	8	7
	AR	4.85	7.92	3.86	6.57	6.68	6.52
	H_E	0.509	0.693	0.571	0.595	0.653	0.489
	P_{HW}	0.024	0.403	0.549	0.067	0.641	0.473
	F_{IS}	−0.017	−0.038	−0.001	0.039	0.0810	−0.159
*Prs*221	*n*	29	25	29	28	40	30
	#A	4	6	4	4	4	4
	AR	4.00	5.92	4.00	3.98	3.60	3.92
	H_E	0.633	0.631	0.649	0.563	0.625	0.444
	P_{HW}	0.615	0.825	0.128	0.480	0.619	1.000
	F_{IS}	−0.178	−0.078	0.203	−0.268	0.040	−0.127
*Lca*43	*n*	29	25	29	28	40	30
	#A	2	2	3	2	2	2
	AR	2.00	2.00	2.97	2.00	2.00	2.00
	H_E	0.177	0.412	0.324	0.194	0.140	0.282
	P_{HW}	1.000	1.000	0.033	1.000	1.000	0.563
	F_{IS}	−0.083	0.028	0.042	−0.102	−0.068	−0.184
*Prs*137	*n*	29	25	29	28	40	30
	#A	5	5	5	5	5	5
	AR	5.00	5.00	4.80	4.71	4.44	4.60
	H_E	0.716	0.733	0.643	0.548	0.603	0.651
	P_{HW}	0.598	0.125	0.212	0.359	0.604	0.990
	F_{IS}	−0.116	−0.201	0.088	−0.043	0.129	−0.025
*Prs*328	*n*	29	25	29	28	40	30
	#A	3	3	3	3	3	3
	AR	3.00	3.00	3.00	2.98	4.19	3.00
	H_E	0.515	0.638	0.557	0.528	0.579	0.579
	P_{HW}	0.305	0.472	0.595	0.847	0.240	0.156
	F_{IS}	0.197	−0.065	0.134	0.121	−0.253	−0.151
*Prs*275	*n*	29	25	29	28	40	30
	#A	8	7	8	7	7	8
	AR	7.70	7.00	7.94	6.84	6.58	7.94
	H_E	0.797	0.828	0.803	0.796	0.799	0.783
	P_{HW}	0.577	0.481	0.081	0.080	0.752	0.001
	F_{IS}	−0.034	0.055	0.061	0.057	−0.016	0.249
*Prs*240	*n*	29	25	29	28	40	30
	#A	4	5	5	4	7	6
	AR	4.00	4.96	4.83	4.00	6.04	5.59
	H_E	0.647	0.687	0.666	0.642	0.702	0.610
	P_{HW}	0.591	0.397	0.052	0.965	0.274	0.017
	F_{IS}	−0.093	0.068	−0.036	−0.058	0.110	0.399

APPENDIX 1

Continued.

Locality		Port Isabel	Port Aransas	Port Fourchon	Tampa	Florida Keys	Tequesta
Microsatellite							
*Lca*91	*n*	29	25	29	28	40	30
	#A	2	3	1	2	2	2
	AR	1.99	2.92	1.00	1.87	2.00	1.96
	H_E	0.073	0.079	0.000	0.036	0.140	0.066
	P_{HW}	1.000	1.000	—	—	1.000	1.000
	F_{IS}	−0.019	−0.011	—	—	−0.068	−0.018
*Lca*22	*n*	29	25	29	28	40	30
	#A	5	5	4	5	6	5
	AR	4.85	5.00	3.83	4.82	5.63	4.92
	H_E	0.659	0.667	0.581	0.595	0.611	0.610
	P_{HW}	0.333	0.633	0.283	0.047	0.648	0.034
	F_{IS}	0.104	0.040	0.169	0.159	0.018	0.180
*Lca*107	*n*	29	25	29	28	40	30
	#A	6	7	9	6	7	7
	AR	6.00	6.96	8.62	5.98	6.60	6.76
	H_E	0.672	0.658	0.768	0.771	0.754	0.697
	P_{HW}	0.433	0.195	0.543	0.273	0.722	0.060
	F_{IS}	−0.032	0.148	0.057	0.027	−0.160	0.139
*Ra*7	*n*	29	25	29	28	40	30
	#A	3	3	3	3	3	3
	AR	3.00	3.00	2.97	2.98	2.60	2.96
	H_E	0.207	0.288	0.223	0.201	0.184	0.314
	P_{HW}	1.000	0.081	0.014	0.114	1.000	0.375
	F_{IS}	−0.069	0.165	0.227	0.289	−0.085	−0.060