Population structure of red snapper (*Lutjanus campechanus*) in U.S. waters of the western Atlantic Ocean and the northeastern Gulf of Mexico

Christopher M. Hollenbeck, David S. Portnoy, Eric Saillant, John R. Gold

SEDAR74-RD58

March 2021

This information is distributed solely for the purpose of pre-dissemination peer review. It does not represent and should not be construed to represent any agency determination or policy.

Fisheries Research 172 (2015) 17–25

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Population structure of red snapper (*Lutjanus campechanus*) in U.S. waters of the western Atlantic Ocean and the northeastern Gulf of Mexico

Christopher M. Hollenbeck[a,*], David S. Portnoy[a,b], Eric Saillant[c], John R. Gold[a,b]

[a] Harte Research Institute, Marine Genomics Laboratory, Texas A&M University-Corpus Christi 6300 Ocean Drive, Corpus Christi, Texas 78412-5869, USA
[b] Department of Life Sciences, Texas A&M University-Corpus Christi, 6300 Ocean Drive, Corpus Christi, Texas 78412-5869, USA
[c] Department of Coastal Sciences, University of Southern Mississippi, Gulf Coast Research Laboratory, 703 East Beach Drive, Ocean Springs, MS 39564, USA

ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form 15 June 2015
Accepted 17 June 2015

Keywords:
Red snapper
Lutjanus campechanus
Atlantic Ocean
Gulf of Mexico
Population structure

ABSTRACT

Population structure of adult red snapper (*Lutjanus campechanus*) from the southeastern coast of the United States (Atlantic) and the northeastern Gulf of Mexico (Gulf) was assessed using genotypes at 16 nuclear-encoded microsatellites and mitochondrial (mt)DNA haplotypes of the NADH dehydrogenase 4 (ND4) gene. Initial tests (F_{ST}-based, hierarchical AMOVA) of spatial genetic homogeneity within and between regions were non-significant, consistent with a single population or stock of red snapper in the Atlantic and Gulf. Inferences derived from other statistical approaches were consistent with genetic and/or demographic differences within and between the two regions. The estimated, average, long-term migration rate between the two regions (0.27%) was well less than the 10% rate below which populations can respond independently to environmental perturbation. Comparisons of global estimates of average, long-term effective size (N_{eLT}) with estimates from individual sample localities indicated genetic heterogeneity within both the Atlantic and Gulf. These results paralleled those of prior genetic studies of red snapper from the Gulf. Future genetics studies and other work on red snapper in both the Atlantic and Gulf should include approaches to identify demographically independent units within each region and assess their size, patterns of connectivity, and contribution to the fishery. Monitoring global and/or local effective size also should be considered.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Red snapper (*Lutjanus campechanus*) have supported important commercial and recreational fisheries in U.S. waters of the western Atlantic Ocean, including the Atlantic coast of the southeastern United States (hereafter Atlantic) and the northern coast of the Gulf of Mexico (hereafter Gulf) for several decades (Hood and Strelcheck, 2007). Commercial landings of red snapper in the Gulf, for example, averaged 1630 metric tons between 2010 and 2012, with an average dockside value of $11.7 million (http://www.st.nmfs.noaa.gov/commercial-fisheries/commercial-landings/annual-landings/index); while recreational fishing in the Gulf in 2011 involved >375,000 targeted trips and $52.8 million in economic impact (GMFMC, 2013). Red snapper in the two regions are currently managed as separate stocks, in part because there is no evidence that red snapper in the Atlantic and Gulf should be managed as a single stock (SEDAR, 2010), and in part because different management councils are responsible for fishery resources in each region. The focus of red snapper management in U.S. waters primarily has been the Gulf where the stock was considered overfished and to be undergoing overfishing since at least the late 1980s (Strelcheck et al., 2007; NOAA, 2012); the Gulf stock is still considered overfished but not undergoing overfishing (SEDAR, 2013). The most recent assessments (SEDAR, 2009, 2010) of red snapper in the Atlantic indicate that the stock in that region is overfished and that overfishing is occurring at several times the sustainable level. Factors impacting red snapper decline in both regions include mortality due to directed fisheries and habitat alteration and degradation. Mortality of juveniles taken as bycatch in the shrimp fishery and seasonal hypoxic zones may also have been important factors in the Gulf (Christman, 1997; Schirripa and Legault, 1999; SEDAR, 2013).

Understanding the nature of stock structure is important in management of exploited marine fishes. Failure to recognize cryptic stocks can lead to over-exploitation and depletion of an undetected stock, potentially resulting in loss of unique genetic

* Corresponding author.
E-mail address: christopher.hollenbeck@tamucc.edu (C.M. Hollenbeck).

http://dx.doi.org/10.1016/j.fishres.2015.06.020
0165-7836/© 2015 Elsevier B.V. All rights reserved.

resources and compromised long-term sustainability of the fishery (Carvalho and Hauser, 1994; Begg et al., 1999; Hilborn et al., 2003). Knowledge of population or stock structure also is critical for rebuilding and restoring depleted stocks, in part to anticipate patterns of recruitment (Ruzzante et al., 1999), and in part to inform management decisions if the future might involve restoration enhancement (Bell et al., 2006), and/or establishment of a protected or restricted fishing areas, e.g., Marine Protected Areas or MPAs (Botsford et al., 2003).

Prior studies of population structure of red snapper in U.S. waters largely have involved genetic markers and samples of adults from the directed fishery in the Gulf. The early work (Camper et al., 1993; Gold et al., 1997; 2001; Heist and Gold, 2000 Heist and Gold, 2000) utilized homogeneity tests of spatial distributions of microsatellite alleles and genotypes and/or haplotypes of mitochondrial (mt)DNA) and found little evidence of population subdivision. Saillant and Gold (2006), however, found significant differences in contemporary (variance) effective size among red snapper sampled from offshore of Alabama, Louisiana, and Texas, and Saillant et al. (2010) found significant heterogeneity in microsatellite allele and genotype distributions among age-0 red snapper sampled at small spatial scales in the western and central Gulf. They (*Ibid*) also found a significant, positive spatial autocorrelation of microsatellite genotypes in age 0 fish within a geographic range of 50–100 km. The lone genetic study of red snapper from the Atlantic (Garber et al., 2004) involved a comparison of sequences of the hypervariable, mtDNA control region among five geographic localities, one in the Atlantic (35 fish) and four in the Gulf (140 fish); no differences in mtDNA haplotype frequencies were detected, consistent with the null hypothesis that red snapper in the Atlantic and Gulf comprise a single, genetic population.

Studies of life history have shown differences in demographics between red snapper in the Atlantic and Gulf (Brown-Peterson et al., 2009) and among red snapper in the Gulf (Fischer et al., 2004; Jackson et al., 2007). Tagging studies to assess movement of red snapper between the Atlantic and the Gulf are limited to studies by Burns et al. (2004, 2008) where more than 5200 red snapper were tagged and released in the Atlantic and Gulf over a 13-year period. Roughly ~40% of the fish were released in the Atlantic. The majority of recaptures were taken within 3 km of the tagging site and only one fish (tagged in the Florida panhandle and recaptured on the Atlantic coast of Florida) moved between regions. Studies based on tagging and/or ultrasonic telemetry of red snapper in the Gulf are equivocal; some have indicated relatively high site fidelity (Szedlmayer, 1997; Schroepfer and Szedlmayer, 2006), while others (Patterson et al., 2001; Patterson and Cowan 2003) have not. The low site fidelity reported, however, may have been due to seasonal conditions and hurricanes (Watterson et al., 1998; SEDAR, 2010). Two tagging studies carried out within the Atlantic indicated relatively limited movement within that region (SEDAR, 2009).

In this study, we used nuclear-encoded microsatellites and sequences of mtDNA to assess genetic population structure of red snapper sampled from five localities in the Atlantic and three localities in the northeastern Gulf. Our primary interests were to test the (null) hypothesis that red snapper in the Atlantic and Gulf are genetically homogeneous and to assess patterns of genetic diversity of red snapper in the Atlantic. We also assessed genetic connectivity between the two regions and genetic effective size at each sample locality and within each region. Information regarding connectivity is important to understanding and managing marine biota whose biogeographic distributions cross political boundaries (Kough et al., 2013), and effective size is important because it provides information regarding standing levels of neutral genetic diversity and a population's capacity to respond to changing or novel environmental pressures (Frankham, 1995; Allendorf and Waples, 1996; Higgins and Lynch, 2001).

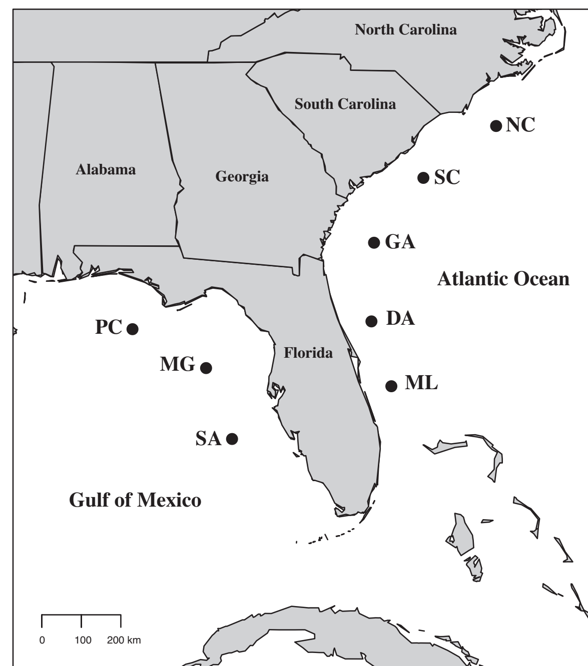

Fig. 1. Approximate sampling localities for red snapper (*Lutjanus campechanus*) in the U.S. Atlantic and eastern Gulf of Mexico. PC: Panama City, MG: Middle Grounds, SA: Sarasota, ML: Melbourne, DA: Daytona, GA: Georgia, SC: South Carolina, NC: North Carolina.

2. Methods

A total of 669 adult red snapper were sampled dockside between 2008 and 2011 from boats fishing offshore of North Carolina (NC), South Carolina (SC), Georgia (GA), Daytona, Florida (DA), and Melbourne, Florida (ML) in the Atlantic, and offshore of Sarasota, Florida (SA), the Florida Middle Grounds (MG), and Panama City, Florida (PC) in the Gulf. Approximate fishing localities are indicated in Fig. 1. Tissue samples (fin clips) were obtained by personnel from several state or federal agencies and fixed in 10% DMSO buffer (Seutin et al., 1991). DNA was extracted following a modified chelex extraction protocol (Estoup et al., 1996); following final centrifugation, 1 μL of the supernatant was used as the template in subsequent polymerase-chain-reaction (PCR) amplification.

All fish were genotyped at 16 nuclear-encoded microsatellites, following multiplex PCR protocols described in Renshaw et al. (2006) and using PCR primers described in Bagley and Geller (1998) and Gold et al. (2001). Amplicons were electrophoresed and visualized on 6% polyacrylamide gels, using an ABI Prism 377 automated sequencer (Applied Biosystems). Allele-calling was conducted manually, using Genescan v.3.1.2 (Applied Biosystems Inc., Warrington, UK) and Genotyper v.2.5 (PerkinElmer). A fragment (590 base pairs) of the mitochondrially-encoded NADH dehydrogenase subunit 4 (ND4) gene was amplified from 20 individuals at each locality, using primers ND4LB (Bielawski and Gold, 2002) and NAP2 (Arèvalo et al., 1994). Thirty microliter PCR reactions consisted of 1x reaction buffer, 1.45 mM $MgCl_2$, 0.25 mM of each dNTP, 30 pmol of each primer, 0.1 U/μL *Taq* polymerase, and 2 μL of DNA template. Reaction conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. Amplified products were purified with ExoSAP-IT^{TM} PCR cleanup kit (GE Healthcare, Piscataway, NJ, USA) and sequenced bi-directionally, using BigDye Terminator v.3.1Cycle Sequencing Kit (Applied Biosystems). Five microliter sequencing reactions consisted of 10–50 ng of template, 0.5 μL of BigDye master mix,

0.875 μL of BigDye 5× reaction buffer, and 32 pmol of forward or reverse primer. Sequencing conditions consisted of denaturation at 96 °C for 1 min followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. Amplifications were electrophoresed on an ABI 3100 Sequencer (Applied Biosystems) through 50 cm capillaries. Sequence chromatograms were aligned and trimmed to a common 590 base pair region, using Sequencher 4.8 (Gene Codes Corporation).

Conformance to expectations of Hardy–Weinberg equilibrium (HWE) was tested for each microsatellite in each locality, using exact tests as implemented in Genepop v.4.0.7 (Raymond and Rousset, 1995; Rousset, 2008). Parameters of the Markov Chain employed in estimation were 10,000 dememorizations, 1000 batches, and 10,000 iterations per batch. Sequential Bonferroni correction (Rice, 1989) was applied for multiple tests. Possible occurrence of scoring error due to stuttering, large allele dropout, and/or null alleles was evaluated for each sample, using Microchecker (Van Oosterhout et al., 2004). Tests of genotypic disequilibrium between pairs of microsatellites within each locality were carried out using Arlequin v.3.5 (Excoffier and Lischer, 2010). Number of alleles, allelic richness, unbiased gene diversity (expected heterozygosity), and the inbreeding coefficient F_{IS} (Weir and Cockerham, 1984) were generated for each microsatellite in each locality, using Fstat v.2.9.3.2 (Goudet, 2001). Homogeneity of allelic richness and unbiased gene diversity among localities was assessed using Friedman rank tests, as implemented in R (R Core Team, 2013). For mtDNA sequences, number of haplotypes, haplotype diversity (h), and nucleotide diversity (π) were obtained for each sample, using Arlequin.

The degree of divergence in microsatellites and mtDNA between pairs of localities was estimated as F_{ST} and Φ_{ST}, respectively, using Arlequin. For mtDNA, Φ_{ST} values were estimated under a Tamura-Nei substitution model (Tamura and Nei, 1993) with a gamma shape parameter of 0.198, as selected by jModelTest v.2.1.1 (Guindon and Gascuel, 2003; Darriba et al., 2012). Significance of F_{ST} and Φ_{ST} values between pairs of localities was assessed by permuting individuals between localities 10,000 times. Hierarchical analysis of molecular variance (AMOVA), as implemented in Arlequin, was conducted for both microsatellites and mtDNA by grouping Atlantic localities (NC, SC, GA, DA, and ML) and Gulf localities (SA, MG, and PC) separately; significance of the between-group component of variance was assessed by permuting sample localities between groups 10,000 times.

Mantel tests, implemented in Arlequin, were used to test for a correlation between genetic distance and geographic distance for both microsatellite genotypes and mtDNA haplotypes. Distance matrices contained pairwise measures of genetic distance, coded as Cavalli-Sforza/Edwards chord distance (Cavalli-Sforza and Edwards, 1967 microsatellites) or Φ_{ST}/1-Φ_{ST} (mtDNA), and approximate coastline geographic distance, and were permuted 10,000 times to assess significance. Hudson's (2000) S_{nn} approach was used to determine whether 'nearest neighbor' mtDNA haplotypes (in terms of sequence identity) were sampled within a region (Atlantic and Gulf) more often than would be expected in a randomly mating population. Last, a median-joining network of mtDNA haplotypes was constructed using Network v.4.6.11 (http://www.fluxus-engineering.com/) in order to visualize spatial relationships among mtDNA haplotypes.

The program Migrate-n v.3.2.16 (Beerli and Felsenstein, 2001; Beerli, 2006) was used to evaluate the marginal likelihood (relative probability) of two different, population-structure models: (i) sampled localities represent a single admixed population (one stock), and (ii) samples were drawn from separate Gulf and Atlantic stocks, with limited migration (gene flow) between the regions. Migrate-n computes the marginal likelihood of pre-defined population models, using factor analysis implemented under a Bayesian coalescent framework (Beerli and Palczewski, 2010); different models can thus be explored and the relative probability of each model compared. Both stock models were run in five random subsamples of 50 individuals, each with two replicates (ten total runs), with a burn-in period of 100,000 steps, followed by 1×10^7 steps, and with trees sampled every 200 steps; a total of 50,000 trees were sampled.

We estimated the average, long-term migration rate (m) between the Atlantic and Gulf, using the Bayesian approach in Migrate-n to estimate the parameter M (mutation-scaled migration rate), where $M = m/\mu$ and μ is the modal mutation rate across all microsatellites per generation. A random subsample ($n = 50$; the smallest individual sample size) was drawn from pooled samples from the Atlantic and pooled samples from the Gulf and replicate runs were combined to generate parameter estimates of M. Estimates of μ were obtained using the Bayesian coalescent approach implemented in Msvar v1.3 (Beaumont, 1999; Storz and Beaumont, 2002); Boa (Smith, 2005) was used to calculate the 95% highest posterior density (HPD) intervals for the modal value of μ. Lower and upper bounds of m were estimated using 95% HPD intervals of M generated by Migrate-n.

Estimates of the effective number of breeders (N_b) were generated for each sample locality, using microsatellite data and the linkage disequilibrium method implemented in LDNe (Waples, 2006; Waples and Do, 2008). Rare alleles below a frequency of 0.02 were excluded from calculations; confidence intervals were obtained by jackknifing. Estimates of average, long-term effective population size (N_{eLT}) for each sample locality were generated using microsatellite data and Migrate-n. A random subsample ($n = 50$) was drawn from each sample locality and replicate runs were combined to generate parameter estimates of theta (θ), where $\theta = 4N_e\mu$; N_e is the average, long-term effective population size (N_{eLT}) and μ is the modal mutation rate across all microsatellites per generation. Estimates of N_{eLT} for the Atlantic (localities pooled), Gulf (localities pooled), and overall (all localities pooled) also were generated from random subsamples ($n = 50$) where replicate runs were combined to generate parameter estimates of θ. Estimates of μ were obtained as above. Lower and upper bounds for N_{eLT} were estimated using 95% HPD intervals of θ generated by Migrate-n and the modal value of μ.

3. Results

Significant deviations from expectations of HWE equilibrium prior to Bonferroni correction were found in 13 of 128 tests; no significant deviations were found following correction. Possible null alleles, as inferred by Microchecker, were detected at *Lca*107 (NC), *Ra*6 (SA), *Lca*43 (MG), and *Prs*221 (PC); no evidence of gel scoring errors caused by stuttering or large allele dropout was detected. Following Bonferroni correction, two pairwise tests of genotypic disequilibrium were significant: *Lca*20-*Lca*107 and *Lca*20-*Prs*328, both in SC. A summary of general statistics for microsatellites and mtDNA is given in Appendix A. For microsatellites, mean (± SE) number of alleles across sample localities ranged from 7.44 ± 1.11(PA) to 9.69 ± 1.12 (ML); mean (± SE) allelic richness ranged from 7.40 ± 1.11 (PC) to 8.12 ± 0.97 (ML); and mean (± SE) gene diversity ranged from 0.583 ± 0.05 (PC) to 0.596 ± 0.05 (ML). Friedman rank tests of homogeneity across sample localities in allelic richness (A_R) and gene diversity (H_E) were non-significant (A_R: $\chi^2_{[7,15]} = 4.01$, $P = 0.778$; H_E: $\chi^2_{[7,15]} = 3.12$, $P = 0.874$). For mtDNA, 39 haplotypes were found among the 160 individuals surveyed. The distribution of haplotypes in the eight sample localities (Appendix B) consisted primarily of two common haplotypes (#2 and #4) and numerous rare haplotypes. A total of 21 haplotypes were unique to the Atlantic, while 12 haplotypes were unique to the Gulf. Previously undescribed haplotypes were submitted

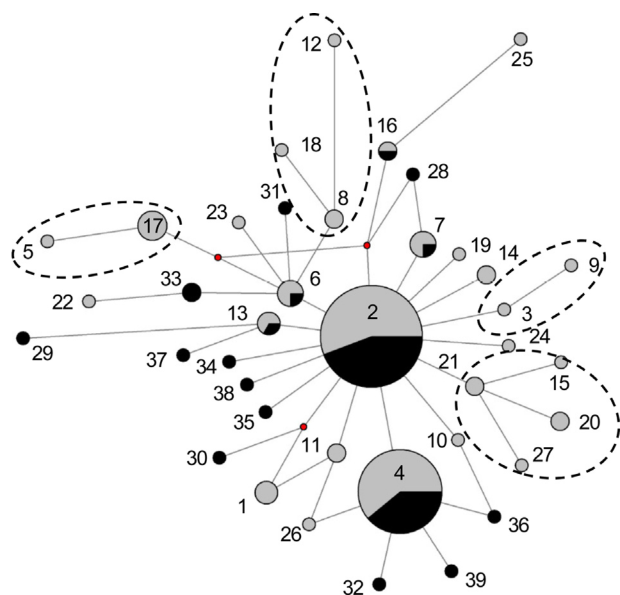

Fig. 2. Median-joining network of ND4 mtDNA haplotypes: gray, haplotypes found in the Atlantic; black, haplotypes found in the Gulf. Each node (small circle) represents a unique haplotype; sizes of nodes are scaled to reflect the relative frequency of each haplotype. Lengths of lines connecting haplotypes reflect number of single-base substitutions between haplotypes; the shortest line is one base-pair substitution. Small nodes indicated by small, unnumbered circles are inferred mtDNA haplotypes. Dotted lines surround putative clades found in the Atlantic.

to GenBank (NCBI), with accession numbers KT201529-KT201542. Estimates of haplotype (h) and nucleotide (π) diversity (Appendix A) ranged from 0.658 (PC) to 0.905 (ML) and 0.050 (MG) to 0.185 (ML), respectively.

Pairwise estimates of F_{ST} (microsatellites) and Φ_{ST} (mtDNA) are given in Table 1; none of the pairwise comparisons of F_{ST} or Φ_{ST} values differed significantly from zero. Results of hierarchical AMOVA (Table 2) indicated that the component of molecular variance attributable to differences between regions (Atlantic versus Gulf) and among localities within regions was non-significant for microsatellites and mtDNA. These results are consistent with previous studies of red snapper adults in the Gulf (Gold et al., 1997, 2001; Heist and Gold, 2000) where significant genetic differences in microsatellite allele/genotype and mtDNA haplotype distributions, using similar analytical approaches, have not been detected.

Mantel tests for correlation between genetic distances and geographic distances were significant for both microsatellites ($P = 0.040$) and mtDNA ($P = 0.023$), indicating that both microsatellite genotypes and mtDNA haplotypes are not distributed randomly across the sampled range. Hudson's nearest-neighbor test, based on mtDNA haplotypes, was significant ($S_{nn} = 0.566$, $P = 0.038$) when pooled samples from the Atlantic were compared with pooled samples from the Gulf, indicating that haplotypes sampled from either region were more closely related in sequence to other haplotypes from the same region than would be expected in a randomly mating population. The median-joining network of mtDNA haplotypes (Fig. 2) grouped by region (Gulf and/or Atlantic) also was consistent with slight differences between regions as at least four putative clades of Atlantic-only haplotypes were recovered, whereas no clades of haplotypes were evident in the Gulf. The difference in number of putative clades may be in part a result of the difference in sample size (60 in the Gulf vs. 100 in the Atlantic), as more unique, rare haplotypes (21 vs. 12) were sampled in the Atlantic. Bayes factor analysis (microsatellite data) yielded mixed results; eight of ten runs indicated that a two-stock model (Atlantic and Gulf) had the highest marginal likelihood ($P > 0.999$) when compared to a one-stock model (all sample localities pooled).

The estimate of average, long-term migration rate (m) from the Atlantic to the Gulf was 0.21% (95% CI: 0.04%–0.77%), while the rate from the Gulf to the Atlantic was 0.33% (95% CI: 0.07%–1.11%). The parameter m is defined as the proportion of individuals in a population that are of immigrant origin and is often expressed as the parameter mN_e, the effective number of migrants entering a subpopulation each generation (Mills and Allendorf, 1996). Because the estimates of m and N_{eLT} (see below) for the two regions did not differ significantly, we used average estimates of m (0.0027) and N_{eLT} (4022) to generate an average, long-term estimate of 10.86 (mN_e), the effective number of migrants per generation moving in either direction from one region to the other.

Mean estimates of the effective number of breeders (N_b) for four of the eight sample localities were infinite, as were upper bounds for seven of the eight (data not shown but available from CMH), suggesting a uniformly large N_b across the localities sampled (Waples and Do, 2010). However, estimates of N_b generated from adult samples are complex and difficult to interpret because they are influenced by the effective number of breeders that generated each cohort in a sample (Waples, 2005). Red snapper can live for over 50 years (Wilson and Nieland, 2001) and mature sexually between age two and four (Schirripa and Legault, 1999; Fitzhugh et al., 2004). Because virtually all of the red snapper sampled in this study were adults, fish in each sample were potentially a mix of overlapping sets of parents in different years, making interpretation of N_b estimates problematic (Waples, 2010).

Estimates of N_{eLT} and 95% confidence intervals for each sample locality, for the Atlantic (localities pooled) and Gulf (localities pooled), and overall (all localities pooled) are given in Table 3. Estimates of N_{eLT} for the eight sample localities ranged from 826.1 (PC) to 2111.3 (SA) and did not differ significantly from one another. The global estimate of N_{eLT} for the Atlantic (3930.1; 95% CI: 3104.3–5,023.76.02) was significantly larger than estimates of N_{eLT} for each of the five sample localities from the Atlantic; while the global estimate of N_{eLT} for the Gulf (4114.1; 95% CI: 3204.5–4,923.5) was significantly larger than estimates of N_{eLT} for two (MG and PC) of the three sample localities in the Gulf (Table 3). The overall global estimate of N_{eLT} (6267.1; 95% CI: 5474.3–7,076.5) was significantly larger than global estimates of N_{eLT} for either region.

Table 1
Estimates of F_{ST} (microsatellites, upper diagonal) and Φ_{ST} (mtDNA, lower diagonal) for pairwise comparisons of sample localities: North Carolina (NC), South Carolina (SC), Georgia (GA), Daytona, FL (DA), Melbourne, FL (ML), Sarasota, FL (SA), Florida Middle Grounds (MG), and Panama City, FL (PC).

	NC	SC	GA	DA	ML	SA	MG	PC
NC	0	0.001	0.001	0.001	0	0	0.002	−0.001
SC	0.046	0	−0.001	−0.001	0	-0.001	0	0.001
GA	−0.023	0.036	0	0.001	−0.001	-0.001	0	0.002
DA	0.005	−0.018	−0.004	0	0	0.002	0	0.002
ML	−0.024	0.02	−0.036	−0.076	0	0	0	−0.001
SA	0.015	0.027	0.011	−0.023	−0.025	0	0.001	-0.001
MG	0.012	-0.028	0.018	−0.012	−0.087	−0.04	0	0
PC	0.092	0.056	0.059	−0.04	−0.025	−0.019	-0.025	0

Table 2
Hierarchical analysis of molecular variance (AMOVA), based on 16 microsatellite loci. Variance components attributed to between regions and among localities within regions were non-significant ($\alpha = 0.05$); d.f. = degrees of freedom; F = fixation index; P = probability that $F = 0$.

Source of Variation	d.f.	Sum of squares	Variance component	% of variation	F	P
Microsatellites						
Between regions (Atlantic and Gulf)	1	5.12	0.0007	0.02	0.0002	0.303
Among localities within regions	6	28.41	0.0003	0.01	0.0001	0.505
Within localities	1330	6227.98	4.6827	99.98	0.0002	0.444
Mitochondrial DNA						
Between regions (Atlantic and Gulf)	1	3.11	0.02	1.21	0.012	0.106
Among localities within regions	6	8.68	-0.02	-1.05	0.002	0.7
Within localities	153	278.73	1.83	99.84	-0.01	0.598

Table 3
Estimates and 95% confidence intervals of average, long-term effective population size (NeLT) for each sample locality, the Atlantic and Gulf (localities in each region pooled), and overall (all localities pooled).

	N_{eLT}		
	2.5	Mode	97.5
NC	717.7	1343.5	1969.4
SC	567.5	1143.3	1719.1
GA	433.9	1009-7	1585.5
ML	901.3	1510.4	2153
DA	884.6	1443.7	2019.5
SA	1168.3	2111.3	3304.6
MG	901.3	1477.1	2036.2
PC	333.8	826.1	1285.1
Atlantic	3104.3	3930.48	5023.7
Gulf	3204.5	4114.1	4923.5
All	5474.3	6267.1	7076.5

4. Discussion

Initial tests (F_{ST}-based, hierarchical AMOVA) of spatial genetic homogeneity within and between regions were non-significant, consistent with a single population or stock of red snapper along the U.S. southeast Atlantic coast and in the northeastern Gulf of Mexico; however, inferences derived from other statistical approaches indicated genetic and/or demographic differences among red snapper within and between the two regions. The significant correlation between genetic and geographic distances for both microsatellites and mtDNA indicated an isolation-by-distance effect, and Hudson's nearest-neighbor test indicated mtDNA haplotypes sampled within a region (Atlantic or Gulf) were more closely related in sequence to other haplotypes from the same region than would be expected in a randomly mating (panmictic) population. Small, mtDNA haplotype clades that were unique to a region were found only in the Atlantic. Bayes factor analysis (microsatellite data) also supported a two-stock (Atlantic/Gulf) model, and the estimated, average, long-term migration between the two regions (0.27%) was well less than the 10% rate below which populations can respond independently to environmental perturbation (Hastings, 1993; Hauser and Carvalho, 2008; Waples, 2010). Finally, differences between local and global estimates of N_{eLT} were consistent with genetic and/or demographic heterogeneity across the sampled area (see below).

The above results are very similar to those of prior genetic studies of red snapper adults from the Gulf where inferences of spatial genetic structure have relied on historical patterns of mtDNA diversification (Pruett et al., 2005), differences in contemporaneous genetic effective size (Saillant and Gold, 2006), or tests of the spatial distribution of microsatellite alleles, including an isolation-by-distance effect, in age-0 fish (Saillant et al., 2010). The isolation-by-distance effect observed by Saillant et al. (2010), however, differs from that found in this study. Their study (*Ibid*) assessed the spatial distribution of age-0 juveniles, presumably reflecting average movement from nursery areas across the region, whereas this study assessed the spatial distribution of adults sampled from the directed fishery. In general, isolation by distance occurs when the rate of gene exchange between distinct subpopulations is a function of geographic distance or when dispersal within a continuously distributed population becomes restricted (Hardy and Vekemans, 1999). The studies of red snapper adults from across the northern Gulf have not detected an isolation-by-distance effect in either microsatellites or mtDNA sampled over a distance of ~1,600 km (Gold et al., 1997; 2001; Saillant and Gold, 2006 Saillant and Gold, 2006), suggesting that genetic divergence in adult red snapper is not necessarily a function of geographic distance. Consequently, we interpret the isolation-by-distance effect observed here to largely reflect limited gene flow between the two regions, given that the distance sampled within each region (~835 km in the Atlantic and ~475 km in the Gulf) is considerably less than that sampled previously across the Gulf.

Our primary interests in the study were to test the (null) hypothesis that red snapper in the Gulf and Atlantic are genetically homogeneous and to assess patterns of genetic diversity of red snapper in the Atlantic. While there were no significant fixation indices between individual sample locations, we did find genetic evidence of different populations in each region, consistent with current management of red snapper resources in U.S. waters (SEDAR 2010, 2013) and with numerous lines of non-genetic evidence. The latter include tag recoveries indicating very limited movement between the regions (Burns et al., 2004; Burns et al., 2004) and low abundance in the Florida Keys, especially along the southeast coast of Florida (Moe, 1963). Levels of genetic variability (allelic richness, gene and haplotype diversity) among the samples from both regions were essentially the same as found previously for adults in the Gulf (Gold et al., 2001; Pruett et al., 2005; Saillant and Gold, 2006), and estimates of average, long-term effective size (N_{eLT}) did not differ among the sample localities. However, the global estimate of N_{eLT} for the Atlantic was significantly larger than estimates of N_{eLT} for the five localities sampled from the Atlantic. Similar results were obtained from the Gulf (the global estimate was significantly larger than estimates for two of the three localities sampled) and overall (the global estimate was significantly larger than the estimates for the Atlantic and Gulf). These comparisons are inconsistent with the null hypothesis that samples from the Atlantic, Gulf, or overall were drawn from a single, well-mixed population, given that N_e in samples drawn from a single, panmictic unit should approximate global N_e, while N_e from a subdivided population should be less than the global N_e (Waples 2010). Inferences from prior genetic studies (Pruett et al., 2005; Saillant et al., 2010) have suggested that genetic patterns among red snapper in the Gulf are consistent with the metapopulation-type structure envisaged by Kritzer and Sale (2006) where connectivity between local populations is variable and where there is geographic asynchrony in demographics across the metapopulation. Data from this study do not refute the existence of this type of metapopulation structure. A conservative management strategy might be to account for this

type population structure, particularly given the economic importance of the species.

The issues associated with management of exploited marine species with a metapopulation-type structure are beyond the scope of this paper and are discussed in Grimm et al. (2003) and Kritzer and Sale (2006). Two points, however, warrant mention: (i) the viability and sustainability of a metapopulation depends on the included local populations and their interactions with one another (Akçakaya et al., 2007), and (ii) unless isolation is essentially complete, the loss of genetic diversity within a metapopulation via genetic drift is determined primarily by the global or metapopulation effective size (N_e) rather than the effective sizes of local subpopulations or demes (Waples, 2010). This suggests that future genetics studies and other work on red snapper in both the Atlantic and Gulf should include approaches to identify demographically independent units within the fishery and assess their demographics (e.g., size, patterns of connectivity, and contribution to the fishery) and to continually monitor global and/or local effective size. The former will not be an easy task because well designed, replicative spatial/temporal sampling will be needed; the importance of doing so is in identifying spatial and temporal units (local populations) that are disproportionally contributing to recruitment and to the fishery. The latter (monitoring global or local N_e) is more straightforward but will require sampling of individuals from the same cohort in different local populations across time in order to utilize recent statistical approaches (Waples et al., 2014) that generate single-sample estimates of contemporaneous N_e.

Genetic and other data obtained to date are consistent with existence of distinct populations of red snapper in U.S. waters (Atlantic and Gulf) which exchange small numbers of migrants over generational time scales. Further identifying discrete subunits within red snapper populations, using microsatellites and mtDNA, will likely be ineffective, in part because of insufficient resolution related to too few markers, but also because of incomplete lineage sorting of ancestral polymorphisms in a species that only recently in evolutionary time colonized its current habitat (Pruett et al., 2005; Orozco-Terwengel et al., 2011). Next-generation sequencing approaches (Davey et al., 2011) that allow genome-wide surveys of thousands of genetic markers, including those that may be associated with fitness on local scales (Nielsen et al., 2009; Allendorf et al., 2010), should be informative in better understanding population dynamics and maintaining genetic and phenotypic diversity in exploited species such as red snapper. One final point to note is that genetic studies, using microsatellites and mtDNA, of four other, exploited snapper species in the Atlantic and Gulf and in U.S. waters of the Northern Antilles also relied on isolation by distance and historical demographics rather than statistical heterogeneity in F_{ST}-based tests to assess population subdivision in each species (Tringali and Higham, 2007; Gold et al., 2009; Carson and Saillant, 2011; Saillant et al., 2012). Snappers (Lutjanidae) are found in tropical and subtropical regions over much of the world (Allen, 1985) and constitute an important food resource, especially in developing countries (Russ and Alcala, 1989; Blaber et al., 2005). A better understanding of population dynamics in red snapper in U.S. waters may thus be useful in conserving marine resources elsewhere.

Acknowledgments

We thank C. Collier and M. Duval (North Carolina Department of Environment and Natural Resources), M. Reichert (South Carolina Department of Natural Resources), K. Knowlton and S. Woodward (Georgia Department of Natural Resources), J. Bogdan, B. Kalmeyer, K. Kowal, B. McMichael, and E. Sanders (Florida Wildlife Research Institute), and K. Brennan and B. Walling (National Marine Fisheries Service) for providing tissue samples, D. Kulaw and J. Puritz for helpful comments on a draft of the manuscript, and B. Sauls for providing information on red snapper catches in southeast Florida. Work was supported by the Marfin Program of the US Department of Commerce (Grant NA10NMF4330114) and Texas AgriLife under Project H-6703. The paper is contribution 7 of the Marine Genomics Laboratory at Texas A&M University-Corpus Christi and Number 101 in the series 'Genetic Studies in Marine Fishes.'

Appendix A

Summary statistics for red snapper (*Lutjanus campechanus*) microsatellite and mtDNA loci at each locality. For microsatellites: n = number of individuals sampled, #A = number of alleles, AR = allelic richness, H_E = expected heterozygosity, P_{HW} = probability of conformance to Hardy–Weinberg expec-

Sample		NC	SC	GA	DA	ML	SA	MG	PC
Microsatellite									
Lca20	n	93	84	101	98	101	48	97	46
	#A	4	5	5	4	5	5	5	3
	A_R	3.8	4.05	3.72	3.3	4.25	4.93	4.03	2.98
	H_E	0.19	0.179	0.208	0.162	0.177	0.196	0.174	0.198
	P_{HW}	0.009	1	0.063	0.115	0.116	0.41	0.101	0.852
	F_{IS}	0.152	−0.067	0.094	0.182	0.049	0.15	−0.009	−0.099
Lca22	n	93	84	101	96	100	47	97	46
	#A	14	12	12	12	13	9	11	8
	A_R	12.06	10.35	11.03	11.1	10.9	8.95	9.45	7.98
	H_E	0.751	0.726	0.727	0.783	0.725	0.693	0.752	0.761
	P_{HW}	0.265	0.629	0.21	0.077	0.304	0.032	0.23	0.661
	F_{IS}	0.055	−0.034	0.087	0.082	0.062	−0.043	0.04	−0.028
Lca43	n	93	85	101	98	101	48	97	46
	#A	7	8	9	9	9	7	8	7
	A_R	6.4	7	7.61	7.58	7.8	6.99	7.34	6.94
	H_E	0.491	0.581	0.539	0.526	0.583	0.56	0.56	0.589
	P_{HW}	0.597	0.127	0.123	0.486	0.366	0.745	0.011	0.196
	F_{IS}	0.08	−0.013	0.026	−0.029	−0.018	0.033	0.19	−0.107
Lca64	n	93	84	101	98	101	48	97	46
	#A	11	12	11	12	9	8	9	6
	A_R	9.17	10.23	8.67	9.48	7.8	7.81	7.62	5.98
	H_E	0.789	0.791	0.782	0.801	0.774	0.713	0.77	0.736
	P_{HW}	0.075	0.792	0.88	0.953	0.808	0.468	0.177	0.384
	F_{IS}	−0.077	0.007	0.013	−0.032	-0.036	0.065	0.023	−0.094

Sample		NC	SC	GA	DA	ML	SA	MG	PC
Lca91	n	93	85	101	98	101	47	97	46
	#A	6	7	6	7	6	6	7	4
	A_R	5.19	5.89	5.65	5.91	5.17	5.96	5.6	3.98
	H_E	0.577	0.565	0.613	0.579	0.573	0.627	0.562	0.557
	P_{HW}	0.31	0.966	0.252	0.982	0.049	0.012	0.514	0.824
	F_{IS}	−0.025	0.042	0.046	−0.041	0.118	0.049	0.028	0.103
Lca107	n	93	84	98	98	101	48	97	46
	#A	9	8	9	10	10	9	10	10
	A_R	8.46	7.98	8.41	9.15	9.22	8.93	9.36	9.96
	H_E	0.776	0.801	0.781	0.814	0.781	0.775	0.8	0.767
	P_{HW}	0.145	0.714	0.034	0.912	0.272	0.119	0.495	0.027
	F_{IS}	0.099	0.019	0.059	0.035	0.023	−0.021	0.021	0.065
Prs55	n	93	84	101	98	101	48	97	46
	#A	7	5	6	4	7	4	6	4
	A_R	5.5	4.06	5.27	3.45	5.19	3.93	4.6	3.98
	H_E	0.271	0.265	0.253	0.256	0.297	0.193	0.251	0.255
	P_{HW}	0.1	0.272	0.753	0.426	0.942	1	0.867	0.611
	F_{IS}	0.166	−0.032	−0.057	0.082	−0.034	−0.077	−0.11	0.062
Prs137	n	93	85	101	98	99	47	97	46
	#A	13	12	11	11	12	12	11	8
	A_R	10.24	9.78	9.09	9.34	9.63	11.74	8.83	7.96
	H_E	0.732	0.68	0.721	0.676	0.72	0.694	0.682	0.676
	P_{HW}	0.013	0.613	0.13	0.513	0.032	0.714	0.054	0.102
	F_{IS}	0.105	0.049	0.066	0.019	0.031	0.049	0.063	0.131
Prs221	n	93	84	101	98	101	48	97	46
	#A	12	12	12	13	15	13	16	11
	A_R	10.67	10.23	10.78	10.93	11.93	12.68	12.54	10.91
	H_E	0.766	0.772	0.8	0.767	0.803	0.807	0.797	0.771
	P_{HW}	0.366	0.36	0.522	0.185	0.532	0.625	0.44	0.388
	F_{IS}	0.032	0.06	0.022	0.082	0.075	−0.007	0.056	0.069
Prs240	n	92	81	101	98	100	47	97	46
	#A	18	18	18	19	18	18	20	18
	A_R	17.03	16.79	15.9	16.61	16.88	17.83	17.13	17.87
	H_E	0.902	0.901	0.889	0.899	0.892	0.902	0.907	0.875
	P_{HW}	0.269	0.298	0.983	0.681	0.85	0.926	0.022	0.042
	F_{IS}	−0.037	0.054	−0.002	−0.022	−0.009	−0.038	0.034	0.056
Prs248	n	93	84	101	98	101	48	97	46
	#A	18	17	20	23	18	14	19	16
	A_R	15.3	14.29	15.5	16.6	14.64	13.8	16.05	15.89
	H_E	0.872	0.868	0.888	0.865	0.877	0.88	0.869	0.879
	P_{HW}	0.644	0.337	0.319	0.952	0.407	0.403	0.906	0.551
	F_{IS}	0.001	−0.056	0.052	−0.05	−0.005	0.029	0.015	0.035
Prs260	n	93	85	101	98	101	48	97	46
	#A	5	3	5	5	3	4	3	3
	A_R	3.93	3	4.52	4.17	3	3.94	3	3
	H_E	0.305	0.397	0.398	0.439	0.394	0.379	0.357	0.393
	P_{HW}	0.064	0.218	0.309	0.017	0.814	1	1	0.4
	F_{IS}	0.118	−0.187	−0.019	−0.047	−0.029	−0.044	−0.011	−0.05
Prs275	n	93	85	101	98	101	48	97	45
	#A	6	8	6	7	10	5	7	5
	A_R	5.34	6.64	5.05	5.75	7.33	4.94	5.36	5
	H_E	0.609	0.579	0.564	0.61	0.632	0.603	0.595	0.608
	P_{HW}	0.837	0.511	0.392	0.272	0.93	0.849	0.539	0.446
	F_{IS}	−0.095	−0.057	0.016	−0.121	−0.05	−0.002	0.099	0.05
Prs282	n	93	85	101	98	101	48	97	46
	#A	12	12	11	11	11	11	11	13
	A_R	9.561	10.688	9.188	9.787	9.677	10.681	10.176	12.912
	H_E	0.599	0.66	0.636	0.64	0.684	0.565	0.655	0.693
	P_{HW}	0.27	0.443	0.399	0.895	0.261	0.63	0.594	0.006
	F_{IS}	0.049	0.091	0.082	−0.004	0.073	0.079	−0.022	0.027
Prs328	n	92	84	101	98	101	48	97	46
	#A	3	4	4	4	6	3	4	3
	A_R	3	3.98	3.45	3.63	4.58	3	3.83	3
	H_E	0.547	0.567	0.568	0.531	0.559	0.516	0.546	0.563
	P_{HW}	0.243	0.487	0.106	0.856	0.93	0.592	0.702	0.842
	F_{IS}	−0.173	−0.008	0.128	0.039	-0.01	0.153	−0.075	−0.042
Prs333	n	93	85	101	98	101	46	97	46
	#A	5	5	7	5	7	5	6	6
	A_R	4.85	4.09	5.08	3.76	5.53	4.98	5.01	5.96
	H_E	0.382	0.277	0.336	0.361	0.395	0.401	0.33	0.414
	P_{HW}	0.104	0.7	0.925	1	0.361	0.424	0.604	0.305
	F_{IS}	−0.04	−0.148	−0.032	−0.019	0.073	0.078	0	0.212

Sample		NC	SC	GA	DA	ML	SA	MG	PC
Ra6	n	92	85	101	98	101	48	97	46
	$\#A$	7	7	7	6	7	7	7	7
	A_R	6.44	5.95	5.72	5.41	6.02	6.88	6.03	6.96
	H_E	0.392	0.401	0.384	0.461	0.355	0.506	0.384	0.409
	P_{HW}	0.202	0.134	0.325	0.994	0.467	0.049	0.126	0.958
	F_{IS}	0.085	0.12	−0.032	−0.018	−0.003	0.218	0.14	−0.169
mtDNA									
ND4	n	20	20	20	20	20	20	20	20
	$\#H$	8	11	7	8	11	10	7	6
	h	0.758	0.884	0.784	0.805	0.905	0.842	0.711	0.658
	π	0.091	0.128	0.106	0.066	0.185	0.095	0.05	0.051

tations, and F_{IS} = inbreeding coefficient. For mtDNA: n = number of individuals sampled, #H = number of unique haplotypes observed, h = haplotype diversity, π = nucleotide diversity.

Appendix B

Spatial distribution of mitochondrial (ND4) haplotypes.

Haplotype	NC	SC	GA	DA	ML	SA	MG	PC
#1	1				2			
#2	9	6	8	7	4	6	10	11
#3	1							
#4	5	4	5	6	5	6	5	5
#5	1							
#6	1	1		1		1		
#7	1			1	1			1
#8	1				1			
#9		1						
#10		1						
#11		2						
#12		1						
#13		1	1			1		
#14		1		1				
#15		1						
#16		1						1
#17			3		2			
#18			1					
#19			1					
#20			1		1			
#21				2				
#22				1				
#23				1				
#24					1			
#25					1			
#26					1			
#27					1			
#28						1		
#29						1		
#30						1		
#31						1		
#32						1		
#33						1	1	
#34							1	
#35							1	
#36							1	
#37							1	
#38								1
#39								1

References

Akçakaya, H.R., Mills, G., Doncaster, C.P., 2007. The role of metapopulations in conservation. In: Macdonald, D.W., Service, K. (Eds.), Key Topics in Conservation Biology. Blackwell Publishing, pp. 64–84.

Allen, G., 1985. FAO Species Catalogue Snappers of the World: An Annotated and Illustrated Catalogue of Lutjanid Species Known to Date, vol. 6. Rome, Food and Agriculture Organization of the United Nations.

Allendorf, F.W., Hohenlohe, P.A., Luikart, G., 2010. Genomics and the future of conservation genetics. Nat. Rev. Genet. 11, 697–709.

Allendorf, F.W., Waples, R.S., 1996. Conservation and genetics of salmonid fishes. In: Avise, J.C., Hamrick, J.L. (Eds.), Conservation Genetics: Case Histories from Nature. Chapman & Hall, pp. 238–280.

Arèvalo, E., Davis, S.K., Sites, J.W., 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst. Biol. 43, 387–418.

Bagley, M., Geller, J., 1998. Characterization of microsatellite loci in the vermilion snapper *Rhomboplites aurorubens* (Percoidei: Lutjanidae). Mol. Ecol. 7, 1089–1090.

Beaumont, M.A., 1999. Detecting population expansion and decline using microsatellites. Genetics 153, 2013–2029.

Beerli, P., 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics 22, 341–345.

Beerli, P., Felsenstein, J., 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. 98, 4563–4568.

Beerli, P., Palczewski, M., 2010. Unified framework to evaluate panmixia and migration direction among multiple sampling locations. Genetics 185, 313–326, http://dx.doi.org/10.1534/genetics.109.112532

Begg, G.A., Friedland, K.D., Pearce, J.B., 1999. Stock identification and its role in stock assessment and fisheries management: an overview. Fish. Res. 43, 1–8.

Bell, J.D., Bartley, D.M., Lorenzen, K., Loneragan, N.R., 2006. Restocking and stock enhancement of coastal fisheries: potential problems and progress. Fish. Res. 80, 1–8.

Bielawski, J.P., Gold, J.R., 2002. Mutation patterns of mitochondrial H-and L-strand DNA in closely related cyprinid fishes. Genetics 161, 1589–1597.

Blaber, S.J.M., Dichmont, C.M., Buckworth, R.C., Badrudin, Sumiono, B., Nurhakim, S., Iskandar, B., Fegan, B., Ramm, D.C., Salini, J.P., 2005. Shared stocks of snappers (Lutjanidae) in Australia and Indonesia: Integrating biology, population dynamics and socio-economics to examine management scenarios. Rev. Fish Biol. Fish 15, 111–127.

Botsford, L.W., Micheli, F., Hastings, A., 2003. Principles for the design of marine reserves. Ecol. Appl. 13, S25–S31.

Brown-Peterson, N., Burns, K., Overstreet, R., 2009. Regional differences in Florida red snapper reproduction. Proc. Gulf Caribb. Fish. Inst. 61, 149–155.

Burns, K.M., Brown-Peterson, N.J., Overstreet, R.M., Gannon, J., Simmons, P., Sprinkle, J., Weaver, C., 2008. Evaluation of the efficacy of the current minimum size regulation for selected reef fish based on release mortality and fish physiology. Mote Tech. Rep., 1176.

Burns, K.M., Parnell, N.F., Wilson, R.R., 2004. Partitioning release mortality in the undersized red snapper bycatch: comparison of depth vs. hooking effects. Mote Tech. Rep., 932.

Camper, J.D., Barber, R.C., Richardson, L.R., Gold, J.R., 1993. Mitochondrial DNA variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Mol. Mar. Biol. Biotechnol. 2, 154–161.

Carson, E., Saillant, E., 2011. Population structure, long-term connectivity, and effective size of mutton snapper (*Lutjanus analis*) in the Caribbean Sea and Florida Keys. Fish Bull. 109, 416–428.

Carvalho, G., Hauser, L., 1994. Molecular genetics and the stock concept in fisheries. Rev. Fish Biol. Fish. 4, 326–350.

Cavalli-Sforza, L., Edwards, A., 1967. Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Genet. 19, 233–257.

Christman, M., 1997. Peer Review of Red Snapper (*Lutjanus campechanus*) Research and Management in the Gulf of Mexico: Statistics Review, Office of Science and Technology, NOAA. Silver Springs, MD.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M., Blaxter, M.L., 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat. Rev. Genet. 12, 499–510.

Estoup, A., Largiader, C.R., Perrot, E., Chourrout, D., 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. Mol. Mar. Biol. Biotechnol. 5, 295–298.

Excoffier, L., Lischer, H., 2010. Arlequin suite ver 3. 5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.

Fischer, A.J., Baker, M.S., Wilson, C.A., 2004. Red snapper (*Lutjanus campechanus*) demographic structure in the northern Gulf of Mexico based on spatial patterns in growth rates and morphometrics. Fish. Bull. 102, 593–603.

Fitzhugh, G.R., Duncan, M.S., Collins, L.A., Walling, W.T., Oliver, D.W., 2004. Characterization of red snapper (Lutjanus campechanus) reproduction: for the

2004 Gulf of Mexico SEDAR. NMFS, SEFSC, Panama City, Florida. Contrib. 1–4. Available at: http://www.sefsc.noaa.gov/sedar/download/SEDAR7_DW35.pdf?id=DOCUMENT

Frankham, R., 1995. Effective population size/adult population size ratios in wildlife: a review. Genet. Res. 66, 95–107.

Garber, A.F., Tringali, M.D., Stuck, K.C., 2004. Population structure and variation in red snapper (*Lutjanus campechanus*) from the Gulf of Mexico and Atlantic coast of Florida as determined from mitochondrial DNA control region sequence. Mar. Biotechnol. 6, 175–185.

Gold, J.R., Pak, E., Richardson, L.R., 2001. Microsatellite variation among red snapper (Lutjanus campechanus) from the Gulf of Mexico. Mar. Biotechnol. 3, 293–304.

Gold, J.R., Saillant, E., Ebelt, N.D., Lem, S., 2009. Conservation genetics of gray snapper (*Lutjanus griseus*) in U. S. waters of the Northern Gulf of Mexico and Western Atlantic Ocean. Copeia, 277–286.

Gold, J.R., Sun, F., Richardson, L.R., 1997. Population structure of red snapper from the Gulf of Mexico as inferred from analysis of Mitochondrial DNA. Trans. Am. Fish. Soc. 126, 386–396.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available at: http://www2.unil.ch/popgen/softwares/fstat.htm

Grimm, V., Reise, K., Strasser, M., 2003. Marine metapopulations: A useful concept? Helgoland Marine Research, pp. 222–228.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

GMFMC, 2013. Red Snapper 2013. Quota Increase and Supplemental Recreational Season Including Environmental Assessment, Regulatory Impact Review, and Regulatory Flexibility Act Analysis. Gulf of Mexico Fishery Management Council. Tampa, FL.

Hardy, O.J., Vekemans, X., 1999. Isolation by distance in a continuous population: Reconciliation between spatial autocorrelation analysis and population genetics models. Heredity 83, 145–154.

Hastings, A., 1993. Complex interactions between dispersal and dynamics: lessons from coupled logistic equations. Ecology 74, 1362–1372.

Hauser, L., Carvalho, G.R., 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 9, 333–362.

Heist, E.J., Gold, J.R., 2000. DNA microsatellite loci and genetic structure of red snapper in the Gulf of Mexico. Trans. Am. Fish. Soc. 129, 469–475.

Higgins, K., Lynch, M., 2001. Metapopulation extinction caused by mutation accumulation. Proc. Natl. Acad. Sci. 98, 2928–2933.

Hilborn, R., Quinn, T.P., Schindler, D.E., Rogers, D.E., 2003. Biocomplexity and fisheries sustainability. Proc. Natl. Acad. Sci. U. S. A. 100, 6564–6568.

Hood, P., Strelcheck, A., 2007. A history of red snapper management in the Gulf of Mexico. In: Patterson III, W.F., Cowan Jr., J.H., Fitzhugh, G.R., Nieland, D.L. (Eds.), Red Snapper Ecology and Fisheries in the U.S. Gulf of Mexico. American Fisheries Society, Bethesda, Maryland, pp. 241–256.

Hudson, R.R., 2000. A new statistic for detecting genetic differentiation. Genetics 155, 2011–2014.

Jackson, M.W., Cowan Jr., J.H., Nieland, D.L., Patterson, W., Cowan, Jhj., Fitzhugh, G.R., 2007. Demographic differences in northern Gulf of Mexico red snapper reproductive maturation: implications for the unit stock hypothesis. Am. Fish. Soc. Symp. 60, 197–206.

Kough, A.S., Paris, C.B., Butler, M.J., 2013. Larval connectivity and the international management of fisheries. PLoS One 8, e64970.

Kritzer, J., Sale, P., 2006. Marine Metapopulations. Academic Press.

Mills, L.S., Allendorf, F.W., 1996. The one-migrant-per-generation rule in conservation and management. Conserv. Biol. 10, 1509–1518.

Moe, M.A., Jr., 1963. A survey of offshore fishing in Florida. Professional Papers Series, Number Four. Florida State Board of Conservation. Available at: http://www.sefsc.noaa.gov/sedar/download/SEDAR24-RD08_Moe1963.pdf?id=DOCUMENT

Nielsen, E.E., Hemmer-Hansen, J., Larsen, P.F., Bekkevold, D., 2009. Population genomics of marine fishes: identifying adaptive variation in space and time. Mol. Ecol. 18, 3128–3150.

NOAA, 2012. Status of stocks: Report on the status of U.S. fisheries for 2011—Report to Congress. Available at: http://www.nmfs.noaa.gov/stories/2012/05/docs/status_of_stocks_2011_report.pdf

Orozco-Terwengel, P., Corander, J., SchlÖtterer, C., 2011. Genealogical lineage sorting leads to significant, but incorrect Bayesian multilocus inference of population structure. Mol. Ecol. 20, 1108–1121.

Patterson III, W.F., Watterson, J.C., Shipp, R.L., Cowan Jr, J.H., 2001. Movement of tagged red snapper in the northern Gulf of Mexico. Trans. Am. Fish Soc. 130, 533–545.

Patterson, W.F., Cowan, J.H., 2003. Site fidelity and dispersion of red snapper associated with artificial reefs in the northern Gulf of Mexico. Am. Fish. Soc. Symp. 36, 181–193.

Pruett, C.L., Saillant, E., Gold, J.R., 2005. Historical population demography of red snapper (Lutjanus campechanus) from the northern Gulf of Mexico based on analysis of sequences of mitochondrial DNA. Mar. Biol. 147, 593–602.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. Available at: http://www.r-project.org/

Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ec.

Renshaw, M.A., Saillant, E., Bradfield, S.C., Gold, J.R., 2006. Microsatellite multiplex panels for genetic studies of three species of marine fishes: red drum (*Sciaenops ocellatus*), red snapper (*Lutjanus campechanus*), and cobia (*Rachycentron canadum*). Aquaculture 253, 731–735.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Rousset, F., 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106.

Russ, G., Alcala, A., 1989. Effects of intense fishing pressure on an assemblage of coral reef fishes. Mar. Ecol. Prog. Ser. 56, 13–27.

Ruzzante, D.E., Taggart, C.T., Cook, D., 1999. A review of the evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. Fish. Res. 43, 79–97.

Saillant, E., Bradfield, S.C., 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 J. Mar. Sci. 67, 1240–1250.

Saillant, E., Gold, J.R., 2006. Population structure and variance effective size of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. Fish. Bull. 104, 136–148.

Saillant, E.A., Renshaw, M.A., Cummings, N.J., Gold, J.R., 2012. Conservation genetics and management of yellowtail snapper, *Ocyurus chrysurus*, in the US Caribbean and South Florida. Fish. Manag. Ecol. 19, 301–312.

Schirripa, M.J., Legault, C.M., 1999. Status of red snapper in the US waters of the Gulf of Mexico: updated through 1998, National Marine Fisheries Service, Southeast Fisheries Science Center. Sustainable Fisheries Division contribution SFD-99/00–75. Miami Laboratory, Miami, Florida. Miami, Florida.

Schroepfer, R.L., Szedlmayer, S.T., 2006. Estimates of residence and site fidelity for red snapper *Lutjanus campechanus* on artificial reefs in the northeastern Gulf of Mexico. Bull. Mar. Sci. 78, 93–101.

SEDAR, 2009. Southeast Data, Assessment, and Review: SEDAR 15 Stock Assessment Report 1 (SAR 1) South Atlantic Red Snapper. North Charleston, SC.

SEDAR, 2010. Southeast Data, Assessment, and Review: SEDAR 24 South Atlantic Red Snapper. North Charleston, SC.

SEDAR, 2013. Southeast Data, Assessment, and Review: SEDAR 31 Gulf of Mexico Red Snapper Stock Assessment Report. North Charleston, SC.

Seutin, G., White, B.N., Boag, P.T., 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69, 82–90.

Smith, B.J., 2005. Bayesian output analysis program (BOA) for MCMC. Available at: http://phase.hpcc.jp/mirrors/stat/R/CRAN/doc/packages/boa.pdf

Storz, J.F., Beaumont, M.A., 2002. Testing for genetic evidence of population expansion and contraction: an empirical analysis of microsatellite DNA variation using a hierarchical Bayesian model. Evolution 56, 154–166.

Strelcheck, A.J., Hood, P.B., Patterson III, W.F., Cowan Jr., J.H., Fitzhugh, G.R., Nieland, D.L., 2007. Rebuilding red snapper: Recent management activities and future management challenges. Am. Fish. Soc. Symp. 60, 353–363.

Szedlmayer, S.T., 1997. Ultrasonic telemetry of red snapper, Lutjanus campechanus, at artificial reef sites in the northeast Gulf of Mexico. Copeia 1997, 846–850.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Tringali, M., Higham, M., 2007. Isolation-by-Distance Gene Flow Among Vermilion Snapper (*Rhomboplites aurorubens* Cuvier, 1829) From the Gulf of Mexico and Southeastern United. Gulf Mex. Sci. 1, 2–14.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.

Waples, R.S., 2005. Genetic estimates of contemporary effective population size: to what time periods do the estimates apply. Mol. Ecol. 14, 3335–3352.

Waples, R.S., 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv. Genet. 7, 167–184.

Waples, R.S., 2010. Spatial-temporal stratifications in natural populations and how they affect understanding and estimation of effective population size. Mol. Ecol. Resour. 10, 785–796.

Waples, R.S., Antao, T., Luikart, G., 2014. Effects of overlapping generations on linkage disequilibrium estimates of effective population size. Genetics 197, 769–780.

Waples, R.S., Do, C., 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol. Ecol. Resour. 8, 753–756.

Waples, R.S., Do, C., 2010. Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evol. Appl. 3, 244–262.

Watterson, J.C., Patterson, W.F., Shipp, R.L., Cowan, J.H., 1998. Movement of red snapper, *Lutjanus campechanus*, in the north central Gulf of Mexico: Potential effects of hurricanes. Gulf Mex. Sci. 16, 92–104.

Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution, 1358–1370.

Wilson, C.A., Nieland, D.L., 2001. Age and growth of red snapper Lutjanus campechanus from the northern Gulf of Mexico off Louisiana. Fish. Bull. 99, 653–664.