North American Journal of Fisheries Management 31:209–223, 2011
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ISSN: 0275-5947 print / 1548-8675 online
DOI: 10.1080/02755947.2011.568864

ARTICLE

Genetic Divergence and Effective Size among Lane Snapper in U.S. Waters of the Western Atlantic Ocean

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Abstract
Population structure of lane snapper *Lutjanus synagris* in U.S. waters in the northern Caribbean Sea was assessed using nuclear-encoded microsatellites and mitochondrial DNA (mtDNA) from samples from four localities in the U.S. Caribbean and one locality in the Florida Keys. Significant heterogeneity was detected for both allele and genotype distributions (microsatellites) and for haplotype distribution (mtDNA). Pairwise comparisons revealed that fish in the Florida Keys differed significantly from fish in the U.S. Caribbean with respect to both microsatellites and mtDNA. A parsimony network of mtDNA haplotypes was consistent with division of the five sample localities into two distinct populations. Genetic diversity at both microsatellites and mtDNA was greater among fish from the Florida Keys. The average, long-term migration rate from the U.S. Caribbean westward to the Florida Keys was approximately 1.75-fold greater than the reverse, suggesting that the elevated genetic variability among fish from the Florida Keys reflects the westward movement of alleles as a function of westward-flowing surface currents in the region. Bayesian coalescent analysis (microsatellites) indicated that each of the two populations has experienced a 10-fold decline in effective population size (N_e). Estimates of long-term effective size, generated using a coalescent, maximum-likelihood method, were 1,671.9 (Florida Keys) and 2,923.2 (U.S. Caribbean). Estimates of contemporaneous effective size, generated using a linkage-disequilibrium approach with minor alleles (those with frequencies of 0.02 or less) being excluded, were 275.6 (Florida Keys) and 668.9 (U.S. Caribbean) and differed significantly from one another. Because the samples contained mixed cohorts, the short-term estimates reflect the effective number of breeders (N_b) that produced the cohort(s) from which the samples were taken. The difference between the long-term and short-term estimates of N_e (or N_b) suggests that the declines in the effective size of both populations are relatively recent and that management concern over lane snapper in the Florida Keys is justified.

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Received July 23, 2010; accepted December 14, 2010

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The lane snapper *Lutjanus synagris* is a lutjanid fish distributed from North Carolina through the Gulf of Mexico and Caribbean Sea to southeastern Brazil (Allen 1985). Although comparatively small in size, with adults averaging approximately 35 cm and approximately 0.45 kg (Bortone and Williams 1986; http://www.flmnh.ufl.edu/fish/Gallery/Descript/LaneSnapper/LaneSnapper.html), lane snapper are among the most economically important lutjanids in the greater Caribbean region (Bortone and Williams 1986; Acosta and Appledoorn 1992; Luckhurst et al. 2000), often accounting for a considerable fraction of the recreational and commercial catch in areas such as south Florida (http://research.myfwc.com/engine/download_redirection_process.asp?file = 37_Lane_snapper_2008.pdf&objid = 5284&dltype = article), Puerto Rico (Matos-Caraballo 2000), and Cuba (Bustamante et al. 2000). In Puerto Rico, for example, commercial landings of lane snapper increased approximately 19.5 times between 1983 and 2005, with total landings in 2005 accounting for nearly 7.9% of all commercial landings (http://sero.nmfs.noaa.gov/sf/pdfs/Commercial%20Landings%20 in%20Puerto%20Rico%20for%201983%20-%202005%20by%20Species.pdf). Because lane snapper in U.S. waters of the Caribbean were considered to be "at risk" by the Sustainable Fisheries Act (SFA) Working Group of the Caribbean Fishery Management Council (CFMC), a 3-month, seasonal closure on fishing for lane snapper in U.S. Caribbean federal waters was implemented in 2005 (CFMC 2005).

In a prior study, Karlsson et al. (2009) identified two genetically distinct populations (stocks) of lane snapper in waters of the continental USA: a western group distributed in the northwestern and north-central Gulf of Mexico and an eastern group distributed along the west coast of Florida, the Florida Keys, and the east (Atlantic) coast of Florida. Bayesian analysis of genetic demography indicated that both groups have experienced a historical decline in effective population size, with the decline being greater in the western group. The spatial discontinuity between the two groups corresponded to a known zone of vicariance in other marine species.

Here, we extend the genetics work on lane snapper to include U.S. waters in the northern Caribbean Sea. The study area was located in the eastern extreme of the Caribbean archipelago and is composed of the Commonwealth of Puerto Rico in the Greater Antilles and the Territory of the U. S. Virgin Islands in the Lesser Antilles island chain; both separate the Caribbean Sea from the western (central) Atlantic Ocean. To date, there is little to no discrete information on stock structure, movement patterns, or demography for any of the exploited shallow-water snappers, including lane snapper, in the U.S. Caribbean. Appledoorn et al. (1992) found evidence of overexploitation and a general decline in all reef fish fisheries (combined) in waters off of both Puerto Rico and the U.S. Virgin Islands, and based on mortality estimates from size-frequency distributions they suggested that growth overfishing was occurring. We employed both nuclear-encoded microsatellites and sequences of mitochondrial DNA (mtDNA) to assess genetic population structure and demography of lane snapper in the region. Knowledge of these genetic variables is critical in the context of species or populations under intensive exploitation because erosion of genetic resources via depletion of unrecognized spawning components can directly impact immediate and long-term recruitment potential (Carvalho and Hauser 1995). Knowledge of demography and effective size (N_e) is critical to conservation and management of exploited natural resources because it potentially provides information on loss of genetic variation and extinction risk (Leberg 2005; Luikart et al. 2010).

METHODS

A total of 497 lane snapper were sampled between 2007 and 2009 from one locality in the Florida Keys and four localities in the U.S. Caribbean (Figure 1). Samples from the Florida Keys were obtained from fish houses or local fishers in or near Marathon, Florida. Samples from the west coast of Puerto Rico (PR-west) were obtained at fish houses in or near Mayaguez, while samples from the east coast (PR-east) were obtained at fish houses in or near Fajardo. Samples from St. Thomas (ST) were obtained at the Gustave Quetel Fish House in Frenchtown (Charlotte Amalie) or from local fishers, while samples from St. Croix (SC) were obtained primarily from catches of nearshore, artisanal fishers landing off the west coast (near Fredericksted) or at the Le Rheine fish market. Duplicate tissue samples, primarily fin clips, were removed from each fish, fixed in 95% ethanol, and returned to the laboratory in College Station.

Whole genomic DNA was extracted from each fish via a phenol-chloroform protocol following digestion with proteinase *K*, after Sambrook et al. (1989). All fish were assayed initially for allelic variation at 17 nuclear-encoded microsatellites, using three multiplex panels (Table 1). Each multiplex panel employed the "Touchdown II" polymerase-chain-reaction (PCR) protocol (Renshaw et al. 2006), with different annealing temperatures utilized for each panel (Table 1). Primers used to amplify individual microsatellites were among those developed by Gold et al. (2001) for red snapper *L. campechanus* (*Lca20*, *Lca22*, *Prs248*, *Prs328*); by Bagley and Geller (1998) for vermilion snapper *Rhomboplites aurorubens* (*Ra1*, *Ra2*, *Ra6*); and by Renshaw et al. (2007) for mutton snapper *L. analis* (*Lan3*, *Lan6*, *Lan11*, *Lan12*, *Lan13*), lane snapper *L. synagris* (*Lsy7*, *Lsy13*, *Lsy14*), and yellowtail snapper *Ocyurus chrysurus* (*Och4*, *Och13*). Microsatellite amplification products were electrophoresed using an ABI 377 automated sequencer (Applied Biosystems Inc., Foster City, California), following manufacturer instructions. Resulting chromatograms were analyzed in Genescan version 3.1.2 (Applied Biosystems); alleles were scored using Genotyper version 2.5 (Applied Biosystems).

A 590 base-pair fragment of the mitochondrially encoded NADH-dehydrogenase subunit 4 (*ND-4*) was PCR-amplified and sequenced from 132 fish (25–32 individuals from each sample locality). The primers NAP-2 (Arevalo et al. 1994) and

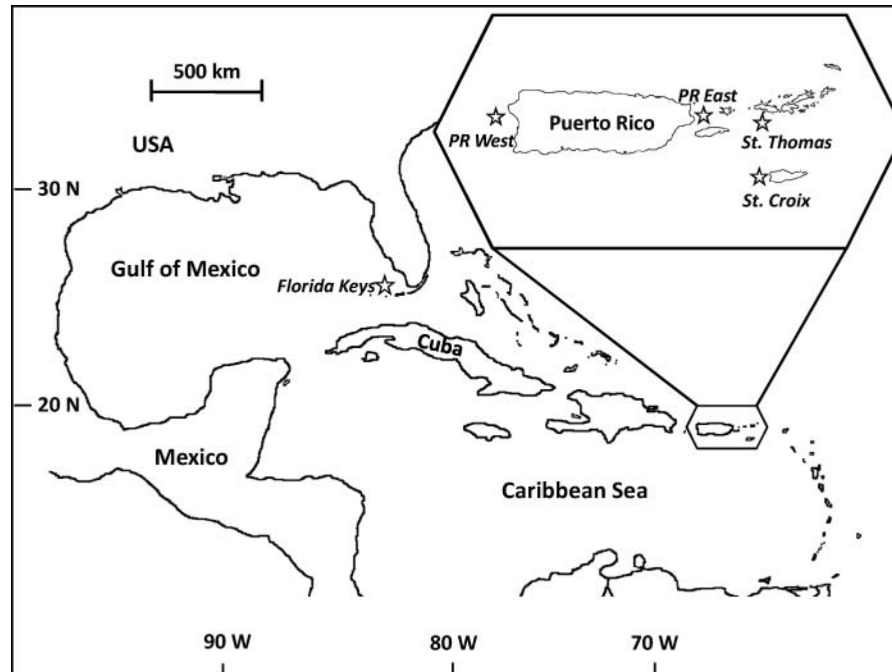

FIGURE 1. Approximate sampling locations (stars) for lane snapper in the Florida Keys and U.S. Caribbean.

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TABLE 1. Multiplex panels used to acquire genotypes at 17 microsatellites from lane snapper. Primer quantities and fluorescent labels (ABI dyes) are given for finalized polymerase chain reaction (PCR) cocktails. Panel-specific annealing temperatures used with the "Touchdown II" PCR protocol (Renshaw et al. 2006) are given in the last column.

Panel	Microsatellite	Primer quantity (pmol)	ABI dye	Annealing temperature (°C)
1	*Och4*	0.8	6-FAM	55
	Lan6	1.5	6-FAM	53
	Lan13	8	HEX	51
	Ra6	1.5	HEX	
	Lan12	1.5	HEX	
	Lan11	1.8	HEX	
2	*Och13*	2.5	6-FAM	54
	Lsy14	4.3	6-FAM	52
	Prs328	4.3	6-FAM	50
	Lca22	5	6-FAM	
	Lsy13	4	NED	
	Lsy7	4.3	NED	
3	*Ra2*	5	6-FAM	52
	Lan3	2	6-FAM	49
	Lca20	5	6-FAM	46
	Ra1	8	HEX	
	Prs248	4.5	NED	

ND4LB (Bielawski and Gold 2002) were used for amplification and sequencing. Protocols for PCR amplifications and DNA sequencing were as described in Karlsson et al. (2009). Sequenced products were separated and visualized on an ABI 3100 capillary sequencer (Applied Biosystems); sequences were aligned and edited using Sequencher 3.0 (Gene Codes Corporation).

Summary statistics for microsatellites, including number of alleles, allelic richness, expected heterozygosity (unbiased gene diversity), and the inbreeding coefficient F_{IS}, were generated using Fstat (Goudet 1995; http://www2.unil.ch/popgen/softwares/fstat.htm). Possible occurrence of null alleles, large allele dropout, or stuttering was evaluated using Microchecker (van Oosterhout et al. 2004). Homogeneity among localities in allelic richness and unbiased gene diversity was tested via Friedman rank tests, using SPSS version 11.0.1 (http://www.spss.com/statistics/); tests between pairs of sample localities were carried out using Wilcoxon's signed rank test (also using SPSS). Tests of conformance of genotypes to Hardy–Weinberg (HW) equilibrium expectations at each microsatellite and tests of genotypic equilibrium between pairs of microsatellites for each sample locality were carried out using exact probability tests, as implemented in Genepop version 3.4 (Raymond and Rousset 1995; http://genepop.curtin.edu.au/) and using a Markov chain approach (Guo and Thompson 1992) that employed 5,000 dememorizations, 500 batches, and 5,000 iterations per batch. Sequential Bonferroni correction (Rice 1989) was applied for all multiple tests performed simultaneously.

Summary statistics for mtDNA included number of haplotypes, haplotype richness, haplotype diversity, and nucleotide diversity. Haplotype richness was computed following El Mousadik and Petit (1996) and using the software Rarefac (available at http://www.pierroton.inra.fr/genetics/labo/Software/Rarefac/index.htm), whereas haplotype diversity and nucleotide diversity were generated using Arlequin version 3.11 (Schneider et al. 2000; http://cmpg.unibe.ch/software/arlequin3/). Homogeneity between pairs of samples in number of mtDNA haplotypes and in haplotype diversity was tested with a bootstrap resampling approach (after Dowling et al. 1996) in which the probability that the number of different haplotypes or haplotype diversity observed in one locality would be observed in a random sample of the same size in another locality is estimated. Pop Tools (a free-ad in software for Excel, available at http://www.cse.csiro.au/poptools/index.htm) was used to randomly sample the number of fish sampled in one locality from another locality. Random sampling was performed 1,000 times, and the average number of observed haplotypes and their upper (0.95) and lower (0.05) percentiles were recorded. Selective neutrality of variation in mtDNA in each of the five samples was tested with Fu's (1997) F_S statistic and Fu and Li's (1993) D^* and F^* statistics, as implemented in the DnaSP package version 5.00.07 (Rozas et al. 2003; http://www.ub.edu/dnasp/). Significance of F_S, D^*, and F^* was assessed from 10,000 coalescent simulations (after Rozas et al. 2003), based on the observed number of segregating sites in each sample. A network of mtDNA haplotypes was constructed using statistical parsimony as described by Templeton et al. (1992) and implemented in TCS version 1.21 (Clement et al. 2000).

Homogeneity of microsatellite allele and genotype distribution and of mtDNA haplotype distribution across localities was tested via exact tests, as implemented in Genepop, and by analysis of molecular variance (AMOVA), as implemented in Arlequin. Exact probabilities were estimated using a Markov chain method that employed the same parameters as used in tests of HW and genotypic equilibrium. Fixation indices (F_{ST}), based on both microsatellites and mtDNA, between pairs of sample localities were estimated as Weir and Cockerham's (1984) θ, as implemented in Fstat (microsatellites) or Arlequin (mtDNA); exact tests, as implemented in Genepop (microsatellites) or Arlequin (mtDNA), were used to test the hypothesis $F_{ST} = 0$.

Estimates of short-term, contemporaneous N_e from single-sample microsatellite variation were generated using LdNe (Waples 2008), which employs the linkage-disequilibrium (LD) approach and implements the bias correction described by Waples (2006). Based on results from homogeneity testing (see Results), N_e was estimated separately for the sample from the Florida Keys and for the four samples (pooled) from the U.S. Caribbean. Alleles with frequencies lower than 0.02 or 0.01 were excluded from the estimation, as recommended by Waples and Do (2010), and 95% confidence intervals (CIs) were estimated using both parametric and jackknife approaches. When samples of individuals contain mixed cohorts, as is the case here, estimates of N_e derived using LdNe reflect the effective number of breeders (N_b) that produced the cohort(s) from which the sample was taken (Waples and Do 2010).

Estimates of long-term N_e were based on maximum-likelihood estimates of the parameter θ, which is defined as $4N_e\mu$ when based on biparental markers such as microsatellites. In this case, N_e represents an average, long-term estimate of effective size integrated over the time to common ancestry of all alleles in the population; μ is the average per-gene mutation rate. Estimates of θ were generated using the coalescent-based program Migrate version 3.03 (Beerli and Felsenstein 2001; http://popgen.scs.fsu.edu/Migrate-n.html), employing Markov chain–Monte Carlo simulations of gene-trees and accounting for migration between populations. An initial run was implemented to generate estimates of θ and M, the mutation-scaled migration rate for each population (see below). These initial estimates were used as starting values for longer Monte Carlo searches that were used to derive final likelihood distributions for θ and M. Final parameters were derived based on the results of three replicate runs. Each replicate run employed 10 initial short chains (4×10^3 trees sampled) and 4 long chains (7.5×10^6 trees sampled). Estimates of N_e were then generated using the 17 microsatellite data set and the average per-gene mutation rates (μ) obtained with the Bayesian coalescent approach of Storz and Beaumont (2002), as implemented in Msvar (see below). Migrate also generates maximum-likelihood estimates of the average long-term (mutation-scaled) migration rate (M). The value M is equal to the migration rate per generation (m) divided by the average, per gene mutation rate (μ). Estimates of long-term m were generated by dividing M by the estimates of μ obtained from Msvar (see below).

Evidence for historical change in effective population sizes was inferred using the Bayesian approach implemented in Msvar version 1.3 (http://www.rubic.rdg.ac.uk/cgi-bin/MarkBeaumont/dirlist1.cgi). This approach (Storz and Beaumont 2002) employs coalescent Monte Carlo–Markov chain simulations to estimate the posterior probability distribution of population parameters, based on the observed distribution of microsatellite alleles and their allelic state (number of repeats). Estimated parameters include current (N_0) and historical or ancestral (N_1) effective size, average mutation rate (μ) across loci per generation, and time (t_a) in generations since the beginning of an expansion or decline phase. Subsampling of chromosomes, assessment of convergence of the Markov chain–Monte Carlo simulations, and means and standard deviations of prior and hyper-prior distributions for all parameters (N_0, N_1, μ, and t_a) were the same as used in Karlsson et al. (2009). Monte Carlo searches employed 2×10^9 steps with the first 4×10^8 discarded as burn-in. A generation time of four years was estimated based on life history data available for lane snapper (Johnson et al. 1997; Luckhurst et al. 2000); age distribution data, inferred from fishery-dependent sampling, indicated fit to a Type II survivorship model, the parameters of which were used to derive generation time, as described in Nunney and Elam (1994).

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RESULTS

Genotypes at 17 microsatellites for all individuals assayed are available by sample locality at http://wfsc.tamu.edu/doc/under the file name "Florida Keys and U.S. Caribbean Lane Snapper Microsatellite Genotypes." Summary statistics for each microsatellite in each sample locality are given in Table A.1. The number of alleles detected ranged from 3–5 at *Lan12* to 23–33 at *Prs248* and was paralleled by allelic richness (from 2.98 to 4.99 at *Lan12* and from 23.00 to 32.86 at *Prs248*) and by unbiased gene diversity (from 0.079 to 0.227 at *Lan12* and from 0.901 to 0.929 at *Prs248*). Across all microsatellites and all sample localities, the average ± SE number of alleles was 9.53 ± 0.65, average allelic richness was 9.51 ± 0.65, and average (unbiased) gene diversity was 0.629 ± 0.020. Allelic richness differed significantly (Friedman's rank test) among the five sample localities ($Q_{[4]} = 18.01$, $P = 0.001$). One-tailed, Wilcoxon's signed-rank tests revealed significant differences in allelic richness prior to sequential Bonferroni correction in 6 of 10 pairwise comparisons between sample localities, one of which (PR-west versus SC; $P = 0.003$) remained significant after Bonferroni correction. However, three of four pairwise comparisons involving PR-west prior to correction differed significantly, and one comparison after correction (PR-west versus ST) had a P-value of 0.007, very close to the Bonferroni adjusted P-value of 0.006. In both comparisons (PR-west versus SC and PR-west versus ST), there were fewer microsatellite alleles overall in ST and SC than in PR-west. Unbiased gene diversity did not differ significantly among sample localities (Friedman's rank test: $Q_{[4]} = 6.46$, $P = 0.167$). However, the rank, by locality, of average allelic richness and unbiased gene diversity generally followed an east–west pattern: Florida Keys > PR-west > SC > PR-East > ST.

Significant departure from Hardy–Weinberg equilibrium expectations for microsatellite genotypes was found in 16 of 85 tests prior to Bonferroni correction. Only one test (*Lca20* in the sample from the Florida Keys) remained significant following correction; the estimated F_{IS} value was 0.212, indicating a deficit of heterozygotes. Analysis using Microchecker indicated the possible occurrence of null alleles at *Prs248* in the sample from the Florida Keys and the possible occurrence of null alleles and (or) stuttering at *Lan13* in the samples from PR-east, PR-west, and SC. All subsequent analyses (below) carried out with or without *Lca20*, *Prs248*, and *Lan13* gave qualitatively identical results; consequently, results that included these three microsatellites are reported. A total of 70 of 680 pairwise tests of genotypic disequilibrium were significant before Bonferroni correction; only one (*Prs248* versus *Ra2* in the sample from the Florida Keys) remained significant after correction.

A total of 31 different mtDNA haplotypes were observed among the 132 individuals sequenced (Table A.2). Haplotype number 5 was the most common in the Florida Keys, occurring in 34.4% of individuals assayed; haplotype 3 was the most common among the remaining four samples, occurring in 60–64% of individuals assayed in each locality. Summary statistics, including number of haplotypes, haplotype richness, haplotype diversity, and nucleotide diversity within each sample locality are given in Table A.1. Estimates for each of these variables were higher in the sample from the Florida Keys than in the four samples from the U.S. Caribbean. Tests of homogeneity in number of mtDNA haplotypes and in haplotype diversity between pairs of samples confirmed significantly greater variation, both in number of haplotypes and haplotype diversity, in the sample from the Florida Keys versus the remaining four sample localities, none of which differed significantly from one another. Based on exact tests of homogeneity (see below), which indicated homogeneity in mtDNA haplotype distribution among the four samples from the U.S. Caribbean, we also tested homogeneity in the number of haplotypes and haplotype diversity between the Florida Keys and the U.S. Caribbean (samples pooled). Resampling of 12 mtDNA haplotypes from the U.S. Caribbean yielded probabilities of 0.049 (number of haplotypes) and 0.001 (gene diversity) that the same or greater number of haplotypes and haplotype diversity as found in the Florida Keys would be found by chance in the U.S Caribbean. Estimates of nucleotide diversity, the average number of nucleotide differences per site between any two DNA sequences chosen at random (Nei and Li 1979), were greater in the samples from the Florida Keys and PR-east than in the remaining three samples; the regional differences, however, were not significant, based on estimated 95% CIs. Estimates of Fu's (1997) F_S statistic (Table 2) were negative but nonsignificant in all five sample localities, consistent with expectations of selective neutrality. Fu and Li's (1993) F^* and D^* statistics (Table 2) also were negative and nonsignificant in all sample localities except for ST, where both

TABLE 2. Fu's (1997) F_S and Fu and Li's (1993) D^* and F^* measures of selective neutrality for lane snapper. Probabilities of significance were estimated from coalescent simulations (Rozas et al. 2003).

Sample	F_S	P	D^*	P	F^*	P
Florida Keys	−3.564	0.101	−1.042	0.184	−1.393	0.108
Puerto Rico–east	−1.660	0.250	−0.054	0.545	−0.418	0.336
Puerto Rico–west	−0.852	0.361	−0.542	0.419	−0.378	0.389
St. Thomas	−2.234	0.170	−2.519	0.049	−2.246	0.032
St. Croix	−2.869	0.029	−1.685	0.078	−1.731	0.072

TABLE 3. Probability that $F_{ST} = 0$ for pairwise comparisons of microsatellite allele distributions (above diagonal) and mtDNA haplotype distribution (below diagonal) between lane snapper from the Florida Keys and U.S. Caribbean. Probability values that differ significantly from zero after Bonferroni correction are in bold italics.

Sample	Florida Keys	Puerto Rico–east	Puerto Rico–west	St. Thomas	St. Croix
Florida Keys		***<0.001***	***<0.001***	***<0.001***	***<0.000***
Puerto Rico–east	***<0.001***		0.199	0.023	0.032
Puerto Rico–west	***<0.001***	0.204		0.377	0.014
St. Thomas	***<0.001***	0.374	0.527		0.500
St. Croix	***<0.001***	0.513	0.903	0.611	

F^* and D^* were significant prior to (but not following) Bonferroni correction.

Exact tests revealed significant heterogeneity among the five sample localities in microsatellite allele and genotype distributions ($P < 0.0001$) and in mtDNA haplotype distribution ($P = 0.000$). Results from AMOVA yielded comparable results; the genetic variance component (F_{ST}) attributable to variation among localities for microsatellites was 0.016 ($P = 0.000$) and was 0.060 ($P < 0.001$) for mtDNA. Exact tests of pairwise comparisons of $F_{ST} = 0$ between sample localities (Table 3) revealed that comparisons between fish from the Florida Keys and fish from the other four localities differed significantly ($P = 0.000$ for both microsatellites and mtDNA) both before and following Bonferroni correction. The parsimony network of mtDNA haplotypes (Figure 2) was consistent with the division of the five sample localities into two distinct groups; haplotypes found in the Florida Keys primarily included haplotype 5 and a small, divergent clade that included haplotypes 2, 7, and 12, whereas haplotypes in the U.S. Caribbean primarily included haplotypes 3 and 19 and their derivatives. Haplotypes in the two groups were not reciprocally monophyletic, suggesting limited, present-day dispersal or historical connectedness.

Exact tests among the four sample localities from the U.S. Caribbean indicated significant genetic heterogeneity in both microsatellite allele ($P = 0.009$) and genotype distributions ($P = 0.046$) but not in mtDNA distribution ($P = 0.445$). Similar results were obtained from AMOVA: $F_{ST} = 0.002$, $P = 0.004$ (microsatellites) and $F_{ST} = -0.009$, $P = 0.658$ (mtDNA). Significant ($P < 0.05$) pairwise comparisons (microsatellites) among the four samples from the U.S. Caribbean prior to Bonferroni correction (Table 3) were PR-east versus ST ($P = 0.023$) and SC ($P = 0.032$) and PR-west versus SC ($P = 0.014$); none of the pairwise comparisons among these four samples, however, differed significantly following Bonferroni correction. In addition, the F_{ST} value for the pairwise comparison between PR-west and SC (which had the lowest P-value) was −0.0002, and results of extended analyses, including spatial analysis of molecular variance or SAMOVA (Dupanloup et al. 2002), as implemented in Samova 1.0 (available at http://web.unife.it/progetti/genetica/Isabelle.samova.html) and spatial autocorrelation analysis (Smouse and Peakall 1999; Peakall et al. 2003), as implemented in GenAlEx 6.0 (Peakall and Smouse 2006), failed to reveal significant genetic heterogeneity among the four sample localities (data available upon request from the senior author).

Estimates of the effective number of breeders (N_b), generated using LdNe, and of long-term effective size (N_e), generated using Migrate, are given in Table 4. Estimates for both parameters (N_b and N_e) are presented for the two populations of lane snapper (Florida Keys and U.S. Caribbean) indicated by the various tests of genetic homogeneity. Estimates of N_b for the population from the Florida Keys were 275.6 and 241.3 (excluding alleles with frequencies < 0.02 and <0.01, respectively), whereas estimates for the population from the U.S. Caribbean were approximately 2.5 times larger (668.9 and 643.8). Based on estimated 95% CIs, the N_b estimates for the sample from the Florida Keys differed significantly from the N_b estimates for the samples from the U.S. Caribbean. Estimates of long-term effective size (N_e) for the two groups also differed significantly and were roughly 6.5 (Florida Keys) and 4.5 (U.S. Caribbean) times the estimates of N_b. Migrate also generated estimates of the average long-term (mutation-scaled) migration rate (M) between the two populations. Estimates of M were 5.751 (Florida Keys to U.S. Caribbean) and 10.086 (U.S. Caribbean to Florida Keys). The average, long-term migration rates (m) between the two groups were estimated using an average estimate of μ (2.74×10^{-4}) across all 17 microsatellites, generated using Msvar (see below). Estimated m for Florida Keys to the U.S. Caribbean was 0.0016 (95% CI = $0.0015 - 0.0026$); estimated m for the U.S. Caribbean to the Florida Keys was 0.0028 ($0.0017 - 0.0030$). These data indicate very limited migration between the two regions, but with a roughly 1.75-fold greater migration rate from the U.S. Caribbean to the Florida Keys.

Summary statistics for the posterior distributions of N_0, N_1, and μ, generated using Msvar, are presented in Table 5. The mode of the posterior distribution of N_0 was 1,992 for the Florida Keys and 1,464 for the U.S. Caribbean, while the mode of N_1 was 27,708 for the Florida Keys and 22,341 for the U.S. Caribbean. Log_{10} values of r, the ratio of N_0/N_1, were –1.143 (Florida Keys) and –1.184 (U.S. Caribbean), indicating that both populations have experienced an order-of-magnitude decline in effective population size. The mode of the posterior distribution of the average mutation rate over all microsatellites was 2.98×10^{-4} (Florida Keys) and 2.50×10^{-4} (U.S. Caribbean), while the

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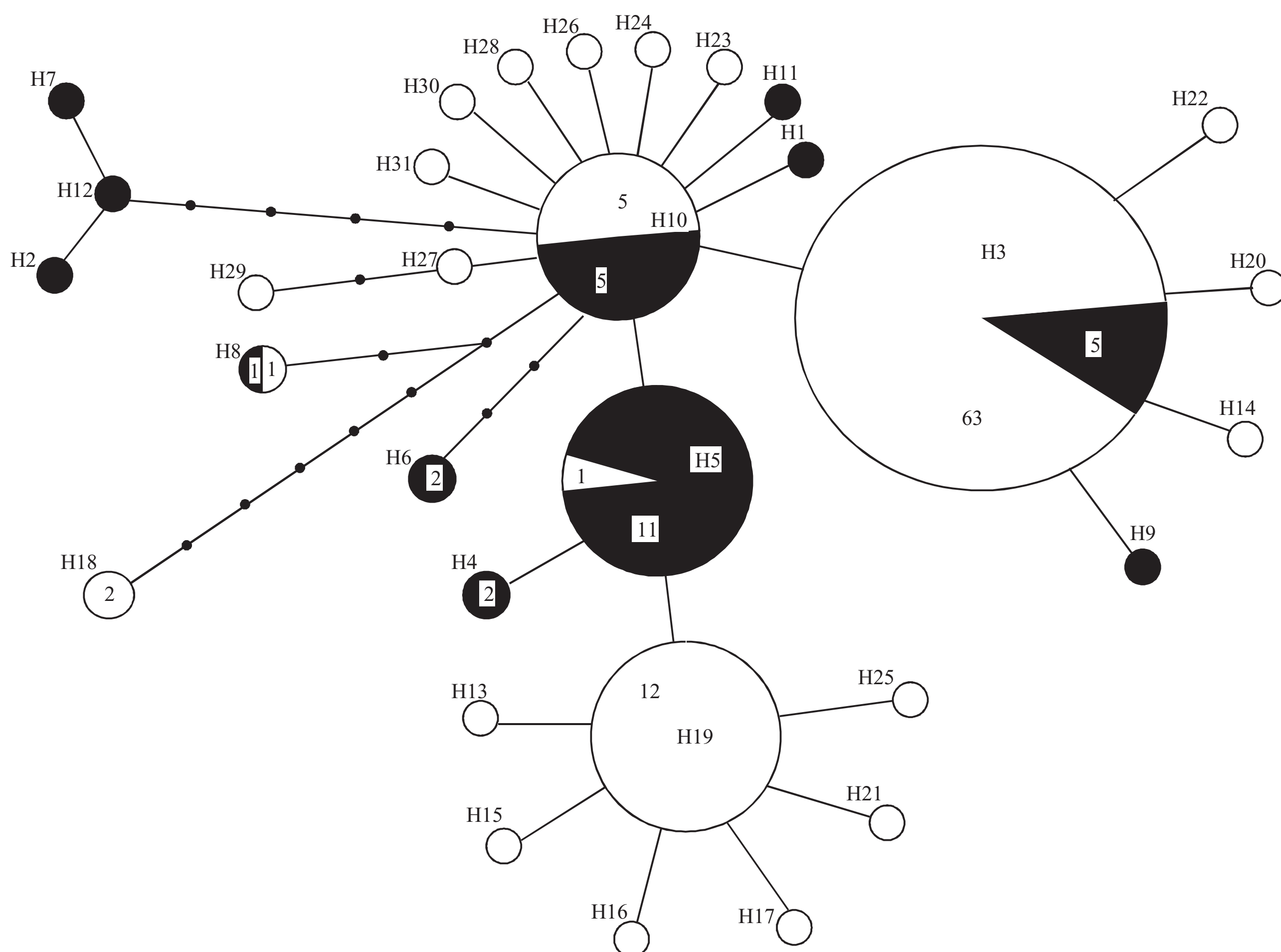

FIGURE 2. Parsimony network of *ND-4* mtDNA haplotypes (H) in lane snapper from the Florida Keys (black) and U.S. Caribbean (white). Haplotype numbers are those given in Table A.1. Sizes of the circles reflect relative haplotype frequencies; number of individuals with a given haplotype is indicated if more than one individual possessed that haplotype. Small, unnumbered circles represent undetected mtDNA haplotypes. Lines between haplotypes represent one base-pair substitution regardless of length.

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mode of the posterior distribution for the time since decline was 7,919 (Florida Keys) and 5,371 (U.S. Caribbean) years.

DISCUSSION

Population Structure

The finding of significant genetic differences between lane snapper in the Florida Keys and the U.S. Caribbean was not unexpected. Similar to most lutjanids, juvenile and adult lane snapper are relatively sedentary, preferring inshore, soft or sandy-bottom habitats or nearshore hard-bottom habitats (Camber 1955; Manooch and Mason 1984; Bortone and Williams 1986; Luckhurst et al. 2000). This life history pattern leads to the expectation that gene flow, which is generally necessary to maintain homogeneity in selectively neutral genetic markers such as microsatellites, would be restricted and limited primarily to hydrodynamic transport of pelagic eggs and larvae. Such transport between the Florida Keys and the U.S. Caribbean seems highly unlikely given the distance between the Florida Keys and the west coast of Puerto Rico (>1,600 km). Cowen et al. (2006) found that larval dispersal distances of ecologically relevant magnitudes for a variety of reef fish species in the Caribbean region were on the scale of only 10–100 km. In addition, the 1-month and 2-month envelopes of potential larval import and export suggested by Roberts (1997) indicated virtually no overlap between the area surrounding Puerto Rico and the U.S. Caribbean and peninsular Florida. Finally, such transport would be largely unidirectional because the major surface current patterns in the Caribbean Sea flow to the west and northwest (Roberts 1997).

Results of tests of genetic homogeneity among the four samples from the U.S. Caribbean were equivocal. Significant heterogeneity was detected in exact tests of microsatellite allele and genotype distributions but not in mtDNA haplotype distributions. Similarly, in AMOVA, the genetic variance attributable to variation in microsatellites was significant, whereas that

TABLE 4. Estimates of (1) effective number of breeders (N_b), generated using a linkage-disequilibrium method, and (2) long-term effective size (N_e), generated using a coalescent-based, maximum-likelihood method, for lane snapper from the Florida Keys and U.S. Caribbean. The values <0.02 and <0.01 for the estimates of N_b refer to analyses excluding minor alleles at the 0.02, and 0.01 frequency levels; 95% confidence intervals given are in parentheses.

	N_b		
Population	<0.02	<0.01	N_e
Florida Keys	275.6	241.3	1, 671.9
	(198.4–434.0)[a]	(189.7–325.5)[a]	(1, 529.9–1, 825.9)
	(188.3–483.4)[b]	(173.4–379.6)[b]	
U.S. Caribbean	668.9	643.8	2, 923.2
	(527.3–893.7)[a]	(531.6–804.6)[a]	(2, 724.1–3, 209.6)
	(499.3–973.4)[b]	(508.4–857.3)[b]	

[a]Parametric.
[b]Jackknife on loci.

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attributable to mtDNA was not. Pairwise tests (microsatellites) between sample localities indicated significant differences before Bonferroni correction (but not after) for the comparisons PR-east versus ST, PR-east versus SC, and PR-west versus SC. Based solely on geographic distance, one might expect a greater degree of genetic divergence between PR-west and SC than between PR-east and either ST or SC. In addition, the F_{ST} value for PR-west versus SC, which had the lowest probability value (−0.014) in exact tests, was less than zero (−0.0002), suggesting that the significant P-values for pairwise comparisons prior to Bonferroni correction could be artifacts. This suggestion was consistent with more extended analyses of population structure, including SAMOVA and spatial autocorrelation, that did not indicate significant population structure among samples from the U.S. Caribbean.

We interpret the forgoing conservatively and conclude that lane snapper from the four localities in the U.S. Caribbean form a single population or stock. However, there are caveats to this hypothesis. The first is that one cannot prove a null hypothesis; a finding that geographic samples do not differ significantly in allele or genotype frequencies could mean simply that each sample has the same parametric allele frequency at each genetic marker yet represent a different entity. A second caveat is that genetic homogeneity may reflect past (historical) rather than present-day population structure. Populations could be isolated at least partially today yet have undergone sufficient gene flow in the recent past such that they remain indistinguishable in allele frequencies. A third caveat is that genetic markers (microsatellites, mtDNA haplotypes) typically employed in population genetic studies are presumed to be selectively neutral and neither influenced by natural selection nor related to genes impacting adaptive traits related to life history or fitness (McKay and Latta 2002). This means that homogeneity in such genetic markers may not necessarily reflect homogeneity in genes affecting life history or fitness traits. This caveat will accompany virtually any assessment of population structure that employs selectively neutral genetic markers such as microsatellites. A final caveat is that confirmation of genetic homogeneity, or heterogeneity for that matter, warrants replicate temporal sampling. The above notwithstanding, the genetic data at hand indicate that the best working hypothesis at present is that there is a single population of lane snapper in waters of the U.S. Caribbean.

Genetic Variation and Effective Size

Allelic richness over all microsatellites differed among the five sample localities, with the significant differences primarily involving PR-west versus ST and SC. Interestingly, average values of both allelic richness and unbiased gene diversity in samples from the U.S. Caribbean generally followed an east to west geographic pattern, which could reflect more or less unidirectional westward movement of alleles as a function of primarily westward flowing surface currents in the region. The main current affecting the U.S. Caribbean would be the Anegada Passage, a channel approximately 65 km wide that

TABLE 5. Summary statistics for the posterior distributions of the parameters N_0 (contemporaneous effective size), N_1 (historical or ancestral effective size), r (ratio of current to ancestral effective size), μ (mutation rate), and t_a (time since the beginning of expansion/decline) for lane snapper from the Florida Keys and U.S. Caribbean. Values given are modes, with the 5th–95th quartiles in parentheses.

Population	N_0	N_1	$\text{Log}_{10}(r)$	μ	t_a (years)
Florida Keys	1,992	27,708	−1.143	2.98×10^{-4}	9,402
	(296–14,595)	(4,496–233,399)		(4.48×10^{-5}–1.73×10^{-3})	(999–105,657)
U.S. Caribbean	1,464	22,341	−1.184	2.50×10^{-4}	13,858
	(199–11,630)	(3,417–188,973)		(4.38×10^{-5}–1.67×10^{-3})	(1,381–130,047)

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connects the Atlantic Ocean with the Caribbean Sea and runs westward between St. Thomas and St. Croix and to the south of Puerto Rico. Johns et al. (2002) estimated total inflow of the Anegada Passage to be 2.5×10^6 m^3/s or greater, consistent with the notion of passive transport of either pelagic eggs and larvae, or even adults, primarily to the west. Allelic richness and gene diversity also were elevated in the sample from the Florida Keys, and estimates of the number of mtDNA haplotypes and haplotype diversity were significantly greater in the sample from the Florida Keys than in samples from the U.S. Caribbean. Consistent with this view are the results of coalescent-based analysis of the microsatellite data, where average, long-term migration rate (m) from the U.S. Caribbean westward to the Florida Keys was approximately 1.75 times that of the reverse. Increased genetic diversity in fish from the Florida Keys also could stem from migrants moving unidirectionally from other populations, particularly those in the southwestern part of the Caribbean Sea, where lane snapper are particularly abundant (Allen 1985). Advective transport from populations in the western Caribbean to Florida has been hypothesized for a number of species (Tester and Steidinger 1997; Johnson et al. 2004) and ostensibly would be largely a function of the Loop Current, where average transport values are estimated to be 30×10^6 m^3/s (Morrison and Nowlin 1977).

Bayesian coalescent analysis (Msvar) of the microsatellite data indicated that the two populations have each experienced a 10-fold decline in effective population size. Estimates of the posterior distributions of the modal contemporaneous effective size (N_0) were 1,992 (Florida Keys) and 1,464 (U.S. Caribbean). Because coalescent-based estimators reflect an average effective size over some number of generations (Leberg 2005) and the approach used in Msvar considers the mutational process, N_0 effectively is a long-term estimate of inbreeding effective size (Wang 2005). Consequently, N_0 is not necessarily an estimate of N_e in the preceding generation, or even a few generations in the past, and the value estimated may have little relationship to current rates of inbreeding (Beaumont 2003). The same, to some extent, also holds for the long-term estimates of N_e generated by the maximum-likelihood approach used in Migrate. Estimates for the two populations, using this approach, were 1,671.9 (Florida Keys) and 2,923.2 (U.S. Caribbean). The long-term estimates of N_e generated by the two approaches are not that dissimilar and indicate a historical perspective against which short-term estimates that measure N_e on a more recent time-scale can be evaluated.

Estimates of short-term effective size, generated using the linkage-disequilibrium approach in LdNe and excluding minor alleles at frequencies of 0.02 and 0.01, were, respectively, 275.6 and 241.3 (Florida Keys) and 668.9 and 643.8 (U.S. Caribbean). Based on 95% CIs, these estimates differed significantly between the two populations regardless of allele-exclusion frequency. In age-structured species such as lane snapper, estimates of N_e derived using the linkage disequilibrium approach provide information about the effective number of breeders (N_b) that produced the cohort(s) from which the sample was taken (Waples and Do 2010). The relationship between N_b and N_e in age-structured species, however, has not been evaluated for the linkage-disequilibrium (or any other single-sample) estimator, and given that the lane snapper in our samples probably came from fewer than the actual number of parental fish in the generation, the estimates of N_b probably are smaller than the parametric effective size (N_e) per generation (Waples and Do 2010; Luikart et al. 2010). Nonetheless, estimates of N_e (or N_b) derived via the linkage-disequilibrium approach measure effective size on a recent time scale (Beaumont 2003) and are useful for early detection of population bottlenecks (Luikart et al. 2010). In addition, estimates of N_e generated by the linkage-disequilibrium approach do provide useful bounds for N_e and can be of interest in conservation and management applications (Waples and Do 2010).

The short-term estimates of N_e (or N_b) for lane snapper in both populations were considerably smaller than the long-term estimates, about 6–7 times for the Florida Keys and about 2–4 times for the U.S. Caribbean. In studies of cyprinids (Alò and Turner (2005) and salmonids (Fraser et al. (2007), coalescent-based, long-term estimates of N_e were higher than short-term estimates, suggesting that the studied populations had experienced relatively recent declines. Given that estimates of N_b may underestimate parametric N_e, apparent recent declines in lane snapper in the two populations may be somewhat less than suggested by differences in the short-term and long-term estimates. One last comment regards the apparent discrepancy between the estimates of genetic variability (seemingly higher in the sample from the Florida Keys) and genetic effective size (seemingly lower in the sample from the Florida Keys). Empirical data from a number of species generally have shown a positive relationship between genetic variation and population size (Frankham 1996). However, this relationship over time depends on a state of equilibrium between genetic drift and gene flow (migration), where the homogenizing effect of migration balances the diversifying effect of genetic drift (Duvernell et al. 2008). In general, populations are rarely in this equilibrium because of widespread, reoccurring demographic instability (Whitlock 1992). Such demographic instability is undoubtedly the case among the lane snapper sampled in this study, in part because of varying exploitation, and in part because patterns of migration appear to be asymmetric. Temporal sampling to determine whether the patterns observed in this study are maintained should be undertaken.

Effective population size is of primary importance to the conservation and management of exploited biological resources in terms of predicting potential extinction risk due to the fixation of deleterious alleles, the loss of adaptive genetic variance, and the potential capacity of a population to respond to either natural selection or environmental perturbation (Franklin 1980; Anderson 2005). In this context, a "50/500" rule (Rieman and Allendorf 2001), where an $N_e < 50$ indicates a population is highly vulnerable to inbreeding depression, and an Ne average

of >500 allows a population to maintain adaptive genetic variation through time, has served as a guide to conservation and management (Franklin 1980; Soulé 1980; but see Traill et al. 2010). The estimates of N_b for the population from the Florida Keys ranged from 241.3 to 275.6, depending on differences in the exclusion of rare alleles, and for both estimates the upper bound of the 95% confidence interval was less than 500. Even considering that N_b in this case is probably less than N_e, further management assessment of lane snapper resources in the Florida Keys would appear to be warranted. This may prove problematic, however, because the status of the lane snapper in the Florida Keys is uncertain; the majority of lane snapper landings in Florida come from the difficult-to-monitor recreational fishery (http://research.myfwc.com/engine/download_redirection_processasp?file=37_Lane_snapper_2008.pdf&objid=5284&dltype=article), making stock assessment uncertain.

Estimates of N_b for lane snapper in the U.S. Caribbean were greater than 500, with the lower bound to 95% confidence limits hovering around 500. Management assessment of lane snapper resources in the U.S. Caribbean potentially would be useful because of the importance of the species in recreational and commercial catches (e.g., in Puerto Rico; Matos-Caraballo 2000) and because species-specific catch data in the U.S. Virgin Islands have been historically absent (http://www.sefsc.noaa.gov/sedar/download/CaribData_Final.pdf?id = DOCUMENT). Based on data we presented here, however, conservation and management activity regarding lane snapper in the Florida Keys would seem the more immediate need.

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ACKNOWLEDGMENTS

We thank the following people for their invaluable assistance in obtaining samples for this study: L. Anibal, J. Leon, H. Lopez, D. Matos-Caraballo, and A. Rosario of the Department of Natural and Environmental Resources Fisheries Research Laboratory in Mayaguez, Puerto Rico; W. Ledee and D. Olsen of the St. Thomas Fisherman's Association; H. Rivera and W. Tobias of the U.S. Virgin Islands Division of Fish and Wildlife; and R. Beaver of the Florida Fish and Wildlife Commission. We also thank E. Carson for generating Figure 1 and for constructive comments on a draft of the manuscript. This work was supported by the Cooperative Research Program of the U.S. Department of Commerce (Grant NA06NMF4540061) and Texas AgriLife Research Project H-6703. This article is number 75 in the series Genetic Studies in Marine Fishes and contribution 193 of the Center for Biosystematics and Biodiversity at Texas A&M University.

REFERENCES

Acosta, A., and R. S. Appledoorn. 1992. Estimation of growth, mortality and yield per recruit for *Lutjanus synagris* (Linnaeus) in Puerto Rico. Bulletin of Marine Science 50:282–291.

Allen, G. R. 1985. Snappers of the world: an annotated catalogue of lutjanid species known to date. FAO (Food and Agriculture Organization of the United Nations) Fishery Synopsis 6.

Alò, D., and T. F. Turner. 2005. Effects of habitat fragmentation on effective population size in the endangered Rio grande silvery minnow. Conservation Biology 19:1138–1148.

Anderson, E. C. 2005. An efficient Monte Carlo method for estimating N_e from temporally spaced samples using a coalescent-based likelihood. Genetics 170:955–967.

Appledoorn, R., J. Beets, J. Bohnsack, B. Bolden, D. Matos, S. Meyers, A. Rosario, Y. Sadovy, and W. Tobias. 1992. Shallow water reef fish stock assessment for the U.S. Caribbean. NOAA Technical Memorandum NMFS-SEFSC-304.

Arevalo, E., S. Davis, and J. Sites Jr. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Systematic Biology 43:387–418.

Bagley, M. J., and J. B. Geller. 1998. Characterization of microsatellite loci in vermillion snapper *Rhomboplites aurorubens* (Percoidei: Lutjanidae). Molecular Ecology Notes 7:1089–1090.

Beaumont, M. A. 2003. Estimation of population growth or decline in genetically monitored populations. Genetics 164:1139–1160.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations using a coalescent approach. Proceedings of the National Academy of Sciences of the USA 98:4563–4568.

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

Bortone, S. A., and J. L. Williams. 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Florida): gray, lane, mutton, and yellowtail snappers. United States Army Corps of Engineers and National Coastal Ecosystems Team, Coastal Ecology Group, Biological Report 82(11.52) TR EL-82-4. Available: www.nwrc.usgs.gov/wdb/pub/species_profiles/82_11–052.pdf. (July 2010).

Bustamante, G., M. Chiappone, J. Kelly, A. Lowe, and K. S. Sealy. 2000. Fish and fisheries in Guantanamo Bay, Cuba: recommendations for their protection. Proceedings of the Gulf and Caribbean Fisheries Institute 51:242–257.

Camber, C. I. 1955. A survey of the red snapper fishery of the Gulf of Mexico, with special reference to the Campeche Banks. Florida Board of Conservation Marine Research Laboratory Technical Series 12.

Carvalho, G. R., and L. Hauser. 1995. Molecular genetics and the stock concept in fisheries. Pages 55–80 in G. R. Carvalho and T. J. Pitcher, editors. Molecular genetics in fisheries. Chapman and Hall, London.

CFMC (Caribbean Fisheries Management Council). 2005. Amendment to the fishery management plans (FMPs) of the U.S. Caribbean to address required provisions of the Magnuson-Stevens Fishery Conservation and Management Act. Available: www.caribbeanfmc.com/SCANNED%20.FMPS/06%20FINAL%20SFA%20-%20MAY%2003,2005/SFA-FMP.htm. (July 2010).

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1660.

Cowen, R. K., C. B. Paris, and A. Srinivasan. 2006. Scaling of connectivity in marine populations. Science 311:522–527.

Dowling, T. E., W. L. Minckley, P. C. Marsh, and E. S. Goldstein. 1996. Mitochondrial DNA variability in the endangered razorback sucker (*Xyrauchen texanus*): analysis of hatchery stocks and implications for captive propagation. Conservation Biology 10:120–127.

Dupanloup, I., S. Schneider, and L. Excoffier. 2002. A simulated annealing approach to define the genetic structure of populations. Molecular Ecology 11:2571–2581.

Duvernell, D. D., J. B. Lindmeier, K. E. Faust, and A. Whitehead. 2008. Relative influences of historical and contemporary forces shaping the distribution of genetic variation in the Atlantic killifish, *Fundulus heteroclitus*. Molecular Ecology 17:1344–1360.

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El Mousadik, A., and R. J. Petit. 1996. High level of genetic differentiation for allelic richness among population of the argan tree (*Argania spinosa* [L.] Skeels) endemic to Morocco. Theoretical and Applied Genetics 92:832–839.

Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10:1500–1508.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135–148 *in* M. E. Soulé and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts.

Fraser, D. J., M. M. Hansen, S. Østergaard, N. Tessier, M. Legault, and L. Bernatchez. 2007. Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. Molecular Ecology 16:3866–3889.

Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics 147:915–925.

Fu, Y.-X., and W.-H. Li. 1993. Statistical tests of neutral mutations. Genetics 133:693–709.

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

Goudet, J. 1995. F-stat, a computer program to calculate F-statistics. Journal of Heredity 86:485–486.

Guo, S., and E. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion from multiple alleles. Biometrics 48:361–372.

Johns, W. E., T. L. Townsend, D. M. Fratantoni, and W. D. Wilson. 2002. On the Atlantic inflow to the Caribbean Sea. Deep-Sea Research Part I Oceanographic and Research Papers 49:211–243.

Johnson, A. G., L. A. Collins, J. Dahl, and S. M. Baker Jr. 1997. Age, growth, and mortality of lane snapper from the northern Gulf of Mexico. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Research Agencies 49(1995):178–186.

Johnson, D. R., H. M. Perry, and W. M. Graham. 2004. Using nowcast model currents to explore transport of non-indigenous jellyfish into the Gulf of Mexico. Marine Ecology Progress Series 305:139–146.

Karlsson, S., E. Saillant, and J. R. Gold. 2009. Population structure and genetic variation of lane snapper (*Lutjanus synagris*) in the northern Gulf of Mexico. Marine Biology 156:1841–1855.

Leberg, P. 2005. Genetic approaches for estimating the effective size of populations. Journal of Wildlife Management 69:1385–1399.

Luckhurst, B. E., J. M. Dean, and M. Reichert. 2000. Age, growth and reproduction of the lane snapper *Lutjanus synagris* (Pisces: Lutjanidae) at Bermuda. Marine Ecology Progress Series 203:255–261.

Luikart, G., N. Ryman, D. A. Tallmon, M. K. Schwartz, and F. W. Allendorf. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conservation Genetics 11:355–373.

Manooch, C. S., III, and D. L. Mason. 1984. Age, growth, and mortality of lane snapper from southern Florida. Northeast Gulf Science 7:109–115.

Matos-Caraballo, D. 2000. Overview of Puerto Rico's small scale fisheries statistics: 1994–1997. Proceedings of the Gulf and Caribbean Fisheries Institute 51:215–231.

McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends in Ecology and Evolution 17:285–291.

Morrison, J., and W. D. Nowlin. 1977. Repeated nutrient, oxygen, and density sections through the Loop Current. Journal of Marine Research 35:105–128.

Nei, M., and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the USA 76:5269–5273.

Nunney, L., and D. Elam. 1994. Estimating the effective population size of conserved populations. Conservation Biology 8:175–184.

Peakall, R., M. Ruibal, and D. B. Lindenmayer. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. Evolution 57:1182–1195.

Peakall, R., and P. E. Smouse. 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Raymond, M., and F. Rousset. 1995. Genepop (version 2.1): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Renshaw, M. A., S. Karlsson, and J. R. Gold. 2007. Isolation and characterization of microsatellites in lane snapper (*Lutjanus synagris*), mutton snapper (*Lutjanus analis*), and yellowtail snapper (*Ocyurus chrysurus*). Molecular Ecology Notes 7:1084–1087.

Renshaw, M. A., E. Saillant, S. C. Bradfield, and J. R. Gold. 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.

Rieman, B. E., and F. W. Allendorf. 2001. Effective population size and genetic conservation criteria for bull trout. North American Journal of Fisheries Management 21:756–764.

Roberts, C. M. 1997. Connectivity and management of Caribbean coral reefs. Science 278:1454–1457.

Rozas, J., J. C. Sanchez-DelBarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd edition. Cold Spring Harbor Laboratory Press, New York.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin version 2.0: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Smouse, P. E., and R. Peakall. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561–573.

Soulé, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pages 151–169 *in* M. E. Soulé and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts.

Storz, J., and M. A. Beaumont. 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.

Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.

Tester, P. A., and K. A. Steidinger. 1997. *Gymnodinium breve* red tide blooms: initiation, transport, and consequences of surface circulation. Limnology and Oceanography 42:1039–1051.

Traill, L. W., B. W. Brook, R. R. Frankham, and C. J. A. Bradshaw. 2010. Pragmatic population viability targets in a rapidly changing world. Biological Conservation 143:28–34.

van Oosterhout, C., W. Hutchinson, D. Will, and P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Wang, J. 2005. Estimation of effective population sizes from data on genetic markers. Philosophical Transactions of the Royal Society B 360:1395–1409.

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

Waples, R. S. 2008. LdNe: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753–756.

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

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Whitlock, M. C. 1992. Temporal fluctuation in demographic parameters and the genetic variance among populations. Evolution 46:608–615.

TABLE A.1. Summary statistics for 17 nuclear-encoded microsatellites and a 590 base-pair sequence of the mitochondrially encoded *ND-4* gene for lane snapper sampled from four localities in the northern Caribbean Sea and one locality in the Florida Keys. For microsatellites the following statistics are reported: sample size (N), number of alleles (A), allelic richness (A_R), gene diversity (expected heterozygosity [H_E]), probability of conforming to expected Hardy–Weinberg proportions (P_{HW}), and an inbreeding coefficient (F_{IS}) measured as Weir and Cockerham's (1984) f. For the *ND-4* gene the following are reported: sample size (N), number of haplotypes (H), haplotype richness (H_R), hypotype (nucleon) diversity (H_D), and nucleotide diversity (π_D).

Locus and statistic	Florida Keys	Puerto Rico-east	Puerto Rico-west	St. Thomas	St. Croix
		Microsatellites			
*Lca*20					
N	99	100	98	100	100
A	13	12	15	12	12
A_R	12.98	11.98	15.00	11.94	12.00
H_E	0.821	0.757	0.798	0.735	0.750
P_{HW}	0.000	0.722	0.233	0.025	0.334
F_{IS}	0.212	0.009	0.092	−0.035	0.080
*Lca*22					
N	98	100	98	100	100
A	9	6	9	8	8
A_R	9.00	6.00	9.00	7.98	7.98
H_E	0.659	0.652	0.693	0.652	0.694
P_{HW}	0.813	0.002	0.468	0.856	0.368
F_{IS}	0.025	0.064	−0.015	−0.089	−0.066
*Lan*3					
N	99	100	98	100	100
A	11	11	12	13	12
A_R	11.00	11.00	12.00	12.96	11.98
H_E	0.851	0.835	0.839	0.851	0.804
P_{HW}	0.577	0.085	0.983	0.088	0.117
F_{IS}	−0.045	−0.018	−0.021	−0.034	−0.058
*Lan*6					
N	99	100	98	100	100
A	11	7	8	6	7
A_R	10.97	7.00	8.00	5.98	6.96
H_E	0.652	0.608	0.627	0.608	0.606
P_{HW}	0.258	0.969	0.947	0.718	0.526
F_{IS}	0.008	−0.037	−0.074	0.029	0.092
*Lan*11					
N	99	100	98	100	100
A	16	13	16	13	15
A_R	15.97	12.88	16.00	12.92	14.88
H_E	0.611	0.570	0.647	0.592	0.590
P_{HW}	0.841	0.977	0.020	0.277	0.757
F_{IS}	−0.025	−0.035	0.039	0.003	−0.119
*Lan*12					
N	99	100	98	100	100
A	5	5	3	3	3
A_R	4.99	4.99	3.00	3.00	2.98
H_E	0.227	0.116	0.079	0.087	0.096
P_{HW}	0.604	1.000	1.000	0.089	0.209
F_{IS}	0.067	−0.039	−0.033	0.199	0.169

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TABLE A.1. Continued.

Locus and statistic	Florida Keys	Puerto Rico-east	Puerto Rico-west	St. Thomas	St. Croix
*Lan*13					
N	99	100	98	100	100
A	6	5	6	6	5
A_R	5.98	4.98	6.00	5.96	4.96
H_E	0.537	0.591	0.602	0.606	0.540
P_{HW}	0.923	0.002	0.025	0.308	0.018
F_{IS}	0.041	0.289	0.135	0.109	0.259
*Lsy*7					
N	99	100	98	100	100
A	10	9	10	10	13
A_R	9.98	9.00	10.00	9.96	12.88
H_E	0.716	0.637	0.667	0.641	0.696
P_{HW}	0.007	0.264	0.378	0.548	0.210
F_{IS}	0.055	−0.051	0.082	0.110	0.037
*Lsy*13					
N	99	100	98	100	100
A	14	12	14	13	11
A_R	13.99	11.92	14.00	12.90	10.94
H_E	0.761	0.742	0.795	0.783	0.798
P_{HW}	0.659	0.496	0.543	0.004	0.164
F_{IS}	−0.022	−0.011	−0.014	0.017	0.035
*Lsy*14					
N	99	100	98	100	100
A	7	4	6	5	4
A_R	6.96	4.00	6.00	4.98	4.00
H_E	0.374	0.633	0.633	0.690	0.667
P_{HW}	0.438	0.837	0.164	0.553	0.424
F_{IS}	−0.027	0.037	−0.015	−0.080	0.014
*Och*4					
N	99	100	98	100	100
A	6	7	8	7	7
A_R	5.99	6.98	8.00	6.98	7.00
H_E	0.614	0.612	0.610	0.653	0.644
P_{HW}	0.206	0.216	0.609	0.893	0.095
F_{IS}	−0.103	0.068	−0.138	0.020	0.036
*Och*13					
N	99	100	98	100	100
A	6	6	6	5	5
A_R	6.00	5.96	6.00	5.00	5.00
H_E	0.432	0.321	0.325	0.228	0.336
P_{HW}	0.912	0.414	0.762	0.209	0.182
F_{IS}	−0.006	−0.090	0.025	−0.051	0.016

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TABLE A.1. Continued.

Locus and statistic	Florida Keys	Puerto Rico-east	Puerto Rico-west	St. Thomas	St. Croix
*Prs*248					
N	98	100	98	100	100
A	23	33	31	30	29
A_R	23.00	32.86	31.00	29.86	28.88
H_E	0.929	0.912	0.913	0.910	0.901
P_{HW}	0.241	0.045	0.080	0.008	0.358
F_{IS}	0.078	0.003	0.061	0.022	−0.010
*Prs*328					
N	99	100	98	100	100
A	7	5	6	8	5
A_R	6.99	5.00	6.00	4.98	7.96
H_E	0.633	0.501	0.433	0.467	0.583
P_{HW}	0.100	0.022	0.387	0.050	0.013
F_{IS}	−0.133	−0.077	0.080	0.058	0.006
*Ra*1					
N	99	100	98	100	100
A	11	9	9	8	9
A_R	10.97	9.00	9.00	7.96	8.96
H_E	0.730	0.742	0.742	0.699	0.726
P_{HW}	0.206	0.595	0.726	0.395	0.477
F_{IS}	0.100	−0.037	0.009	−0.015	0.104
*Ra*2					
N	99	100	98	99	100
A	7	8	7	7	6
A_R	7.00	7.96	7.00	6.98	6.00
H_E	0.704	0.780	0.767	0.734	0.723
P_{HW}	0.242	0.876	0.368	0.160	0.056
F_{IS}	−0.062	0.025	0.009	0.005	0.091
*Ra*6					
N	99	100	98	100	100
A	5	5	5	5	5
A_R	4.99	5.00	5.00	5.00	5.00
H_E	0.552	0.659	0.611	0.544	0.541
P_{HW}	0.059	0.061	0.045	0.307	0.011
F_{IS}	0.103	0.135	0.049	−0.046	0.045
MtDNA					
N	32	25	26	25	25
H	12	9	6	8	8
H_R	9.38	8.00	4.88	7.00	7.00
H_D	0.845	0.597	0.597	0.590	0.637
π_D	0.0046	0.0044	0.0024	0.0029	0.0024

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TABLE A.2. Spatial distribution of mtDNA haplotypes among lane snapper (*Lutjanus synagris*) from one locality in the Florida Keys and four localities in the Caribbean Sea.

MtDNA Haplotype	Florida Keys	Puerto Rico-east	Puerto Rico-west	St. Thomas	St. Croix	GenBank Accession #
#1	1					EU676013
#2	1					EU676016
#3	5	16	16	16	15	EU025741
#4	2					EU025748
#5	11		1			EU025734
#6	2					EU025745
#7	1					EU025746
#8	1			1		EU025747
#9	1					HM369112
#10	5	1	2		2	EU025744
#11	1					HM369113
#12	1					EU025752
#13		1				HM369114
#14		1				HM369115
#15		1				HM369116
#16		1				HM369117
#17		1				HM369118
#18		2				HM369119
#19		1	5	3	3	HM369120
#20			1			HM369121
#21			1			HM369122
#22				1		EU676017
#23				1		HM369123
#24				1		HM369124
#25				1		HM369125
#26				1		HM369126
#27					1	HM369127
#28					1	HM369128
#29					1	HM369129
#30					1	HM369130
#31					1	HM369131

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