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# Population Structure, Long-Term Connectivity, and Effective Size of Mutton Snapper (*Lutjanus Analis*) In the Caribbean Sea and Florida Keys

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**Abstract**—Genetic structure and average long-term connectivity and effective size of mutton snapper (*Lutjanus analis*) sampled from offshore localities in the U.S. Caribbean and the Florida Keys were assessed by using nuclear-encoded microsatellites and a fragment of mitochondrial DNA. No significant differences in allele, genotype (microsatellites), or haplotype (mtDNA) distributions were detected; tests of selective neutrality (mtDNA) were nonsignificant after Bonferroni correction. Heuristic estimates of average long-term rate of migration (proportion of migrant individuals/generation) between geographically adjacent localities varied from 0.0033 to 0.0054, indicating that local subpopulations could respond independently of environmental perturbations. Estimates of average long-term effective population sizes varied from 341 to 1066 and differed significantly among several of the localities. These results indicate that over time larval drift and interregional adult movement may not be sufficient to maintain population sustainability across the region and that there may be different demographic stocks at some of the localities studied. The estimate of long-term effective population size at the locality offshore of St. Croix was below the minimum threshold size considered necessary to maintain the equilibrium between the loss of adaptive genetic variance from genetic drift and its replacement by mutation. Genetic variability in mutton snapper likely is maintained at the intraregional level by aggregate spawning and random mating of local populations. This feature is perhaps ironic in that aggregate spawning also renders mutton snapper especially vulnerable to overexploitation.

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## Population structure, long-term connectivity, and effective size of mutton snapper (*Lutjanus analis*) in the Caribbean Sea and Florida Keys

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An understanding of the genetic structure of exploited, reef-associated marine fish species is important for their effective conservation and management. Many such species are known to have long larval phases (Boehlert, 1996)—a trait associated with either genetic cohesion or connectivity across regional geographic scales (Shulman and Bermingham, 1995; Rocha et al., 2002). However, many of these species also form stable spawning aggregations where assemblages of individuals gather in large densities with the specific purpose of reproducing, generally at approximately the same time and place each year (Domeier and Colin, 1997). For geographically widespread species, multiple spawning aggregations could tend to minimize connectivity at regional scales if adult movements are intraregionally localized. This effect would be pertinent to management of heavily exploited groups such as snappers and groupers in which spawning aggregations are common (Claro and Lindeman, 2003). Because fishing efforts commonly target spawning

aggregations, participating species are at elevated risk of overexploitation and rapid population depletion (Domeier and Colin, 1997; Domeier, 2004; Sadovy de Mitcheson et al., 2008). Knowledge of population structure is thus of importance because separate management of subregional stocks, should they exist, is critical both to avoid over-exploitation and to maintain potentially adaptive genetic variation (Carvalho and Hauser, 1995; Hauser and Ward, 1998). Recent population-genetic studies of species that participate in spawning aggregations have included assessment of genetic variation (Rhodes et al., 2003) and the relationship between effective population size ( $N_e$ ) and census size (Bekkevold et al., 2002).

In this study, we assessed genetic structure and average long-term connectivity and effective size of mutton snapper (*Lutjanus analis*) sampled from four localities in the northeastern Caribbean Sea and one locality in the Florida Keys. Mutton snapper are an important component of commercial fisheries in this region

(Matos-Caraballo et al., 2004), and fillets often sell for as much as US \$12 per pound in Miami seafood markets (Watanabe, 2001). Landings of mutton snapper, however, have declined over the past decade in Puerto Rico (Matos-Caraballo et al., 2004; Cummings, 2007a, 2007b) and in southern Florida. In the latter fishery, commercial landings between the years 2006 and 2009 dropped from 127.0 to 53.6 metric tons ([http://www.st.nmfs.noaa.gov/st1/commercial/landings/annual\\_landings.html](http://www.st.nmfs.noaa.gov/st1/commercial/landings/annual_landings.html), accessed July 2011). Although mutton snapper are not considered overfished in U.S. waters of the western Atlantic Ocean and Caribbean Sea (Federal Register, 2005), concern regarding the condition of the fishery has prompted both seasonal and permanent closures off southwest Florida, Puerto Rico, and the U.S. Virgin Islands (<http://www.edf.org/article.cfm?contentid=443>; <http://sero.nmfs.noaa.gov/sf/ClosedAreaCoordinates.htm>, accessed March 2011). Finally, aggregate spawning of mutton snapper is well documented (Claro, 1981; Domeier et al., 1996), with known aggregations occurring at Riley's Hump in the Dry Tortugas National Park, Florida (Domeier, 2004; Burton et al., 2006), Gladden Spit, Belize (Graham et al., 2008), numerous sites along the coast of Cuba (Claro and Lindeman, 2003), Turks and Caicos (Mueller, 1994; Domeier et al., 1997), off the southwest coast of St. Croix in the U.S. Virgin Islands, (SEDAR, 2007), and at La Parguera shelf along the southwest coast of Puerto Rico (Esteves, 2005). Other, less well-documented aggregations occur in the Cayman Islands and the Bahamas (Heyman<sup>1</sup>).

In a recent study by Shulzitski et al. (2009), variation at eight nuclear-encoded microsatellites was used to investigate population structure in mutton snapper sampled from localities off the Florida Keys, two localities in the western Caribbean Sea (Belize and Honduras), and the west coast of Puerto Rico. No evidence of genetic heterogeneity was found, leading these authors to suggest that larval dispersal or long-distance migration of adults maintained genetic homogeneity over such a broad geographic scale. However, simulation studies based on prevailing currents in the Caribbean Sea have indicated that larval transport of reef-associated species in most areas in the region is limited, with average distances of 145 and 212 km for one- and two-month periods of larval dispersal, respectively (Roberts, 1997), and with ecologically relevant larval dispersal distances in the 10–100 km range (Cowen et al., 2000, 2006). In addition, empirical studies in the region have shown that species with the capacity for long-range larval dispersal often exhibit high levels of larval retention (Taylor and Hellberg, 2003) and that the degree of dispersal can differ substantially between windward (high dispersal) and leeward (low dispersal) sides of islands (Swearer et al., 1999). Finally, the few data that exist (Beaumarriage, 1969; Mueller, 1995; Farmer, 2009) indicate that

movement of adult mutton snapper is generally limited to only a few kilometers.

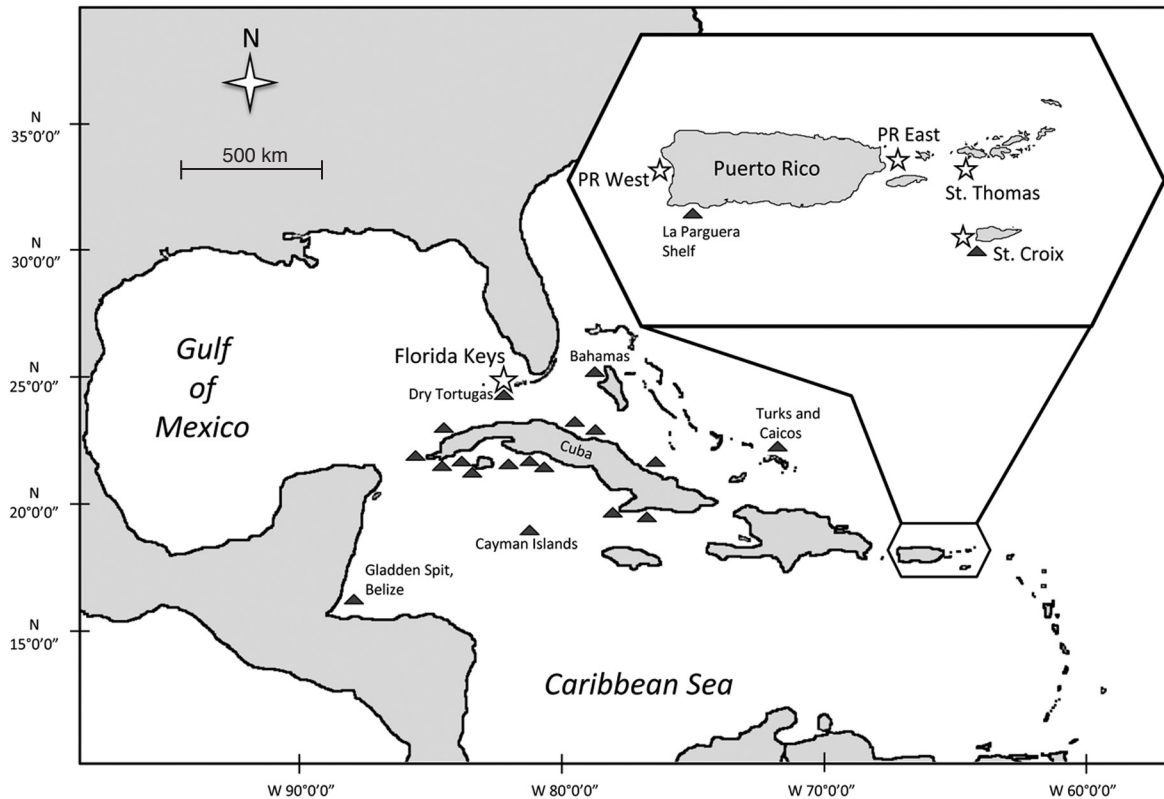
The possibility of limited larval transport and long-distance adult movement among mutton snapper in the region may indicate that the genetic homogeneity observed by Shulzitski et al. (2009) at markers (microsatellites) presumed to be selectively neutral may obscure other differences that impact local population sustainability. The goal of the present study was to examine this possibility further by using genetic data to assess both long-term connectivity (migration) and effective population size ( $N_e$ ) among the sampled localities. Populations with homogeneous allele frequencies at selectively neutral loci do not necessarily have the same effective sizes (Saillant and Gold, 2006) and differences in  $N_e$  could signal populations with reduced sustainability and capability to respond to environmental pressures such as over-exploitation or habitat degradation (Frankham, 1995).

## Materials and methods

A total of 498 mutton snapper were sampled between 2007 and 2009 from four localities in the northern Caribbean Sea and one locality in the Florida Keys (Fig. 1). The locality in the Florida Keys is near a now annually protected mutton snapper spawning aggregation in the Dry Tortugas; the locality off the west coast of Puerto Rico is near several marine protected areas (MPAs) and a mutton snapper spawning aggregation off the southwest coast; the locality off the south coast of St. Thomas is near several MPAs; and the locality off the southwest coast of St. Croix is a seasonally protected mutton snapper spawning aggregation area. Samples from the Florida Keys (FK) were obtained from local fishermen or fish houses in or near Marathon, Florida. Samples from the west coast of Puerto Rico (PR-west) were procured from fish houses in or near Mayaguez, whereas samples from the east coast of Puerto Rico (PR-east) were obtained at fish houses in or near Fajardo. Samples from St. Croix (SC) were obtained as part of an ongoing project of the Caribbean Fishery Management Council (Kojis and Quinn<sup>2</sup>), and samples from St. Thomas (ST) came from the Gustave Quétel Fish House in Frenchtown (Charlotte Amalie) or local fishermen. Sample sizes were as follows: FK (118), PR-east (96), PR-west (94), ST (97), and SC (93). Except for samples from St. Croix (SC), small pieces (4–5 mm<sup>3</sup>) of caudal fin were removed from each fish and fixed in 95% ethanol. Samples from St. Croix primarily were internal organs fixed in DMSO storage buffer (Seutin et al., 1991). DNA

<sup>1</sup> Heyman, W. D. 2010. Personal commun. Department of Geography, Texas A&M University, College Station, Texas 77843-3148.

<sup>2</sup> Kojis, B. L., and N. J. Quinn. 2011. Validation of a spawning aggregation of mutton snapper and characterization of the benthic habitats and fish in the mutton snapper seasonal closed area, St. Croix, U.S. Virgin Islands. [Available at <http://www.caribbeanfmc.com/pdfs%202011/Mutton%20Snapper%20Report%20for%20CFMC%20-%202014%20Feb%2011%20, Final.pdf>, accessed July 2011.]



**Figure 1**

Map of mutton snapper (*Lutjanus analis*) collection sites and known sites of spawning aggregations in the Caribbean Sea and Florida Keys. Collection sites are represented by stars; known aggregation sites are represented by triangles.

was extracted by using a standard phenol-chloroform protocol (Sambrook et al., 1989).

Seventeen microsatellites were surveyed in three multiplex panels: panel 1 (*Lan6*, *Lan11*, *Lan12*, *Lan13*, *Och4*, and *Ra6*), panel 2 (*Lan9*, *Lca22*, *Lca64*, *Lsy8*, *Lsy13*, and *Ra2*), and panel 3 (*Lan3*, *Lca20*, *Lsy4*, *Prs248*, and *Ra1*). Touchdown polymerase-chain-reaction (PCR) protocols were as follows: 95°C for 3 min; 7 cycles at 95°C for 30 sec, annealing at  $T_{A1}$  for 1 min, and 72°C for 4 min; 7 cycles at 95°C for 30 sec, annealing at  $T_{A2}$  for 1 min, and 72°C for 4 min; and 28 cycles at 95°C for 30 sec, annealing at  $T_{A3}$  for 1 min, and 72°C for 4 min, with a final extension of 10 min at 72°C. Annealing temperatures  $T_{A1}$ ,  $T_{A2}$ , and  $T_{A3}$ , respectively, were 55°C, 53°C, 51°C (Panel 1), 54°C, 52°C, and 50°C (Panel 2), and 52°C, 49°C, and 46°C (Panel 3). PCR primer sequences for individual microsatellites may be found as follows: *Ra1*, *Ra2*, and *Ra6* (Bagley and Geller, 1998); *Lca20*, *Lca22*, *Lca64*, and *Prs248* (Gold et al., 2001); and *Lan3*, *Lan6*, *Lan9*, *Lan11*, *Lan12*, *Lan13*, *Lsy4*, *Lsy8*, *Lsy13*, and *Och4* (Renshaw et al., 2007). Details regarding fluorescent labeling of primers and amplification are described in Renshaw et al. (2006, 2007). An ABI Prism 377 DNA sequencer (Applied Biosystems Inc., Foster City, CA) was used to separate and visualize amplification products. Gel analysis was

performed with Genescan Analysis, vers. 3.1.2® (Applied Biosystems), with allele-calling performed with Genotyper® software, vers. 2.5 (Applied Biosystems). Genotypes at the 16 polymorphic microsatellites assayed may be found at <http://wfsc.tamu.edu/doc/>, accessed July 2011, under the file name "Microsatellite genotypes of mutton snappers."

A 590 base-pair (bp) fragment of the mitochondrial NADH-dehydrogenase subunit 4 (ND-4) gene was amplified and sequenced for 134 individuals (25–29 from each locality). The primers NAP-2 (Arevalo et al., 1994) and ND4LB (Bielawski and Gold, 2002) were used for fragment amplification, and ND4BL was used for sequencing. Polymerase chain reaction amplifications were run in 30  $\mu$ L reaction volumes, with ~100 ng whole genomic DNA, 1 $\times$  GoTaq Flexi Buffer (Promega, Madison, Wisconsin), 1.5 mM MgCl<sub>2</sub>, 0.5  $\mu$ M of each primer, 250  $\mu$ M of each dNTP, and 1.7U GoTaq Taq polymerase (Promega). The PCR protocol consisted of a 95°C initial denaturation, followed by 35 cycles of 95°C for 30 sec, 50°C for 45 sec, and 72°C for 1 min, with a 10 minute final extension at 72°C. Sequencing reactions were carried out with a Big Dye terminator kit®, vers. 3.1 (Applied Biosystems), according to the manufacturer's recommendations; products were separated and visualized on an ABI 3100 capillary sequencer



(Applied Biosystems) and sequences were aligned and edited in Sequencher, vers. 4.0 (Gene Codes Corp., Ann Arbor, MI).

For microsatellites, summary statistics, including number of alleles, allelic richness, unbiased gene diversity (expected heterozygosity), and the inbreeding coefficient  $F_{IS}$ , measured as  $f$  of Weir and Cockerham (1984), were generated in FSTAT (Goudet, 1995; vers. 2.9.3.2, <http://www2.unil.ch/popgen/softwares/fstat.htm>, accessed July 2011). Homogeneity in allelic richness and gene diversity among the five locations were assessed by using Friedman rank tests, with SPSS software (<http://www-01.ibm.com/software/analytics/spss/products/statistics/>, accessed July 2011). Departure from Hardy-Weinberg (HW) equilibrium expectations for each locality was tested with exact probability tests in Genepop (Raymond and Rousset, 1995; vers. 3.4, <http://genepop.curtin.edu.au/>, accessed March 2011), by using a Markov Chain approach (Guo and Thompson, 1992), with 10,000 dememorizations, 500 batches, and 5000 iterations per batch. Global and pairwise (between localities) exact tests of homogeneity of allelic (genic) and genotypic distributions also were conducted in Genepop; genetic homogeneity among locations was further tested by using analysis of molecular variance (AMOVA), with the program Arlequin (Excoffier and Lischer, 2010; vers. 3.5.1.2, <http://cmpg.unibe.ch/software/arlequin35/>, accessed March 2011). Sequential Bonferroni correction (Rice, 1989) of  $P$  values was applied for all simultaneous tests. Microchecker (van Oosterhout et al., 2004) was used to determine whether genotype scores at each locus were compromised by the presence of null alleles, stuttering, or genotyping errors.

For mitochondrial ND-4 DNA sequences, number of haplotypes and haplotype diversity were determined by using DnaSP, vers. 5.10.01 (Rozas et al., 2003; <http://www.ub.edu/dnasp/>, accessed March 2011); haplotype richness and nucleotide diversity for each sample locality were estimated following El Mousadik and Petit (1996) and using the software Rarefac (available at <http://www.pierroton.inra.fr/genetics/labo/Software/Rarefac/>, accessed July 2011) and Arlequin, respectively. A bootstrap resampling method (Dowling et al., 1996) was used to test homogeneity of haplotype number and diversity among localities. The observed haplotype number and diversity at each locality was compared to the distribution in 1000 bootstrap samples of a comparable size drawn from the entire population (all samples pooled); resampling was conducted in PopTools, a free add-in to Microsoft Excel and available at <http://www.poptools.org/>, accessed July 2011. Differences in average nucleotide diversity were considered significant if pairwise comparisons differed by more than two standard errors. Homogeneity in mtDNA haplotype distributions among localities was tested using global exact tests and analysis of molecular variance (AMOVA), as implemented in Arlequin. Pair-wise (between locations) estimates of  $\Phi_{ST}$ , an analogue of  $F_{ST}$ , were generated by using Arlequin;  $\Phi_{ST}$  estimates were based on pair-wise genetic distances, with significance determined by exact tests

(Raymond and Rousset, 1995; Goudet et al., 1996), as implemented in Arlequin. Selective neutrality of mtDNA variation in each sample was tested by calculating Fu's (1997)  $F_S$  statistic and Fu and Li's (1993)  $D^*$  and  $F^*$  statistics, as implemented in the DnaSP program. Significance of  $F_S$ ,  $D^*$ , and  $F^*$  was assessed in FSTAT by using 10,000 coalescent simulations (after Rozas et al., 2003) based on the observed number of segregating sites in each sample.

The coalescent-based program Migrate (Beerli and Felsenstein 2001; vers. 3.2.6, <http://popgen.sc.fsu.edu/Migrate/Migrate-n.html>, accessed July 2011) was used to generate maximum-likelihood estimates of both the average long-term (mutation-scaled) migration rate ( $M$ ) between pairs of localities and the parameter theta ( $\Theta$ ) for each locality. Because the model used in Migrate explicitly accounts for migration, estimates of  $\Theta$  can be generated for individual subpopulations within a larger metapopulation (Waples, 2010). The average long-term migration rate ( $m$ ) between localities was estimated as  $M = m/\mu$ , where  $\mu$  is the average, per gene mutation rate. Values of theta ( $4N_e\mu$ ) were used to estimate average long-term effective population size ( $N_e$ ) of each locality. To obtain estimates of  $m$  and  $N_e$ , the modal mutation rate ( $\mu$ ) of the microsatellite data set was obtained by using the Bayesian coalescent approach of Beaumont (1999) and Storz and Beaumont (2002) and the software MSVAR (vers. 0.4.1b, <http://www.rubic.rdg.ac.uk/~mab/software.html>, accessed July 2011). Because simulations in Migrate are computationally demanding, all parameter estimates were based on a random sample of 25 individuals from each location (125 individuals total). A preliminary analysis (short run) established initial estimates for both  $M$  and  $\Theta$  that were then used as starting values in final long runs. Parameter estimates were obtained by averaging three replicate long runs that included 40 short Monte Carlo Markov chains (MCMC,  $10^4$  gene trees sampled) and three long chains ( $2.5 \times 10^6$  gene trees sampled). To ensure parameter stability, the first  $1 \times 10^4$  steps of each chain were discarded as burn-in.

## Results

Summary statistics for microsatellites are presented in Table 1. Microsatellite *Lca64* was monomorphic for a 111-bp allele (scored with primers as 151 bp) and was excluded from further analysis. Of the remaining 16 microsatellites, the number of alleles ranged from 2–3 at *Lsy8* to 19–25 at *Prs248*. Allelic richness ranged from 2.00–2.86 at *Lsy8* and from 19.00–23.67 at *Prs248*, and expected (unbiased) gene diversity ranged from 0.050–0.118 at *Lsy8* and from 0.860–0.901 at *Lan11*. Across all microsatellites and localities, number of alleles, allelic richness, and gene diversity averaged ( $\pm$ standard error [SE]) 10.05 (0.26), 9.80 (0.22), and 0.594 (0.01), respectively. No significant differences (Friedman's rank tests) in allelic richness ( $P=0.232$ ) or gene diversity ( $P=0.373$ ) were found among localities.

**Table 1**

Summary statistics for 16 nuclear-encoded microsatellites and a 590 base-pair sequence of the mitochondrially encoded ND-4 gene for mutton snapper (*Lutjanus analis*) sampled from four localities in the northeastern Caribbean Sea and one locality in the Florida Keys. For microsatellites,  $n$  is sample size, #A is number of alleles,  $A_R$  is allelic richness,  $H_E$  is gene diversity (expected heterozygosity),  $P_{HW}$  is the probability of conforming to expected Hardy-Weinberg genotypic proportions, and  $F_{IS}$  is an inbreeding coefficient (measured as  $f$  of Weir and Cockerham, 1984). For mitochondrial DNA (mtDNA):  $n$  is sample size, # $H_{obs}$  is observed number of haplotypes,  $H_R$  is haplotype richness,  $H_{Dobs}$  is observed haplotype (nucleon) diversity, and  $\pi_D$  is nucleotide diversity. # $H_{exp}$  and  $H_{Dexp}$  are expected haplotype number and diversity (95% confidence interval [CI]), respectively, as determined by bootstrap resampling of the entire population.

Microsatellite	St. Croix	St. Thomas	Puerto Rico-east	Puerto Rico-west	Florida Keys
<i>Lan3</i>					
$n$	81	97	96	94	118
#A	14	14	15	13	13
$A_R$	13.98	13.81	14.58	12.84	12.78
$H_E$	0.849	0.857	0.829	0.838	0.857
$P_{HW}$	0.274	0.502	0.011	0.875	0.377
$F_{IS}$	0.026	-0.035	0.020	0.061	-0.009
<i>Lan6</i>					
$n$	93	97	96	94	114
#A	20	17	18	19	18
$A_R$	19.54	16.75	17.80	18.78	17.11
$H_E$	0.854	0.835	0.882	0.897	0.868
$P_{HW}$	0.033	0.282	0.776	0.838	0.676
$F_{IS}$	0.030	-0.024	0.032	0.016	-0.010
<i>Lan9</i>					
$n$	93	97	96	94	118
#A	13	12	12	12	12
$A_R$	12.69	11.56	11.80	11.93	11.75
$H_E$	0.776	0.774	0.781	0.746	0.795
$P_{HW}$	0.628	0.236	0.477	0.322	0.230
$F_{IS}$	0.058	-0.039	-0.027	-0.012	-0.003
<i>Lan11</i>					
$n$	91	96	96	94	116
#A	21	20	22	21	24
$A_R$	20.50	19.54	21.54	20.06	22.25
$H_E$	0.889	0.869	0.886	0.860	0.901
$P_{HW}$	0.002	0.766	0.743	0.968	0.151
$F_{IS}$	0.110	0.005	-0.070	-0.027	-0.004
<i>Lan12</i>					
$n$	93	97	96	94	118
#A	5	5	5	5	6
$A_R$	4.86	5.00	4.95	4.98	5.86
$H_E$	0.595	0.646	0.643	0.611	0.642
$P_{HW}$	0.208	0.823	0.193	0.080	0.057
$F_{IS}$	0.151	-0.022	0.011	-0.045	0.023
<i>Lan13</i>					
$n$	93	97	96	94	118
#A	4	4	5	3	5
$A_R$	3.98	3.83	4.81	3.00	4.36
$H_E$	0.295	0.297	0.378	0.380	0.341
$P_{HW}$	0.083	0.198	0.337	0.234	0.863
$F_{IS}$	0.161	0.064	0.063	0.076	-0.044

*continued*

Table 1 (continued)

Microsatellite	St. Croix	St. Thomas	Puerto Rico-east	Puerto Rico-west	Florida Keys
<i>Lca20</i>					
<i>n</i>	93	96	95	94	117
#A	12	14	13	14	12
A <sub>R</sub>	11.84	13.80	12.96	13.64	11.86
H <sub>E</sub>	0.589	0.646	0.683	0.677	0.659
P <sub>HW</sub>	0.115	0.610	0.039	0.388	0.125
F <sub>IS</sub>	0.032	-0.015	0.076	0.010	0.041
<i>Lca22</i>					
<i>n</i>	91	97	96	94	118
#A	5	4	5	4	6
A <sub>R</sub>	4.88	3.83	4.83	3.85	5.35
H <sub>E</sub>	0.546	0.465	0.483	0.481	0.507
P <sub>HW</sub>	0.106	0.744	0.600	0.710	0.440
F <sub>IS</sub>	0.074	0.003	0.072	0.005	0.013
<i>Lsy4</i>					
<i>n</i>	93	95	95	93	118
#A	4	4	4	4	4
A <sub>R</sub>	4.00	4.00	4.00	4.00	4.00
H <sub>E</sub>	0.270	0.331	0.386	0.329	0.355
P <sub>HW</sub>	0.682	0.308	0.867	0.806	0.932
F <sub>IS</sub>	-0.077	0.078	-0.090	-0.077	-0.050
<i>Lsy8</i>					
<i>n</i>	93	96	95	94	118
#A	3	2	2	3	2
A <sub>R</sub>	2.86	2.00	2.00	2.85	2.00
H <sub>E</sub>	0.083	0.118	0.052	0.102	0.050
P <sub>HW</sub>	1.000	1.000	1.000	1.000	1.000
F <sub>IS</sub>	-0.034	-0.062	-0.022	-0.046	-0.022
<i>Lsy13</i>					
<i>n</i>	91	96	96	94	118
#A	10	7	7	7	7
A <sub>R</sub>	9.42	6.81	6.95	6.81	6.65
H <sub>E</sub>	0.733	0.716	0.739	0.728	0.742
P <sub>HW</sub>	0.007	0.875	0.037	0.010	0.633
F <sub>IS</sub>	0.105	-0.018	-0.015	0.065	-0.073
<i>Och4</i>					
<i>n</i>	93	97	96	94	118
#A	5	4	4	4	5
A <sub>R</sub>	4.98	4.00	4.00	4.00	4.68
H <sub>E</sub>	0.559	0.576	0.535	0.553	0.565
P <sub>HW</sub>	0.334	0.318	0.495	0.238	0.004
F <sub>IS</sub>	0.115	-0.127	0.085	-0.039	0.160
<i>Prs248</i>					
<i>n</i>	80	97	94	91	117
#A	19	22	25	24	24
A <sub>R</sub>	19.00	21.28	23.67	22.48	21.57
H <sub>E</sub>	0.860	0.886	0.886	0.870	0.870
P <sub>HW</sub>	0.011	0.583	0.507	0.190	0.818
F <sub>IS</sub>	0.084	0.000	0.014	-0.015	0.007

continued

Table 1 (continued)

Microsatellite	St. Croix	St. Thomas	Puerto Rico-east	Puerto Rico-west	Florida Keys
<i>Ra1</i>					
<i>n</i>	93	97	96	94	118
#A	5	4	5	6	6
$A_R$	4.84	3.97	4.67	5.83	5.25
$H_E$	0.592	0.560	0.558	0.606	0.567
$P_{HW}$	0.877	0.327	0.150	0.308	0.410
$F_{IS}$	0.037	0.043	0.142	-0.019	0.059
<i>Ra2</i>					
<i>n</i>	93	94	96	94	118
#A	19	17	18	19	17
$A_R$	18.52	16.51	17.13	18.63	16.04
$H_E$	0.780	0.767	0.753	0.778	0.781
$P_{HW}$	0.345	0.873	0.466	0.549	0.436
$F_{IS}$	0.008	-0.013	0.073	-0.012	-0.020
<i>Ra6</i>					
<i>n</i>	93	97	92	94	118
#A	2	4	4	3	3
$A_R$	2.00	3.65	3.87	2.85	2.90
$H_E$	0.053	0.109	0.134	0.111	0.105
$P_{HW}$	1.000	0.106	1.000	1.000	1.000
$F_{IS}$	-0.022	0.050	-0.052	-0.051	-0.046
mtDNA					
<i>n</i>	27	26	29	25	27
# $H_{obs}$	11	11	11	11	10
# $H_{exp}$	9.6 (7–12)	9.9 (7–13)	9.5 (7–12)	9.6 (7–12)	9.7 (7–13)
$H_R$	10.97	10.99	10.92	11.00	9.97
$H_{Dobs}$	0.832	0.812	0.865	0.873	0.721
$H_{Dexp}$	0.835	0.818	0.873	0.818	0.835
(95% CI)	(0.732–0.915)	(0.709–0.900)	(0.788–0.934)	(0.693–0.897)	(0.731–0.916)
$\pi_D$	0.003	0.004	0.004	0.004	0.003

Significant deviations from Hardy Weinberg equilibrium were found before Bonferroni correction in nine of 80 tests (Table 1). Only one microsatellite (*Lan11*) at one locality (SC) deviated significantly ( $P=0.0019$ ) from expectation after correction. Analysis with Microchecker indicated an excess of homozygotes, indicating possible null alleles, at *Lan11* (in SC) and *Och4* (in FK), a finding reflected in the  $F_{IS}$  values of 0.11 and 0.16 (Table 1), respectively, for these microsatellites at those localities. Sizes of observed alleles were compatible with the stepwise mutation model (SMM) for all microsatellites, except sizes of *Lca20*, *Lsy13*, and *Prs248*. Very rare alleles at *Lca20* (allele 231) and *Prs248* (allele 227) that differed by one base from their “regular” dinucleotide repeat were excluded from the analysis (Migrate) where a SMM was assumed. Two such alleles (alleles 118 and 120) were found at *Lsy13*; consequently, *Lsy13* also was excluded from analysis with Migrate.

A total of 25 mtDNA haplotypes were observed among the 134 individuals sequenced. The number and distribution of mtDNA haplotypes across localities are

given in Table 2; summary statistics for mtDNA may be found in Table 1. Haplotype richness ranged from 9.97 (FK) to 11.00 (PR-west), and haplotype diversity ranged from 0.72 (FK) to 0.87 (PR-west). Results of bootstrap resampling analysis indicated that observed haplotype number and diversity at each locality did not deviate significantly from expectations in random subsamples of the overall data set (Table 1). Estimates of haplotype richness and nucleotide diversity were essentially identical at all localities (Table 1).

Exact tests of homogeneity of both microsatellite allele and genotype distributions among localities were nonsignificant ( $P=0.225$ , alleles;  $P=0.288$ , genotypes), and the among-localities component of molecular variance (all microsatellites combined), estimated by AMOVA, did not differ significantly from zero ( $\Phi_{ST}=-0.0001$ ,  $P=0.644$ ). Nearly identical results were obtained for mtDNA; an exact test of homogeneity of haplotype distribution was nonsignificant ( $P=0.590$ ) and the among-locality component of molecular variance (from AMOVA) did not differ significantly from zero ( $\Phi_{ST}=-0.010$ ,



**Table 2**

Distribution of individual haplotypes in 134 mutton snapper (*Lutjanus analis*) sampled from four localities in the northeastern Caribbean Sea and one locality in the Florida Keys. Numbers below each locality indicate observed occurrence of each of 25 haplotypes identified across all localities. GenBank® is a genetic sequence database, available at <http://www.ncbi.nlm.nih.gov/genbank/>, accessed July 2011).

Haplotype	St. Croix	St. Thomas	Puerto Rico-east	Puerto Rico-west	Florida Keys	GenBank no.
1	1	0	1	0	1	JF514891
2	10	10	9	5	14	JF514892
3	1	0	0	1	0	JF514893
4	0	0	0	1	0	JF514894
5	0	0	0	1	0	JF514895
6	0	1	2	2	0	JF514896
7	0	0	1	0	0	JF514897
8	5	6	4	7	4	JF514898
9	0	1	0	0	1	JF514899
10	0	0	0	0	1	JF514900
11	1	0	0	0	0	JF514901
12	0	0	0	1	0	JF514902
13	1	0	0	0	0	JF514903
14	2	1	0	0	1	JF514904
15	1	0	1	0	0	JF514905
16	0	0	1	0	0	JF514906
17	0	1	0	1	1	JF514907
18	0	2	2	0	2	JF514908
19	3	1	5	4	1	JF514909
20	0	1	1	1	0	JF514910
21	0	0	0	0	1	JF514911
22	0	0	0	1	0	JF514912
23	1	0	0	0	0	JF514913
24	1	1	2	0	0	JF514914
25	0	1	0	0	0	JF514915
Total	27	26	29	25	27	

$P=0.785$ ). Pair-wise exact tests (between samples) for both microsatellites and mtDNA also were nonsignificant (data not shown but available from E. W. Carson). Finally, nine of 15 tests of selective neutrality were significant before Bonferroni correction; none remained significant after Bonferroni correction.

Estimates of average long-term mutation-scaled migration ( $M$ ) between geographically adjacent pairs of sample localities are presented in Table 3. Estimates of  $M$  between adjacent localities were generated by averaging bidirectional estimates from Migrate, in part because bidirectional estimates generally were equivalent, and in part because confidence intervals for estimates of  $m$ , generated with Migrate, are generally compromised (Abdo et al., 2004). Based on a modal mutation rate ( $\mu$ ) over all microsatellites of  $2.51 \times 10^{-4}$ , generated with MSVAR, estimates of average long-term migration rate ( $m$ ) between adjacent localities varied from 0.0033 (PR-west vs. PR-east) to 0.0054 (SC vs. ST). Higher estimates of  $m$  were found between ST and SC and between ST and PR-east (Table 3).

Estimates of average long-term, effective population size ( $N_e$ ) for each locality (Table 3) were derived from

$\Theta$  values generated in Migrate ( $N_e = \Theta/4\mu$ ), with  $\mu$  equal to the modal mutation rate of  $2.51 \times 10^{-4}$  obtained from the Bayesian coalescent approach of Beaumont (1999) and Storz and Beaumont (2002). Initial Migrate runs revealed that the sample from SC had by far the lowest estimate of  $N_e$ . Because genotypes at microsatellite *Lan11* did not conform to Hardy-Weinberg expectations in the sample from SC (Table 1), values reported in Table 3 reflect Migrate runs without *Lan11*. Average long-term  $N_e$  among the five localities varied from a low of 341 (SC) to a high of 1066 (FK). Estimates from Migrate of 95% confidence intervals (CIs) indicate significant differences in average long-term  $N_e$  among localities (Table 3); the lower values for PR-west and SC are especially relevant because these two localities are close to known mutton snapper spawning aggregation sites in the U.S. Caribbean.

## Discussion

Analysis of microsatellite and mtDNA variation in mutton snapper sampled from localities in the north-

eastern Caribbean Sea and the Florida Keys revealed no evidence of either genetic heterogeneity or population subdivision. Shulzitski et al. (2009) found similar results in their study of mutton snapper from the west coast of Puerto Rico, the Florida Keys, and localities in Belize and Honduras. Because two of the localities (one in the Florida Keys and one along the west coast of Puerto Rico) sampled by Shulzitski et al. (2009) were very near two of the localities sampled in this study, it appears that mutton snapper from the Leeward (northeastern) Islands in the Lesser Antilles to the Central American coast to the eastern Gulf of Mexico may be homogeneous in frequencies of alleles at microsatellite markers.

Shulzitski et al. (2009) suggested that genetic homogeneity among mutton snapper in the region stemmed from long-distance larval dispersal or adult migration to spawning aggregations. Estimates of long-term migration rates ( $m$ ) in our study between geographically proximal localities, some separated by less than 100 km, ranged from 0.33% to 0.54%. These estimates of  $m$ , however, should be viewed as heuristic, in part because Migrate tends to underestimate  $m$  and because confidence intervals for  $m$  are generally unreliable (Abdo et al. 2004), and in part because of potential bias introduced by the necessity of running subsets of data owing to the computational demands of Migrate (Palstra et al., 2007). On the other hand, even if our estimates of  $m$  were 20 times higher, there still could be independent response of local populations to environmental or other (e.g., fishing) perturbations (Hastings, 1993, Hauser and Carvalho, 2008). Because the genetic markers used here and in Shulzitski et al. (2009) are presumed to be selectively neutral, genetic homogeneity in this case could be decoupled from genetic factors that affect adaptability and sustainability of local populations. That is, patterns of variation in genes affecting traits influenced by natural selection do not necessarily follow the same patterns as selectively neutral genes (or genetic markers) and geographic differences in adaptively useful genes (or alleles) can be maintained even in the face of substantial gene flow (Conover et al., 2005). Our estimates of average long-term migration also are consistent with the argument of Roberts (1997) that regional currents in the Caribbean Sea are insufficient for larval dispersal across the region.

The estimates of average long-term effective size varied three-fold among the localities sampled and the lowest and highest effective size was found in the samples from St. Croix ( $N_e=341$ ) and the Florida Keys ( $N_e=1066$ ), respectively. Briefly,  $N_e$  is the number of breeding individuals in an ideal population that experience the same amount of genetic drift and show the same dispersion of allele frequencies or inbreeding as the population under consideration (Wright, 1931) and is of importance as a measure of a population's response to evolutionary and ecological forces (Waples, 2010). For the conservation and management of exploited biological resources, effective size reflects fixation of deleterious alleles, loss of adaptive genetic variance, and the capacity to respond to either natural selection or

**Table 3**

Estimates of average long-term mutation-scaled migration ( $M$ ) and rate of migration ( $m$ , proportion of migrant individuals/generation), and of average long-term effective size ( $N_e$ ) for mutton snapper (*Lutjanus analis*). Estimates of  $M$  and  $m$  are presented for pair-wise comparison of geographically adjacent sample localities; distance (in km) between pairs of localities is approximate. Estimates of  $N_e$  and 95% confidence intervals (CI) are presented for each of five sample sites. PR=Puerto Rico.

Comparison	$M$	$m$	Distance
St. Croix and St. Thomas	21.46	0.0054	60
St. Croix and PR-east	15.16	0.0038	90
St. Thomas and PR-east	35.11	0.0053	90
PR-east and PR-west	13.27	0.0033	200
PR-west and Florida Keys	15.07	0.0038	>1,600

Site	$N_e$	Lower 95% CI	Upper 95% CI
St. Croix	341	314	372
St. Thomas	922	847	1007
PR-east	828	766	896
PR-west	646	607	689
Florida Keys	1066	987	1155

environmental perturbation (Franklin, 1980; Anderson, 2005). Long-term estimates of  $N_e$  represent a harmonic mean of  $N_e$  over approximately  $4N_e$  generations (Hare et al., 2011), meaning that 1) smaller values (that may have occurred either in the past or recently) will have a greater weight on average values, and 2) the time over which long-term  $N_e$  in mutton snapper was estimated ranged between ~1500 and >4000 generations. Because of the time period usually involved, estimates of long-term  $N_e$  are not necessarily reliable indicators of contemporary  $N_e$  but do provide a baseline for evaluating management planning (Hare et al., 2011). Differences in long-term  $N_e$ , however, do indicate possible differences in long-term demographic dynamics that potentially affect the number of individuals over time that produce surviving offspring (and hence population sustainability). Demographic factors that generate differences in effective size are difficult to assess empirically and can stem from varying numbers of breeding individuals across generations or from variance in reproductive success of either or both sexes (Charlesworth, 2009). In both cases, a number of factors including food availability, habitat quality, predation, or mortality are likely involved (Saillant and Gold, 2006).

The low effective size observed for the sample of mutton snapper taken off the southwest coast of St.

Croix is of possible concern for several reasons. First, the average long-term  $N_e$  estimate of 341 is below the upper bound of the “50/500” rule (Rieman and Allendorf, 2001), where an effective size of 500 or greater is needed to maintain the equilibrium between the loss of adaptive genetic variance from genetic drift and its replacement by mutation (Franklin, 1980; Schultz and Lynch, 1997). A potential consequence of sustained low effective size over time would be loss of adaptive genetic variance and reduced capacity to respond to perturbation (including exploitation). Second, this sample of mutton snapper came from a known spawning aggregation site that currently is under a joint territorial and federal closure during the spawning season (<http://fw.dpnr.gov/vi/fish/Docs/Fisheries%20Master%20Plan/Sections/Appendix3.pdf>, accessed July 2011). The estimate of effective size certainly indicates that the closure is appropriate and timely. Finally, St. Croix is near the northeastern edge of the Lesser Antilles and the spawning aggregation site is located on the leeward side of the island. Surface currents in the area are almost all to the west (Roberts, 1997) and include the Anegada Passage, a fairly wide channel that connects the Atlantic Ocean with the Caribbean Sea and runs westward between St. Thomas and St. Croix (Johns et al., 2002). Both the prevailing currents and the observation (Swearer et al., 1999) that leeward-island sites are prone to larval retention and less affected by larval immigration than windward locations would indicate that immigration into the spawning aggregation from locations outside of St. Croix could be limited. Limited immigration into St. Croix waters from outside potentially could impede recovery if the spawning aggregation becomes depleted. These inferences also are consistent with the findings of Wares and Pringle (2008) who found that  $N_e$  may be reduced in populations where there is unidirectional transport of individuals away from natal grounds.

The differences in long-term  $N_e$  among the samples of mutton snapper further indicate that at least historically there may have been distinct demographic stocks within the region. In addition to the low estimate of  $N_e$  for the sample from St. Croix, the estimate for the sample from St. Thomas ( $N_e=922$ ) was nearly three times as large as the estimate for St. Croix, yet the distance between the two localities (60 km) is substantially less than the larval-dispersal ranges of Roberts (1999) and the ecologically relevant larval dispersal distances of Cowen et al. (2000, 2006). Sailant and Gold (2006) in their study of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico defined demographic stocks as geographic samples that differed in dynamics that potentially affected  $N_e$  and the number of individuals that produce surviving offspring. In their study, estimates of  $N_e$  were negatively correlated with several critical fishery parameters, including size at age, maximum size, proportion of smaller and younger fish, and size and age of females at sexual maturity, reported by Fischer et al. (2004) and Woods et al. (2003), respectively. Similar age and

growth and reproductive studies on mutton snapper in the U.S. Caribbean are clearly warranted.

At present, mutton snapper in the U.S. Caribbean (Puerto Rico, St. Thomas/St. John, and St. Croix) are managed as a single management unit, although island-specific management is under consideration. Based on data on prevailing surface currents, low probability of larval input, and restricted movements of adults, the life-history subgroup of a recent stock assessment (SEDAR, 2007) indicated a two-stock hypothesis, with one stock on the Puerto Rican platform (Puerto Rico and St. Thomas/St. John) and a second stock around St. Croix. The estimates of long-term  $N_e$  are consistent with the hypothesis that mutton snapper off St. Croix may represent a different demographic stock. In addition, the estimate of long-term  $N_e$  for mutton snapper of the west coast of Puerto Rico ( $N_e=646$ ) is less than the estimate for the east coast of Puerto Rico ( $N_e=828$ ) and nearly 1.5-fold less than the estimate for St. Thomas. This could indicate that there are different demographic stocks of mutton snapper on the Puerto Rican platform. Further study of mutton snapper off the west coast of Puerto Rico is likely justified because our sample locality is near a known spawning aggregation (Esteves, 2005). Finally, stock structure of mutton snapper in the U.S. Caribbean may follow metapopulation models suggested by Kritzer and Sale (2002), Hellberg et al. (2002), and Østergaard et al. (2003) where 1) subpopulations (stocks) may be asynchronous demographically but display homogeneity at selectively neutral (genetic) markers, and 2) subpopulations may be independent in terms of recruitment events and yet show no genetic differences because of sporadic gene flow.

## Conclusions

Results of our study indicate that mutton snapper across the Caribbean Sea to the Florida Keys may be subdivided into a number of demographic stocks that differ in aspects that impact effective size and hence local sustainability. These differences could easily be both genetic and environmental, and in the future it will be of interest to apply new genomic tools (Allendorf et al., 2010) that allow identification of specific genomic regions responding to local adaptation. A second implication of our results is that neither larval drift nor inter-regional adult movement may be sufficient over time to offset these differences. Critical genetic variability in mutton snapper at the intraregional level, consequently, is likely maintained by aggregate spawning and random mating of local populations. It is perhaps ironic that the life-history characteristic (aggregate spawning) that makes mutton snapper especially vulnerable to overexploitation also could be a critical asset in maintaining local genetic diversity. This characteristic elevates the importance of securing the vitality of spawning aggregations in species such as mutton snapper. Protective measures for spawning aggregations, including seasonal closures and appropriately placed marine protected areas (MPAs),

are clearly critical steps to help ensure sustainability of species with this life history in the Caribbean Sea and Florida Keys.

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