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Abstract—Determining patterns of population connectivity is critical to the evaluation of marine reserves as recruitment sources for harvested populations. Mutton snapper (*Lutjanus analis*) is a good test case because the last known major spawning aggregation in U.S. waters was granted no-take status in the Tortugas South Ecological Reserve (TSER) in 2001. To evaluate the TSER population as a recruitment source, we genotyped mutton snapper from the Dry Tortugas, southeast Florida, and from three locations across the Caribbean at eight microsatellite loci. Both *F*-statistics and individual-based Bayesian analyses indicated that genetic substructure was absent across the five populations. Genetic homogeneity of mutton snapper populations is consistent with its pelagic larval duration of 27 to 37 days and adult behavior of annual migrations to large spawning aggregations. Statistical power of future genetic assessments of mutton snapper population connectivity may benefit from more comprehensive geographic sampling, and perhaps from the development of less polymorphic DNA microsatellite loci. Research where alternative methods are used, such as the transgenerational marking of embryonic otoliths with barium stable isotopes, is also needed on this and other species with diverse life history characteristics to further evaluate the TSER as a recruitment source and to define corridors of population connectivity across the Caribbean and Florida.

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The elucidation of patterns of population connectivity and the determination of sources of recruiting larvae are central concerns among ecologists and are critical for the implementation of spatially explicit management strategies. The marine environment provides a particularly complex backdrop for studies of connectivity because absolute barriers to dispersal are rare and ocean currents can be temporally and spatially heterogeneous. In addition, the majority of marine organisms have a pelagic larval phase, and because of the small sizes and patchy distributions of individuals at this stage, it is nearly impossible to directly observe dispersal events (Leis, 1991). Genetic markers have often been used to infer dispersal scale and connectivity (Hellberg, 2007). One advantage of this approach is that it provides information about effective dispersal among populations (i.e., only migrants that go on to reproduce in their new population will contribute gene copies). A second advantage is that population genetic structure reflects an average, over many generations, of migration events that are likely to vary substantially over time, and therefore it provides an estimate of population connectivity that is relevant over the long term.

Studies with genetic markers have increased our understanding of population connectivity in the Caribbean region. Shulman and Bermingham (1995) found weak but significant population subdivision across the Caribbean basin for three out of eight fish species using restriction endonuclease analyses of mitochondrial DNA (mtDNA). Taylor and Hellberg (2003) examined mtDNA haplotypes of the sharknose goby (*Elacatinus evelynae*) and demonstrated extremely restricted dispersal among populations. Analyses of genotypes at multiple microsatellite loci in elkhorn coral (*Acropora palmata*) indicated two distinct genetic groups, corresponding to western and eastern Caribbean sampling locations (Baums et al., 2005). In a recent study, Purcell et al. (2006) found weak genetic structure and yet a significant pattern of isolation-by-distance using microsatellite markers for the French grunt (*Haemulon flavolineatum*). In contrast, the bluehead

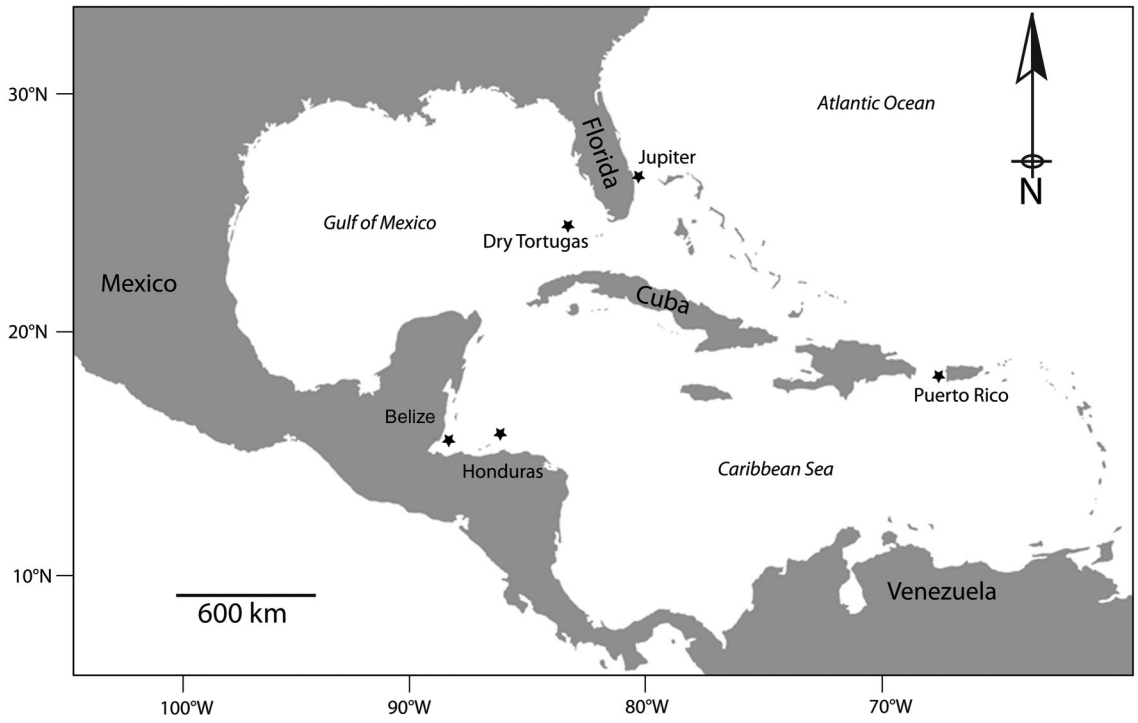

Figure 1

Map of sampling locations for mutton snapper (*Lutjanus analis*) collected across Florida and the Caribbean for microsatellite analysis. Stars denote sampling sites. Collection dates were as follows: Belize=May 2003; Dry Tortugas, FL=June–December 2003; Honduras=April 2004; Jupiter, FL=October 2004; and Puerto Rico=September 2004–February 2005. Adult fish were collected with hook and line; juvenile fish (Jupiter site only) were collected by seining.

wrasse (*Thalassoma bifasciatum*) lacks structure even at the scale of the entire Caribbean basin. Similarly, the slippery dick (*Halichoeres bivittatus*) shares mtDNA haplotypes across biogeographical provinces and locations separated by more than 2000 km (Rocha et al., 2005).

The results of these studies indicate similar opportunities for evaluating patterns of connectivity among reef fish populations in the Dry Tortugas, southeast Florida, and the Caribbean basin. In July 2001, the Tortugas South Ecological Reserve (TSER) was established approximately 110 km southwest of Key West, Florida, encompassing an historical fishing area known as Riley's Hump. This topographic feature was instrumental in the delineation of the reserve boundaries because it serves as a spawning site for various commercially and recreationally important snapper and grouper species (Lindeman et al., 2000). The high site fidelity and temporal stability exhibited by these spawning aggregations (Domeier and Colin, 1997) has led to their heavy exploitation and rapid decline on Riley's Hump (Burton, 2002). In fact, this site represents the last known major spawning aggregation for the mutton snapper (*Lutjanus analis*) in U.S. waters, making this species of particular interest to both conservationists and fishery managers.

In this study, we used *L. analis* as a focal species to examine eight high-resolution genetic markers to estimate connectivity among populations in the TSER, in southeast Florida, and at other sites throughout the Caribbean, with the ultimate goal of identifying larval source populations.

Materials and methods

Sample collection and genotyping

Mutton snapper tissue samples were obtained between May 2003 and February 2005 from five geographic locations and stored in salt-saturated dimethyl sulfoxide (DMSO). Samples of adult fish came from Gladden Spit, Belize (BZ); Roatan, Honduras (HN); Dry Tortugas (DT), Florida; and Mayaguez, Puerto Rico (PR) (Fig. 1); in addition, a sample of juvenile fish (standard length [SL]: 34–233 mm) from Jupiter (JP), Florida, was collected from Jupiter Inlet in October 2004 to serve as a downstream population. Genomic DNA was isolated according to a modification of the "rapid isolation of mammalian DNA" protocol of Sambrook and Russell (2000).

Two-hundred and forty-five individuals were genotyped at eight microsatellite loci (Table 1). Amplifications (15 µL) contained 5–20 ng template DNA, 15 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 mM each dNTP, 0.5 uM each primer, and 0.75 U

of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Cycling parameters began with a hot start of 10 minutes at 95°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at the optimized annealing temperature (see Table 1), and 60 seconds at 72°C, and a final extension of 30 minutes at 72°C. Polymerase chain reaction products were visualized on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA) and examined further with GENESCAN 3.7 software (Applied Biosystems, Foster City, CA). In GENOTYPER 3.7 (Applied Biosystems, Foster City, CA) peaks were labeled and binned into allele-size categories.

Data analysis

Each population was tested for departures from Hardy-Weinberg equilibrium (HWE) at each locus with GENEPOP vers. 3.4 software (Raymond and Rousset, 1995a) by using a probability test and a Markov chain method to obtain the unbiased exact *P*-value (Guo and Thompson, 1992). In a similar manner, all loci and population pairs were tested for linkage equilibrium. Because sample sizes varied from 40 to 55 individuals, allelic richness (the number of alleles present in populations independent of sample size) was calculated for each population-locus combination and overall with FSTAT vers. 2.9.3 software (Goudet, 1995). This parameter is an estimate of the expected number of alleles for a subsample of genes equal in size to that of the smallest sample.

In order to assess allelic and genotypic distributions across populations, an exact probability test (Raymond and Rousset, 1995b) and a log-likelihood based exact test (Goudet et al., 1996) were performed in GENEPOP to evaluate genic and genotypic differentiation, respectively. Both tests employed a Markov chain method to calculate an unbiased estimate of the *P*-value.

An unbiased estimator, θ (Weir and Cockerham, 1984), of Wright's (1921) fixation index (F_{ST}), a measure of among-population subdivision, was calculated with the GENETIX vers. 4.02 program (Belkhir et al., 2001) for each locus, as well as for each pairwise population comparison; permutation tests (1000 randomizations) were used to estimate *P*-values. An estimator of R_{ST}, ρ (Slatkin, 1995), was also calculated by using FSTAT (Goudet, 1995). This analog of F_{ST} takes into account allelic size by assuming that alleles of a similar size are more closely related, given that loci adhere to a stepwise mutation model (Slatkin, 1995). An additional measure of pairwise genetic differention, ϕ_{ST}, has been reported to be more appropriate in the case of highly polymorphic loci such as microsatellites, and was calculated with Geno-

Table 1

Mutton snapper (*Lutjanus analis*) microsatellite loci isolated for this study. T_m = annealing temperature; bp = base pairs; n = number of individuals genotyped; A = number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; test for Hardy-Weinberg equilibrium: * $P<0.05$, *** $P<0.001$.

Locus	Repeat motif of clone	Primer sequences (5′ to 3′)	T_m (°C)	Product size range (bp)	n	A	H_E	H_O
La25	$(ATCT)_{19}$	F: GGA GGA ACC TCC TGG AAT GT R: GTT TGC ACT TGA AGA AAA AGG GTG A	63	137–235	241	36	0.942	0.926
La39	$(TG)_{11}$	F: TGC TGA GGA GCA TTT GCT TT R: GTT TAA AGT CAC ATA AAC GGG GAC T	65	143–179	226	15	0.802	0.603***
La18a	$(ATGG)_6$ GTGGATGG(ATAG)$_{21}$	F: CCT CAC TTT TGT GTG AGA CAG C R: GTT ATG ATT GCT AGG AGC ATC TGG	65	188–340	239	37	0.964	0.963
La27a	$(AGAT)_{22}$	F: CTT AGC AAG CCA ACA AAC AAT G R: GTT TCC AAG GTC CAT TGA TCT TTA GTG	65	176–312	184	39	0.939	0.856*
La34a	$(AGAT)_{14}(AGTG)$ $(GAGT)(GAGA)$	F: TGT CTC TTC GAA ATC AAA CAC AA R: GTT TGA GGC TTA TCT GCC CCT CTC	57	239–303	235	17	0.914	0.908
La45a	$(TCCA)_{15}$	F: AAC CAC ATC TGG CTC AAT CA R: GTT TAG CCC CAG AGT AGG GTG AGA	62	194–254	238	15	0.880	0.871
La49a	$(TATC)_{12}(CATC)(TATC)$	F: GCT GAG GCA GAA ATC ACA C R: GTT ATG TCC ACT GAT GCC TCA AAA	65	224–312	235	19	0.905	0.840
LaC-16	$(TC)_{11}(AC)_8$	F: GGT GTT GAT TGG TCC TCT GG R: GTT TGG GGT TGG TAT TCA TCC AGT	66	146–164	238	10	0.853	0.834

Dive software (Meirmans, 2006). Finally, genetic chord distance, D_{CE} (Cavalli-Sforza and Edwards, 1967), between each population pair was calculated in GENETIX with permutation tests (1000 randomizations) used to estimate *P*-values.

Individual-based analyses may be more suited to questions of dispersal and connectivity because in these analyses, information contained in each individual multilocus genotype is used. By comparison, in population-based analyses, allele frequencies and heterozygosities are calculated for each population. Three different individual-based analyses were employed in this study.

The frequency-based assignment method of Paetkau et al. (1995) was implemented in GENECLASS software (Piry et al., 2004). Populations were determined *a priori* by sampling locations, and GENECLASS generated allele frequencies for each population, excluding the individual to be assigned in the given procedure (Waser and Strobeck, 1998). The expected frequency of each individual's genotype at each locus across all populations was calculated and each individual was assigned to the population from which its multilocus genotype most likely originated. Alleles that were absent from a population were designated a frequency of 0.001.

Genotypes were also analyzed by a Bayesian procedure implemented in the program STRUCTURE (Pritchard et al., 2000). STRUCTURE uses a Markov Chain Monte Carlo (MCMC) algorithm to cluster individuals into populations that each exhibit Hardy-Weinberg and linkage equilibrium (HWLE), without prior definition of the number or geographic location of these populations. Five runs were performed at each value of K genetic clusters, with K varied from 1 to 5, to ensure proper mixing in the MCMC chain of iterations and consistent results. For all runs we used a burn-in period of 10^6 iterations and followed it by 10^6 MCMC iterations. We assumed an admixture model, in which individuals may have mixed ancestry and correlated allele frequencies, which could account for similarity between closely related populations.

As a third method for inferring genetic structure in *L. analis*, we also used the landscape genetics program Geneland, available in the R statistical package (Guillot et al., 2005a, 2005b). This software operates like STRUCTURE in using Bayesian inference of Mendelian populations in HWLE. But unlike STRUCTURE, Geneland incorporates geographic coordinates of the samples into the prior parameters of the estimation procedure. Recent applications (e.g., Galarza et al., 2009) show promise for inferring structure at low levels of genetic differentiation between marine populations. For spatial coordinates, we ran separate analyses with and without a variable "uncertainty" factor—roughly interpretable as encompassing the home range of an individual fish and appropriate in the case of highly mobile animals (Guillot et al., 2005a). Each run comprised 10^5 MCMC iterations with a thinning set at 100 and K genetic clusters varying from 1 to 10; Dirichlet (uncorrelated) allele frequency distributions were assumed and null allele frequencies were explicitly considered (Guillot et al., 2008a, 2008b). Ten independent runs under each set of conditions were launched to check for convergence on K populations. Once a reliable estimate of K was found, a run with this value fixed was used to estimate and map posterior probabilities of population membership.

Results

High levels of polymorphism were observed in all five populations of mutton snapper at the eight microsatellite loci. The number of alleles detected per locus ranged from nine to 32, and expected and observed heterozygosities ranged from 0.771 to 0.968 and 0.500 to 0.982, respectively (Table 2). Predictably, populations with larger sample sizes exhibited slightly increased levels of allelic diversity; there were 149 alleles present in the JP population and only 135 in the DT population. Also, only two private alleles (i.e. alleles present in only a single population) were present in the DT population, whereas all other populations contained seven or eight. However, there were no apparent trends towards reduced heterozygosity in populations with smaller sample sizes, and estimations of allelic richness indicated that no single population was particularly deficient in genetic diversity across loci.

Seven out of 40 tests indicated significant departures from HWE ($0<P<0.05$). These significant tests were distributed evenly across populations, yet four of the significant tests involved locus La39 (Table 2). After the implementation of sequential Bonferroni corrections (Rice, 1989), only three tests, those involving La39, remained significant. Pairwise locus-population tests of linkage disequilibrium yielded six out of 140 significant comparisons ($0.01<P<0.05$); however, no test remained significant after sequential Bonferroni corrections. Three out of 80 tests indicated significant heterogeneity in allelic distribution between population pairs ($0.01<P<0.05$). Tests of genotypic distributions between population pairs indicated significant heterogeneity in two out of 80 tests ($0.01<P<0.05$). After sequential Bonferroni corrections, no test of genic or genotypic differentiation remained significant.

Values of F_{ST} can range from zero to one with zero indicating the absence of population substructure; values for estimators of this parameter can also be negative, indicating greater heterozygosity within than between populations. In this study estimates of F_{ST} (θ) for each locus ranged from −0.005 to 0 and estimates of R_{ST} (ρ) ranged from −0.009 to 0.005. Pairwise estimates of F_{ST} (θ) ranged from −0.0035 to 0.0022 and pairwise genetic distances, D_{CE}, ranged from 0.012 to 0.018; none of these pairwise comparisons were significant. To address the effect of high variation on estimates of F_{ST}, Meirmans (2006) has developed, ϕ_{ST}, a standardized measure of genetic variation based on the analysis of molecular variance (AMOVA) framework. As with F_{ST} (θ), all pairwise estimates of ϕ_{ST} were negative, ranging from −0.033 to −0.059.

Table 2

Genetic diversity at eight microsatellite loci in five sampled populations of mutton snapper (*Lutjanus analis*). n = number of genotyped individuals; A = number of alleles; $\hat{A}$ = allelic richness; a = number of private alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; test for Hardy-Weinberg equilibrium: * $P<0.05$, ** $P<0.01$, *** $P\leq0.001$.

	Locus								
Population	La25	La39	La18a	La27a	La34a	La45a	La49a	LaC-16	Overall
Belize (BZ)									
n=49									
A	21	11	31	25	14	13	14	10	139
$\hat{A}$	18.9	9.6	27.0	22.3	13.4	11.5	13.0	9.4	125.1
A	0	0	3	2	0	0	2	0	7
H_E	0.943	0.806	0.968	0.945	0.910	0.856	0.911	0.864	
H_O	0.939	0.750	0.957	0.872*	0.875	0.898	0.896	0.896*	
Honduras (HN)									
n=53									
A	27	13	28	26	17	14	12	10	
$\hat{A}$	21.5	11.3	24.5	22.3	15.6	12.3	11.5	9.5	128.5
a	5	1	0	2	0	0	0	0	8
H_E	0.942	0.813	0.962	0.939	0.926	0.887	0.905	0.847	
H_O	0.868	0.585***	0.962	0.825	0.942	0.865	0.827	0.830	
Puerto Rico (PR)									
n=46									
A	25	12	27	22	16	12	15	10	
$\hat{A}$	21.0	10.6	23.7	21.0	14.9	11.2	13.9	9.3	
a	3	0	0	1	0	1	2	0	7
H_E	0.934	0.771	0.956	0.937	0.911	0.873	0.908	0.846	
H_O	0.957	0.622**	0.957	0.906	0.976	0.884	0.762*	0.841	
Dry Tortugas (DT)									
n=39									
A	24	11	31	19	16	13	12	9	135
$\hat{A}$	21.8	10.3	28.1	19.0	15.4	12.0	11.3	8.7	126.6
a	0	1	0	1	0	0	0	0	2
H_E	0.942	0.794	0.964	0.920	0.915	0.876	0.894	0.859	
H_O	0.895	0.500***	0.947	0.862	0.921	0.821	0.789	0.846	
Jupiter (JP)									
n=55									
A	25	11	32	29	16	12	14	10	149
$\hat{A}$	21.2	9.7	26.8	23.9	14.8	11.4	12.8	9.3	129.9
a	2	0	2	2	0	0	1	0	7
H_E	0.949	0.802	0.966	0.945	0.917	0.891	0.916	0.858	
H_O	0.964	0.537***	0.982	0.841	0.836	0.873	0.891	0.759	

Only 46 individuals (18.8%) were correctly assigned to their source populations with the frequency-based assignment test (Table 3). Individuals were assigned to the population in which their multilocus genotypes exhibited the highest likelihood of occurrence. However, for most individuals, likelihoods of occurrence were similar across populations and success for assignments to origin was similar across populations. Thus, we were unsuccessful at detecting the origin of an individual based on its multilocus genotype and population allele frequencies—not surprising given the similarity in allele frequencies across population samples.

With the Bayesian methods applied in the program STRUCTURE we identified a single genetic cluster with a posterior probability of 1.0. Posterior probabilities were essentially zero for the presence of two, three, four, or five clusters. In the absence of prior information regarding the geographic origin of individuals, we did not detect any genetic structure corresponding to geographic location.

Results from the Geneland analysis broadly agreed with those from STRUCTURE, with subtle differences. Ten independent runs incorporating no uncertainty, or with uncertainty of 120 km^2 in geographic position of

Table 3

Individual assignments based on the frequency test. Values represent the number (%) of individuals from the source populations in each assigned population. BZ = Belize; DT = Dry Tortugas; HN = Honduras; JP = Jupiter; PR = Puerto Rico.

Source population	Assigned population					Total
	BZ	DT	HN	JP	PR	
BZ	11 (22.0)	11 (22.0)	13 (26.0)	11 (22.0)	4 (8.0)	50 (20.4)
DT	7 (17.5)	9 (22.5)	12 (30.0)	8 (20.0)	4 (10.0)	40 (16.3)
HN	11 (20.8)	10 (18.9)	7 (13.2)	15 (28.3)	10 (18.9)	53 (21.6)
JP	10 (18.2)	14 (25.5)	14 (25.5)	11 (20.0)	6 (10.9)	55 (22.4)
PR	9 (19.1)	8 (17.0)	13 (27.7)	9 (19.1)	8 (17.0)	47 (19.2)
Total	48 (19.6)	52 (21.2)	59 (24.1)	54 (22.0)	32 (13.1)	245

individual fish, all converged on a clear mode at K=1, but extensive mixing around this value showed nonzero probabilities of K=2. When geographic uncertainty was increased to 1° on each axis, 3 out of 10 runs converged on K=2. Nevertheless, maps of posterior probabilities from runs with K fixed at 2 (a representative one shown in Fig. 2) showed that the regions encircling our five sampling sites fell into a single genetic cluster at P=1, produced no strong discontinuities in the genetic landscape, and showed that no map regions from which fish were collected grouped with genetic cluster 2 (not shown). Hence the results from both Geneland and STRUCTURE analyses support the existence of a single interbreeding population across the sampled range of *L. analis* (Fig. 2).

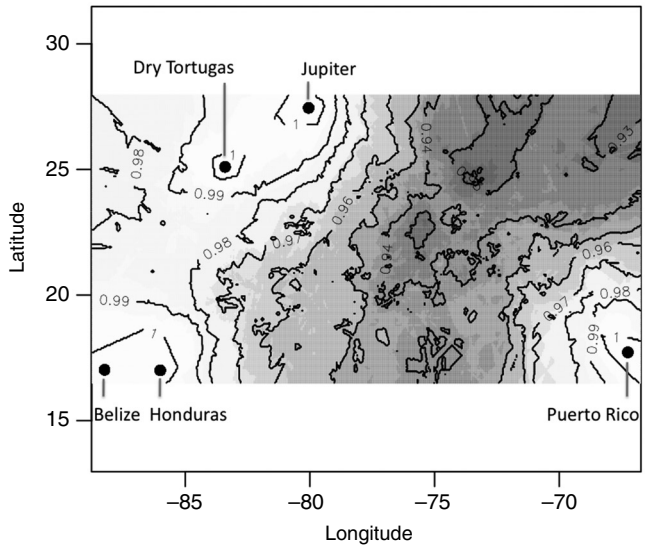

Figure 2

Map of the population membership of mutton snapper genotypes in genetic cluster 1, estimated by the Geneland software package (Guillot et al., 2005b). Contours enclose regions at a given posterior probability of membership. Note that sampled locations all group with genetic cluster 1 at P=1.

Discussion

Based on the results of this study, we cannot reject the null hypothesis that the sampled mutton snapper populations constitute a single panmictic unit. Population genetic substructure is absent from the five sample locations, ranging across approximately 2000 km. Further, because we could not differentiate genetically among the potential sources, it was not possible to ascertain the relative contributions of potential sources to "downstream" populations.

Given these results there are two possible scenarios for the population-level genetic structure. The first is that genetic structure does in fact exist among mutton snapper populations but could not be resolved in this study. If so, future efforts to estimate the relative contributions of mutton snapper spawning aggregations to downstream populations must be explicitly designed to detect such weak structure (e.g., Johansson et al., 2008). This research could require increasing the number of microsatellite loci; in particular, models show that loci exhibiting moderate allelic diversity (i.e., 6 to 10 alleles) are most efficient for population-assignment techniques in weakly differentiated populations (Bernatchez and Duchesne, 2000). Thus, future microsatellite applications to mutton snapper could target these loci in place of the highly polymorphic loci used in our study. In addition, sampling of populations between the relatively distant sites we sampled, as well as outlying populations in the eastern Caribbean, would allow improved evaluation of the genetic landscape of this species.

The geographic scale covered in our study does seem sufficient to recover genetic structure, if it is present. Yellowtail snapper (*Ocyurus chrysurus*) in Belize are genetically differentiated across a

biogeographic boundary from populations along the coast of Brazil (Vasconcellos et al., 2008). Along the Pacific coast of the U.S., copper rockfish (*Sebastes caurinus*) exhibit a pattern of isolation-by-distance over 1400 km, and along the Oregon coast, populations appear to be further structured by habitat and oceanographic barriers (Johansson et al., 2008). Finally, significant genetic structure is present across Barbados, Panama, and Belize in the barred hamlet (*Hypoplectrus puella*) and the black hamlet (*Hypoplectrus nigricans*) (Puebla et al., 2008).

A second, alternative scenario indicates that mutton snapper across the Caribbean region do in fact represent a single panmictic population. Genetic homogeneity across large distances is not an uncommon pattern in marine systems—even with the use high-resolution microsatellite markers. Wreckfish (*Polyprion americanus*) lack genetic structure within both the North Atlantic and South Pacific basins, although interoceanic differences are apparent (Ball et al., 2000). Weak structure across a major biogeographic boundary off the coast of northern Australia indicates high levels of gene flow for the mangrove jack (*Lutjanus argentimaculatus*) (Ovenden and Street, 2003). Heist and Gold (2000) found genetic homogeneity across 1500 km of the Gulf of Mexico for the red snapper (*Lutjanus campechanus*). And bluehead wrasse (*Thalassoma bifasciatum*) lack genetic structure across the entire Caribbean basin (Purcell et al., 2006).

Larval dispersal has long been recognized as a homogenizing force in marine systems. Given a pelagic larval duration (PLD) of 27 to 37 days for mutton snapper, it is reasonable to imagine dispersal by ocean currents across large geographic distances. The PLD of mutton snapper is similar to that of the red snapper (28 to 30 days), a species that exhibits genetic homogeneity across geographic distances comparable to those in the present study (Heist and Gold, 2000). For snapper species the four- to five-week period of time spent in the water column may facilitate long distance dispersal and, thus, genetic homogenization. Although reef fishes shown to exhibit significant population structure tend to have shorter PLDs (beaugregory [*Stegastes leucostictus*]: 19–21 days, slippery dick: 22–30 days, and French grunt: 13–20 days), there are numerous exceptions. Early life history traits, such as PLD, are often unable to satisfactorily explain patterns of genetic structure found across species (e.g., Galarza et al., 2009). Alternatively, the contribution of the larval stage to the maintenance of genetic homogeneity may be due to the interaction of species-specific larval behaviors with the physical environment, rather than a direct function of PLD. The complexity of this interaction could explain the inconsistency in studies that relate early life history traits to broad-scale genetic patterns.

In addition to the pelagic larval stage, dispersal in the adult stage is likely to be a route for gene flow among mutton snapper populations. In fact, annual migrations of tens to hundreds of kilometers to large spawning aggregations may serve to homogenize allele frequencies across a given region (e.g., Florida reef tract) and hence the adult stage may serve to homogenize genotypes at a regional scale, with larval dispersal maintaining genetic connectivity among regions.

Management and conservation implications

In the face of genetic homogeneity across large geographic distances, it is crucial to differentiate between ecological and evolutionary time scales. Boundaries between populations on an ecological scale are not necessarily congruent with those on an evolutionary scale. Our ability to detect ecologically significant population structure with genetic techniques continues to improve, yet remaining challenges limit the inferences we can draw from such a data set. The present study indicates that mutton snapper populations across the study area may be extensively connected over evolutionary time. Yet very little effective migration per generation can prevent genetic divergence among regions (Slatkin, 1987) even while the number of fish arriving from a distant location may be insignificant from the management (i.e., ecological) perspective (Cowen et al., 2007). Thus, ecologically significant dispersal between populations could still be slight, even given the genetic homogeneity observed in the present study. One well-studied example comes from bluehead wrasse, where genetic homogeneity of populations throughout the Caribbean (Purcell et al., 2006) contrasts with results obtained with natural elemental signatures recorded in otoliths (Swearer et al., 1999). Retention signatures found in 89% of summertime recruits to the leeward side of St. Croix indicate high levels of local retention despite the maintenance of genetic homogeneity on a large scale. Thus, future work must continue to try and bridge the distinction between evolutionary and ecological time scales.

The hydrographic environment of the Tortugas South Ecological Reserve, including the formation of the Tortugas Gyre, supports the potential for retention of larvae spawned on Riley's Hump (Lee et al., 1994). Although there is evidence that oceanographic processes in this region can lead to the delivery of these larvae to reefs and nursery habitats of the Florida Keys and southeast Florida shelf (Limouzy-Paris et al., 1997), there is also evidence that some of these same processes can actually advect larvae offshore (D'Alessandro et al., 2007). Thus, at the present time, the pathways of larval dispersal and patterns of connectivity among populations, and the relative contributions of larval sources to fisheries in the Dry Tortugas, Florida Keys, and southeast Florida, remain unclear. Domeier (2004) has provided indirect evidence of a recruitment pathway originating at the TSER that may deliver larvae to the Florida reef tract and to nursery habitats as far north as Palm Coast, Florida. Drifter vials were released over a mutton snapper spawning aggregation site in the TSER for two consecutive years. Based on vial returns, the overall range of dispersal was similar across years, yet the pattern of concentration of returns

was variable. Although drifter vials cannot provide a simulation of larval behavior and only provide beginning and end points in the dispersal process, this study is significant in showing that passive propagules originating in the TSER can in fact be transported to suitable nearshore habitats in a time period similar to average PLDs for reef fishes. This lends support to the idea that genetic homogeneity among mutton snapper populations across the Caribbean does not preclude a substantial contribution of the TSER as a source population for mutton snapper to the Florida Keys and the southeast Florida shelf.

In cases where genetic homogeneity exists in the absence of substantial recruitment from distant populations it will be critical to employ alternative methods to quantify levels and patterns of demographic connectivity among locations. The transgenerational marking of embryonic otoliths with barium stable isotopes is one emerging technique that could directly assign marked larvae and newly recruited juveniles to an adult spawning source (Thorrold et al., 2006). Reef fishes that form large spawning aggregations, such as snapper and grouper, are particularly suited for this application because numerous adults can be marked at once. The barium isotopic signature has been shown to be effectively transmitted to embryonic otoliths in both benthic- and pelagic-spawning fishes (Thorrold et al., 2006). Larvae or juveniles sampled from downstream locations that exhibit this signature in the core of their otoliths can be unequivocally traced back to the spawning source. Although this approach is costly, the information it provides could substantially increase our understanding of demographic connectivity and could be used to gauge the contribution of larval sources.

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