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MANAGEMENT BRIEF

Genetic Variation of Gray Triggerfish in U.S. Waters of the Gulf of Mexico and Western Atlantic Ocean as Inferred from Mitochondrial DNA Sequences

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Abstract

Gray triggerfish *Balistes capriscus* is a reef fish exploited by recreational and commercial fisheries in the southeastern United States. Recent stock assessments indicated that the species is overfished, and a rebuilding plan is in progress. The U.S. fishery is currently managed as a single stock owing to the absence of reliable information on stock structure. We sequenced a 617-base-pair fragment of the *ND4* mitochondrial gene in a total of 150 specimens from five localities (South Texas, Louisiana, West Florida, southeastern Florida, and South Carolina) encompassing the exploited range of the species in the USA. Analysis of molecular variance, spatial analysis of molecular variance, and spatial autocorrelation analysis did not reveal significant spatial heterogeneity in haplotype distributions within the studied range. Significant departure from neutrality was inferred from neutrality tests and may reflect the signature of a rapid population expansion following the recent glacial epochs, an inference that was supported by the results of Bayesian coalescent analysis. While the present results are consistent with management of the species as a single stock, development of additional genetic markers is needed to increase the resolution in genetic analyses and evaluate fine-scale genetic stock structure in the region.

The gray triggerfish *Balistes capriscus* is a reef fish that frequents temperate and tropical waters of the western and eastern Atlantic Ocean, including the Mediterranean Sea (Robins and Ray 1986; Sazonov and Galaktionova 1987). In the USA, the species is exploited mostly in the northern Gulf of Mexico and off the southeastern coast of Florida, the eastern part of this range generating the highest contribution to the total catch. Gray triggerfish are targeted primarily by recreational fishers, although a commercial fishery that uses handlines, longlines, traps, and trawls contributes a small proportion of the landings (Goodyear and Thompson 1993). The species was not considered a desirable catch until recently but has been increasingly targeted since the 1980s, probably owing to the decline of other reef fishes (e.g., red snapper *Lutjanus campechanus*) and the implementation of more restrictive regulations on those species (Harper and McClellan 1997; SEDAR-9 2006).

Gray triggerfish catches have steadily declined since the early 1990s, from an average yearly total catch of 970,315 kg for the period 1990–1992 to 396,114 kg for the period 2007–2009 (National Marine Fisheries Service, Fisheries Statistics Division, personal communication). The stock assessment conducted in 2001 (Valle et al. 2001) indicated that overfishing was probably occurring and that mortality rates were nonsustainable. The species has been managed since 1984 under the aggregate bag limit regulation of the Reef Fishery Management Plan implemented by the Gulf of Mexico Fishery Management Council. A minimum size of 12 in (30.5 cm) fork length (FL) for the species was added in 1999. New regulations established in 2008 (http://www.gpoaccess.gov/fr/index.html) to end overfishing and rebuild the stock included a minimum retention size of 14 in (35.6 cm) FL, annual catch limits, and accountability

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measures that use average annual landings for the recreational sector and in-season adjustments for the commercial sector.

The first assessment report of the Southeast Data Assessment and Review (SEDAR-9 2006) on gray triggerfish emphasized the definition of management criteria and stock benchmarks. The validity of considering multiple geographic subunits of the stock was left unclarified owing to erratic variation in life history and phenotypic traits over small geographic scales. Because these variations potentially reflect, in part, differing fishing pressures among subregions, definition of multiple stocks on the basis of phenotypic and life history traits appeared to be problematic. In consequence, current management of gray triggerfish does not distinguish multiple stocks throughout the exploited range in southeastern U.S. waters. Whether multiple stocks (e.g., northeastern and northwestern Gulf of Mexico or Atlantic Ocean and Gulf of Mexico) exist is, however, a possibility that remains to be tested rigorously. Knowledge of stock structure is essential to fisheries management because failure to recognize existing subunits of an exploited stock may lead to overexploitation and depletion of local populations or stocks (Carvalho and Hauser 1995; Begg and Waldman 1999). Another consequence is that unique (genetic) characteristics of local stocks may be lost, reducing biodiversity at the metapopulation level (Waples 1995). These genetic resources contribute to the metapopulation long-term evolutionary potential and its capability to sustain environmental changes (Hilborn et al. 2003). Thus, identification and conservation of genetically distinct local populations should be a primary focus in stock assessment.

Here we report a first assessment of genetic stock structure in gray triggerfish using sequence variation at a coding mitochondrial gene. Genetic variation was assessed in multiple localities along the U.S. coasts of the Gulf of Mexico and western Atlantic basins thereby encompassing the main portion of the range where the species is exploited in the USA. We also examined population historical growth rate and neutrality of variation at the locus and thereby evaluated the potential impact of these demographic processes on patterns of genetic variation and population structure exposed by the mitochondrial marker.

METHODS

Sampling.—Gray triggerfish specimens were collected during the summer and fall of 2008 and 2009 offshore of five localities along the northern Gulf of Mexico and the southeast Atlantic coast (Figure 1). Samples from south Texas and Louisiana were collected by trawling in conjunction with the National Marine Fisheries SEAMAP surveys. Samples from the vicinity of Panama City, Florida, were collected at recreational fishing docks or using traps during fishery-independent reef fish monitoring programs. Samples from southeast Florida were obtained via hook and line fishing. Finally samples from South Carolina were collected by trawling in conjunction with the MARMAP survey implemented by the South Carolina Department of Natural Resources. Sample sizes ranged between 27 and 32 gray triggerfish per locality. Tissue samples (fin clips, muscle tissue, or both) were collected on fresh or frozen specimens and preserved in 95% ethanol at room temperature until DNA isolation, except for the samples from South Carolina that were preserved in a sarkosyl urea lysis buffer (1% *N*-lauroylsarcosinate, 20 mM NaPO4, 8 M urea, 1 mM EDTA) until DNA isolation.

Laboratory procedures.—The DNA was extracted from tissue samples using a standard phenol-chloroform protocol (Sambrook et al. 1989) following proteinase *K* digestion. A 694-base-pair fragment of the ND4 subunit of the NADH dehydrogenase gene encoded by mitochondrial DNA was amplified by using the primers ND4LB (Bielawski and Gold 2002) and NAP2 (Arevalo et al. 1994). Polymerase chain reactions (PCRs) were conducted in a 25-μL volume solution containing 25–50 ng

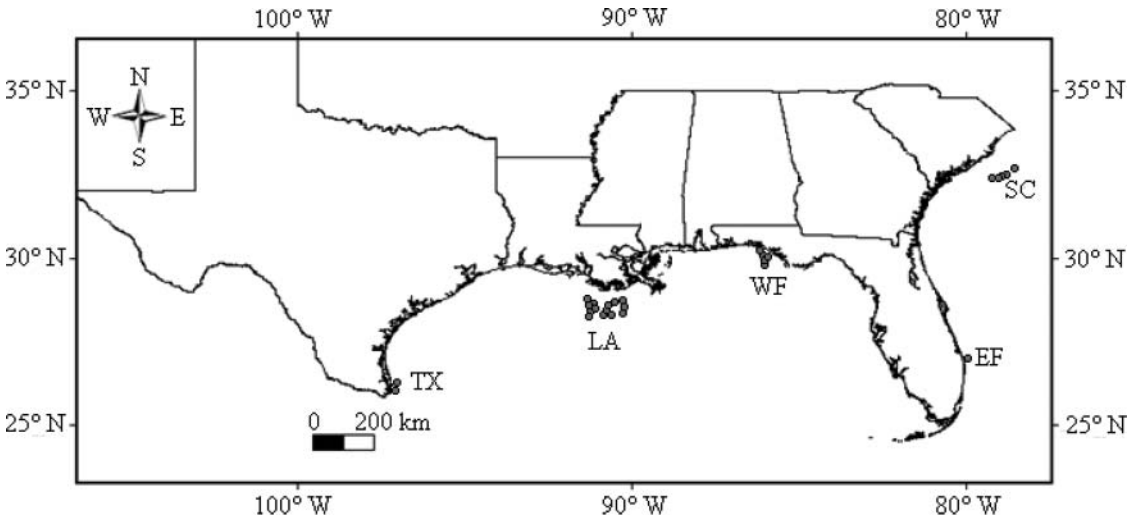

FIGURE 1. Sampling localities for gray triggerfish: Texas (TX), Louisiana (LA), West Florida (WF), East Florida (EF), and South Carolina (SC). The circles represent individual sampling stations.

of genomic DNA, 10 pmol of each primer, 1.25 U of TAQ polymerase (Promega Inc., Madison, Wisconsin), 5 nmol of deoxynucleotide triphosphates, 37.5 nmol of $MgCl_2$, and 1× buffer (Promega). Amplification by PCR consisted in an initial denaturation at 95°C for 3 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1.5 min, and a final extension of 15 min at 72°C.

The PCR products were purified using the Exo-SAP-IT PCR clean-up kit (GE Healthcare, Piscataway, New Jersey) and sequenced using the Big-Dye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, California) following the manufacturer's protocol. Sequencing reaction products were run on a ABI-3730XL capillary sequencer (Applied Biosystems) at the High-Throughput Genomics Unit of Seattle or on an ABI-3130 capillary sequencer in our laboratory.

Data analysis.—Sequences were edited in the software Sequencher version 4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan) and aligned in MEGA version 4.1 (Tamura et al. 2007). A 617-base-pair fragment could be reliably scored in all individuals examined and was used for data analysis. A statistical parsimony network of haplotypes was generated in TCS version 1.21 (Clement et al. 2000). Summary statistics including number of haplotypes, haplotype diversity and nucleotide diversity were computed in DNAsp version 5.10 (Rozas et al. 2003).

Homogeneity among samples in the number of mtDNA haplotypes and haplotype diversity was tested via a bootstrap resampling approach (Karlsson et al. 2008). Under the null hypothesis of homogeneity, the number of different haplotypes or haplotype diversity statistics observed in one locality sample does not differ significantly from that in a random sample of the same size from the overall data set (all localities pooled). Pop Tools (a free-add in software for Excel, available at http://www.cse.csiro.au/poptools/index.htm) was used to generate random samples of 27–32 haplotypes from the overall data set. Random sampling was performed 10,000 times, and the average number of observed haplotypes and haplotype diversity statistics and their upper (0.975) and lower (0.025) percentiles were recorded. Observed statistics in individual localities were significantly different from expected under the null hypothesis of homogeneity if they lay outside the bounds of the 95% bootstrap confidence interval (CI) obtained.

Genetic homogeneity among localities was tested by analysis of molecular variance (AMOVA; Excoffier et al. 1992) as implemented in ARLEQUIN 3.01 (Excoffier et al. 2005) by using the number of pairwise differences between haplotypes as a molecular distance. Significance of the genetic variance among localities and Φ_{CT} was assessed using 10,000 permutations of haplotypes. Population subdivision was further evaluated using spatial analysis of molecular variance (SAMOVA; Dupanloup et al. 2002), which employs a simulated annealing algorithm to optimize allocation of N geographic populations into K groups ($2 \leq K < N$) in order to maximize the proportion of total genetic variance due to genetic variation among the inferred groups. The software SAMOVA 1.0 (http://web.unife.it/progetti/genetica/Isabelle/samova.html) was used in computations. We used 100 simulated annealing processes to determine optimal allocation of the five geographic samples into two, three, or four groups.

The possible occurrence of restricted gene flow and isolation by distance was evaluated using spatial autocorrelation analysis (Smouse and Peakall 1999; Peakall et al. 2003), as implemented in GenAlEx 6.3 (Peakall and Smouse 2006). The spatial autocorrelation coefficient (r) was computed based on a linear, one-dimensional coastline geographic distance and the number of pairwise differences between haplotypes as a genetic distance, as above. With isolation by distance, the estimated value of r among proximal localities (small distance classes) is significantly greater than zero and decreases with increasing distance between localities. Because estimation of spatial autocorrelation is influenced by the size of distance classes, r was computed (following Peakall et al. 2003) based on a series of increasing distances between pairs of localities. Distance-classes were determined so that at least two pairs of localities per distance class would be included in the computation of r. Significance of r was determined via 10,000 random permutations of haplotypes among localities and by using a one-tailed test (i.e., the observed r is significantly greater than zero if it lies beyond the upper 95% bound of the distribution of r in randomized data sets).

Neutral evolution of the mtDNA locus was tested via Fu's (1997) F_S statistic and Fu and Li's (1993) D^* and F^* statistics, as implemented in the Dna-SP package (Rozas et al. 2003). Significance of F_S, D^*, and F^* was assessed using 10,000 coalescent simulations in DNA-SP.

The demographic history of gray triggerfish was examined using the Bayesian skyline plot (BSP) approach (Drummond et al. 2005). The BSP is a method for estimating past population dynamics (i.e., change in effective population size, N_e) through time from a sample of molecular sequences without dependence on a prespecified parametric model of demographic history. The method allows discovering novel demographic signatures that are not readily described by simple demographic models. The BSPs of gray triggerfish effective population size through time were constructed using a Monte Carlo–Markov chain (MCMC) sampling algorithm, as implemented in BEAST version 1.4.8 (Drummond and Rambaut 2007) available at http://beast.bio.ed.ac.uk/. The underlying population size function of the BSP was fitted using a piecewise linear function of population size change based on 10 control points in BEAST. Gene trees were inferred using a general time-reversible substitution model with gamma-distributed variation in substitution rate among sites and a proportion of invariant sites. Substitution rates were estimated allowing for rate variation along the branches of the gene tree (relaxed molecular clock uncorrelated setting), rates in each branch being drawn from a lognormal distribution. Three MCMC chains were run, each involving 1.6×10^8 steps. The software Tracer version 1.4.1 available

at http://tree.bio.ed.ac.uk/software/tracer/was used to visualize trace files generated in BEAST and construct the skyline plot. Examination of the time series of the MCMC plot and of the effective sample sizes for each parameter computed in Tracer, as well as the overall consistency of the three MCMC chains, indicated that runs had converged to the posterior distribution. The first 2×10^7 to 3×10^7 steps were discarded to avoid influence of starting values on the estimated posterior distributions. The three runs gave consistent results and were combined to derive final posterior distributions and BSP for the data set. Assignment of a time scale to the population size estimates was achieved by calibrating the rate of molecular evolution. A range of substitution rates of 1–1.5% per million years (MY) was considered based on molecular-clock calibrations of the mitochondrial cytochrome *c* oxidase (1.2%/MY) and *NADH-2** and *ATPase6** (1.3%/MY) genes developed for several geminate species pairs of fishes (Bermingham et al. 1997). Computations were performed at the Computational Biology Service Unit at Cornell University.

RESULTS

Thirty-eight unique mtDNA haplotypes (Table 1) were found among the 150 sequences obtained. There were 43 base substitutions among the 38 haplotypes, of which 32 were synonymous and 11 were nonsynonymous changes with 42 transitions and 1 transversion. The statistical parsimony network revealed a star-like phylogeny of haplotypes that included three closely related haplotypes, representing 59% of the samples, and 23 singleton haplotypes (Figure 2). Haplotype diversity and nucleotide diversity were similar among localities and ranged between 0.815 and 0.907 for haplotype diversity, 0.00345–0.00451 for nucleotide diversity (Table 1). The number of haplotypes and haplotype diversity observed in each individual locality were all included in the 95% CI obtained during bootstrap resampling, indicating that observed values did not differ significantly from those expected in random subsamples of the overall data set.

The AMOVA did not reveal the occurrence of a significant genetic variance among localities ($\Phi_{CT} = -0.0091, P = 0.805$). The molecular variance among groups of populations was maximized in SAMOVA when four groups were considered; the sample from Louisiana and Texas were associated in one group and the other three localities—West Florida, East Florida, and South Carolina—were inferred to constitute distinct groups. The estimated molecular variance component among groups was, however, negative (−0.08% of the total molecular variance) and did not differ significantly from zero ($P = 0.404$). Spatial autocorrelation coefficients did not differ significantly from zero for any of the distance class considered (data not shown). Based on these results, data from the five localities were pooled for subsequent analyses.

Fu's F_S and Fu and Li's D^* and F^* statistics were all significant ($F_S = -33.157$, $P < 0.0001$; $D^* = -4.59$, $P < 0.02$; $F^* = -4.24$, $P < 0.02$), indicating departure from the patterns expected under neutral genetic variation and mutation-drift equilibrium.

The mean of the posterior distribution obtained during Bayesian skyline reconstruction suggested the occurrence of an initial rapid increase in historical population size followed by a phase of slower population growth and a third, recent growth phase (Figure 3). Considering mutation rates of 1.0% per MY and 1.5% per MY, the initial growth phase would have occurred between 67,000 and 33,000 years ago (at 1.0%) or between 100,000 and 50,000 years ago (at 1.5%). The third phase would have begun 6,700 (at 1%) to 10,000 (at 1.5%) years ago and would have corresponded to a slight increase in population growth rate in recent times. The 95% highest posterior density interval, however, also included support for a reduction in population size during the latter phase (Figure 3).

DISCUSSION

Genetic Diversity and Spatial Genetic Variation

We surveyed genetic variation at a 617-base-pair fragment of the ND4 subunit of NADH dehydrogenase encoded by mitochondrial DNA in five localities along the northern Gulf of Mexico and the western Atlantic Ocean. Genetic diversity indices were relatively high (38 haplotypes detected and a haplotype diversity index averaging 0.876); these estimates are in the range of values reported in other reef fishes in the region for the same mitochondrial gene: average $H_e = 0.79$ for red snapper in the northern Gulf (Pruett et al. 2005); average $H_e = 0.89$ for lane snapper *Lutjanus synagris* in the eastern Gulf and southeast Florida (Karlsson et al. 2009). Bootstrap resampling analysis did not reveal significant differences in haplotype diversity or number of haplotypes among localities. In addition, no significant heterogeneity in haplotype distributions was detected among localities or groups of localities during AMOVA and SAMOVA. Spatial autocorrelation analysis did not reveal significant spatial structuring of haplotypes either, thus providing no support for variation according to an isolation-by-distance model. Altogether, these results indicate homogeneity in the distributions of genetic variants among geographic populations of the northern Gulf of Mexico and the South Atlantic region of the USA. This tentatively suggests that gray triggerfish constitute a single stock across the sampled range. Tag and recapture studies indicated that gray triggerfish are highly sedentary as adults, owing to territorial behavior (Ingram 2001), and suggesting that adult migration is not a likely mechanism promoting gene flow. On another hand gene flow could be promoted at the larval and juvenile stage. Gray triggerfish larvae are known to utilize the pelagic habitat (Richards and Lindeman 1987; Leis 1991), and juveniles are found associated with seaweed and flotsam (mostly *Sargassum* spp.; Aiken 1983), where they can stay for a relatively long time: from a few weeks to a few months with lengths reaching up to 8.0 cm standard length in the study of Wells and Rooker (2004) and 12.7 cm FL in the study of Franks

TABLE 1. Haplotype distributions and summary statistics for gray triggerfish samples collected from five offshore localities along the U.S. Gulf of Mexico and South Atlantic coasts.

Haplotype	GenBank accession number	Texas	Louisiana	West Florida	East Florida	South Carolina
BC001	HQ599468	2	5	5	4	4
BC002	HQ599469	6	7	5	5	5
BC003	HQ599470	2	1	0	0	1
BC004	HQ599471	8	11	6	8	8
BC005	HQ599472	1	1	0	1	0
BC006	HQ599473	0	1	0	0	0
BC007	HQ599474	2	1	0	0	1
BC008	HQ599475	1	1	1	1	2
BC009	HQ599476	0	1	0	0	0
BC010	HQ599477	0	1	0	0	0
BC011	HQ599478	0	1	0	0	0
BC012	HQ599479	0	0	1	0	0
BC013	HQ599480	0	0	1	0	0
BC014	HQ599481	0	0	1	0	0
BC015	HQ599482	0	0	1	0	0
BC016	HQ599483	0	0	1	0	0
BC017	HQ599484	0	0	1	0	0
BC018	HQ599485	0	0	1	0	1
BC019	HQ599486	0	0	1	0	0
BC020	HQ599487	1	0	1	1	3
BC021	HQ599488	0	0	1	0	0
BC022	HQ599489	0	0	0	2	1
BC023	HQ599490	0	0	0	0	1
BC024	HQ599491	0	0	0	0	2
BC025	HQ599492	0	0	0	0	1
BC026	HQ599493	1	0	0	0	0
BC027	HQ599494	1	0	0	0	0
BC028	HQ599495	1	0	0	0	0
BC029	HQ599496	2	0	0	0	0
BC030	HQ599497	1	0	0	1	0
BC031	HQ599498	1	0	0	1	0
BC032	HQ599499	0	0	0	1	0
BC033	HQ599500	0	0	0	1	0
BC034	HQ599501	0	0	0	1	0
BC035	HQ599502	0	0	0	2	0
BC036	HQ599503	0	0	0	1	0
BC037	HQ599504	0	0	0	1	0
BC038	HQ599505	0	0	0	1	0
Number of haplotypes		16	14	11	12	15
Haplotype diversity		0.89195	0.81505	0.87957	0.90726	0.88736
Nucleotide diversity		0.00451	0.00345	0.00368	0.00403	0.00393

et al. (2007). While local recruitment could still be promoted if pelagic *Sargassum* aggregations remain near spawning grounds, dispersal over broad geographic distances may also occur depending on the direction and strength of oceanic currents encountered by the *Sargassum* during passive drift. As an example, *Sargassum* could be captured in the Gulf of Mexico and transported to waters off the U.S. East Coast by the Loop Current (Ulanski 2008; Gower and King 2008), thereby promoting gene flow between Gulf and Atlantic gray triggerfish. Prediction of the overall direction and distance of such transport is, however, a priori difficult considering that surface currents patterns that may be involved are diverse and probably vary in direction and

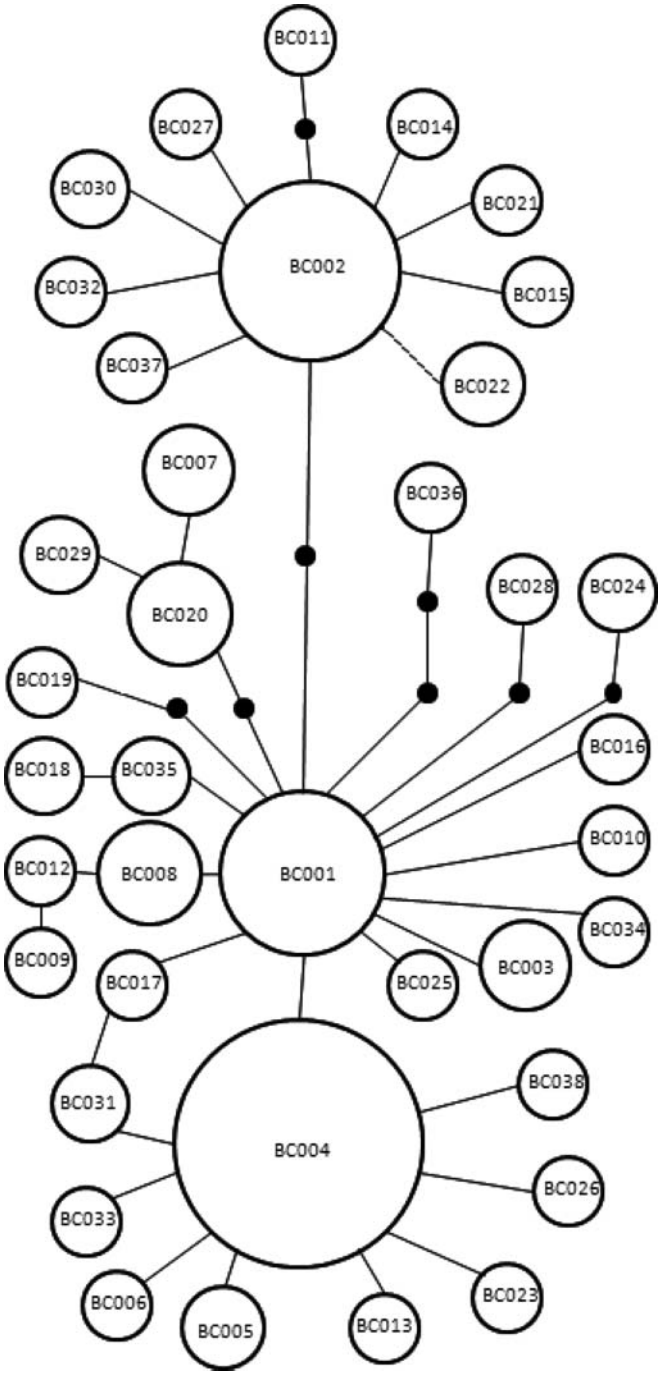

FIGURE 2. Statistical parsimony network of haplotypes detected in gray triggerfish from South Carolina to Texas. The codes within the circles correspond to those used for the individual haplotypes in Table 1; the size of the circle reflects the frequency of occurrence of that haplotype. Solid lines between haplotypes, regardless of their length, correspond to single-base-pair substitutions, and filled circles represent unsampled intermediate haplotypes in the cladogram.

strength during the course of the protracted spawning season of gray triggerfish.

The spatial genetic homogeneity of gray triggerfish suggested by our results contrasts with findings on the population-genetic

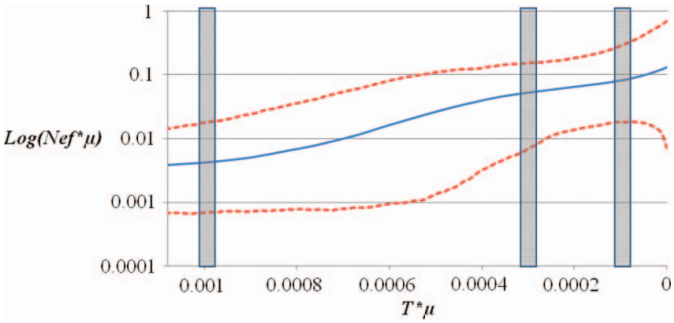

FIGURE 3. Bayesian skyline plot of $\log_{10}$ transformed effective population size (N_{ef}) of female gray triggerfish through time (T [number of generations]) scaled by the substitution rate (μ [percent per million years]). The thick solid line represents the mean of the posterior distribution and the dashed lines the upper and lower limits of the 95% highest posterior density interval. The shaded vertical strips mark the historical changes in the population growth rate. [Figure available in color online.]

structure of other marine and coastal fishes in the region. Divergence between Atlantic and Gulf populations of several species was reported by Avise (1992) and was hypothesized to stem from a variety of factors including historical processes, discontinuous habitats, current patterns, life history, and behavior. More recently, significant structuring among geographic populations of gray snapper *Lutjanus griseus* and lane snapper was reported within the sampled range by Gold et al. (2009) and Karlsson et al. (2009). Divergence involved the eastern versus western Gulf of Mexico (gray snapper and lane snapper) and the Gulf of Mexico versus the east coast of Florida (gray snapper). Adults of these two snapper species are sedentary, as are gray triggerfish, but differ in dispersal potential during early life stages. Lane and gray snapper larvae are pelagic but do not use the *Sargassum* habitat, and they probably disperse over shorter periods than gray triggerfish larvae and juveniles. Indeed, the planktonic phase of gray and lane snapper larvae is estimated to last at most 20–40 d before settlement on benthic habitats occurs (Lindenman et al. 2000). Higher connectivity among gray triggerfish populations thus suggests that dispersal through transport by the *Sargassum* is effective at promoting gene flow and connectivity across broader geographic ranges. Further study encompassing a wider geographic area is needed to determine the spatial scale of this connectivity.

There are a number of caveats to the hypothesis that gray triggerfish form a single stock in U.S. waters. Spatial genetic homogeneity could stem from high gene flow within the area, but also from a nonequilibrium situation, where regional populations would currently be partially isolated, but there would not have been sufficient time since isolation to allow for accumulation of detectable genetic differences. Distinguishing between the two hypotheses is difficult because both may result in homogeneous present-day haplotype frequencies. Another caveat to the single stock hypothesis is that very limited level of gene flow (only a few migrants every generation) is sufficient to maintain genetic homogeneity (Waples 1998). Thus, populations may be

demographically independent while displaying no detectable genetic divergence. Our study is limited by sample sizes, and the use of only one locus may not provide enough resolution to reveal subtle genetic differences (if they exist) among populations. Further analysis of gene flow and isolation by distance using increased sample size and additional molecular markers, such as hypervariable microsatellite loci, will therefore be necessary to achieve a more robust assessment of stock structure in gray triggerfish.

Historical Demography

Historical demography was examined using Bayesian skyline reconstruction. The mean of the posterior distribution converged towards a rapid increase in population growth rate beginning 67,000–10,000 years ago. This result is consistent with the hypothesis that gray triggerfish expanded and increased in population size as habitat availability increased following the last glacial epoch. Similar inferences were made in other reef fishes of the Gulf of Mexico that use the same habitat as gray triggerfish, including red snapper (Pruett et al. 2005) and lane snapper (Karlsson et al. 2009). However, the lower 95% bound of the Bayesian skyline plot included support for a reduction in population size for the most recent period of inference (6,700–10,000 years ago). A recent significant reduction in effective population size may indeed have occurred due to overfishing, as mentioned earlier. Caution needs therefore to be exerted regarding interpretations of inference based on the mean of the posterior distribution with regards to recent and/or current demographic processes in gray triggerfish. As illustrated by Rogers' (1996) simulation study, the star-like phylogeny of haplotypes signature of population expansion exposed by the mitochondrial marker may not be affected by reductions in population size subsequent to the expansion. Consequently, the present data set may not be appropriate to infer recent demographic history, especially recent population bottlenecks experienced by gray triggerfish. Analysis of faster-evolving markers such as microsatellites is thus warranted to address this question and may bring to light recent demographic processes not detected by the present data set, as was reported during studies in other fishes (e.g., Saillant et al. 2004, 2010). All three neutrality tests indicated significant departure of observed variation from neutral expectations. A significant Fu's F_S test is more likely reflecting population expansion or genetic hitchhiking, while Fu and Li's (D^* and F^*) tests are more powerful in the case of background selection (Fu 1997). While the relative role of each factor in generating departure from neutrality in gray triggerfish is not clear, the results of the Fu's F_S test are consistent with the historical population growth inferred during Bayesian skyline reconstruction. Examination of genetic variation at additional markers (including nuclear loci) would be useful to determine the origin(s) of the departure from neutrality at the mitochondrial locus and potential impacts on inference regarding population structure.

In summary, this study did not reveal significant spatial genetic heterogeneity among the gray triggerfish within the exploited range in the USA, a result that is consistent with the current management of the species as a single stock. Caution should be exercised with this assessment owing to the limits of the present data set outlined above, and further study is warranted using additional genetic markers that provide higher resolution during genetic analysis.

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