Evaluating the demographic significance of genetic homogeneity using a coalescent-based simulation: a case study with gag (*Mycteroperca microlepis*)

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Abstract: Gag (*Mycteroperca microlepis*) from the eastern Gulf of Mexico and northwestern Atlantic are managed as separate stocks, although evidence for their demographic isolation remains equivocal. Several hundred individuals were genotyped at 11 microsatellite loci and it was found that gag across these regions were genetically indistinguishable (F_{ST} < 0.001). A coalescent-based computer simulation was employed to quantitatively assess the relative importance of dispersal rate, population size, and time since divergence on gag genetic homogeneity. Using empirical estimates of long-term effective population size (16 500), a range of dispersal rates and divergence times (500 to 500 000 generations) was modeled, and it was concluded that present-day genetic homogeneity must be maintained by dispersal rates of at least 20–30 migrants per generation and up to hundreds or thousands per generation. This study also documents the absence of significant temporal genetic structure and inbreeding in the Atlantic when comparing cohorts separated in time from weeks to 20 years. This suggests that the long-standing practice of overfishing gag has yet to manifest as an inbreeding effect. Overall, gag dispersal rates remain uncertain, and consequently, this study can neither support nor refute management schemes that independently regulate gag in the eastern Gulf of Mexico and northwestern Atlantic.

Résumé : Les badèches baillou (*Mycteroperca microlepis*) de l'est du golfe du Mexique et du nord-ouest de l'Atlantique sont gérées comme des stocks distincts, bien que les données sur leur isolement démographique restent équivoques. Nous avons déterminé le génotype à 11 locus microsatellites de plusieurs centaines d'individus et nous n'arrivons pas à distinguer génétiquement les badèches baillou dans l'ensemble de ces régions (F_{ST} < 0,001). Une simulation sur ordinateur basée sur la coalescence nous à servi à évaluer quantitativement l'importance relative du taux de dispersion, de la taille de la population et du temps depuis la divergence pour l'homogénéité génétique de la badèche. En utilisant des estimations empiriques de la taille effective de la population à long terme (16 500), nous avons modélisé une gamme de taux de dispersion et de temps depuis la divergence (500 à 500 000 générations); il en ressort que l'homogénéité génétique actuelle doit être maintenue par des taux de dispersion d'au moins 20–30 migrants par génération jusqu'à des centaines ou des milliers de migrants par génération. Notre étude note aussi l'absence de structure génétique temporelle significative et de consanguinité dans l'Atlantique dans des comparaisons de cohortes séparées dans le temps par des périodes allant de semaines à 20 années. Cela indique que la pratique de longue date de surpêche de la badèche baillou ne s'est pas encore manifestée comme un effet de consanguinité. Globalement, les taux de dispersion de la badèche demeurent incertains et, en conséquence, notre étude ne peut ni appuyer, ni rejeter les propositions de gestion qui contrôlent de façon indépendante les badèches baillou de l'est du golfe du Mexique et du nord-ouest de l'Atlantique.

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Introduction

Fisheries managers use several criteria to determine whether species should be managed as a single stock or as multiple units (Begg and Waldman 1999; Fraser and Bernatchez 2001). From the biological perspective, management units (or populations and stocks) are groups of individuals that are demographically independent from others (Waples and Gaggiotti 2006). However, identifying which groups are demographically isolated depends on describing patterns of connectivity across space (Fogarty and Botsford 2007), which itself is extremely difficult for most marine populations because of the biological and oceanographic complexity inherent to dispersal processes (Shanks 1995).

One approach to describing patterns of connectivity is to measure gene flow, which has the distinct advantage of al-

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lowing scientists to evaluate patterns of effective dispersal across multiple generations (Hauser and Carvalho 2008). Unfortunately, the genetic approach often yields ambiguous interpretation when fisheries species exhibit weak differentiation across broad spatial scales (Ward et al. 1994; Waples 1998). This is because it can be difficult to tease apart the primary demographic and evolutionary causes of genetic homogeneity: stabilizing selection, nonequilibrium between drift and migration since subpopulations diverged, and ongoing gene flow. Moreover, even modest levels of gene flow (hundreds of individuals per generation) can yield values of differentiation (or F_{ST}) that are swamped by the sampling error of most studies (Waples and Gaggiotti 2006; Cano et al. 2008). To address these limitations, novel genetic and analytical methods are needed to help tease apart the causes of the weak genetic differentiation that characterizes many fisheries species (Selkoe et al. 2008).

The gag (*Mycteroperca microlepis*; Goode and Bean 1879) is a large (maximum ~1.2 m total length), protogynous grouper commonly found in the northwestern Atlantic, Gulf of Mexico, and off the coast of Brazil (Heemstra et al. 2002), and is one of the most commercially and recreationally important grouper species in the southeastern United States (Bullock and Smith 1991). Gag are currently managed as two separate stocks: the eastern Gulf of Mexico (Gulf) and the northwestern Atlantic off the southeastern US coast (Atlantic), with the delineation at the Florida Keys. Two studies have employed molecular techniques to address gag population genetic structure. Jue (2006) sampled the Gulf and documented no temporal or spatial genetic differentiation across several yearly cohorts. Chapman et al. (1999) found patchy genetic differentiation among sampling locations for gag from the Atlantic and the Gulf, but the number of samples and genotyped loci were modest and preclude strong statistical inference.

Eleven microsatellite loci were genotyped to address three main objectives: (*i*) to determine whether spatial and temporal genetic differences exist among yearly cohorts of gag taken from different regions within the Atlantic; (*ii*) to determine whether spatial differentiation exists between the Atlantic and Gulf; and (*iii*) to employ a coalescent-based simulation approach to evaluate the biological significance of the lack of genetic differentiation between the Gulf and Atlantic. These analytical simulations explicitly address the degree to which nonequilibrium processes or ongoing gene flow are responsible for genetic homogeneity, an issue that confounds many studies of marine fisheries genetics.

Materials and methods

Sample collection and DNA isolation

Four sets of spatially and temporally segregated samples were collected: offshore Atlantic adult and subadult gag from several year classes, 2005 year-class juvenile gag from North Carolina estuaries, 1985 year-class gag postlarvae (~15 mm) from South Carolina estuaries, and offshore adult Gulf gag from several unknown year classes (Table 1; Fig. 1). Tissue samples from Atlantic adult and subadult gag were collected in April and May of 2005 from offshore North Carolina, South Carolina, and the eastern coast of Florida by the Marine Resources Monitoring, Assessment, and Prediction (MARMAP) Program of the South Carolina Department of Natural Resources (SCDNR), with the help of hook-and-line commercial (bandit reel) and recreational (rod and reel) fisherman. Young-of-the-year gag were collected from North Carolina estuaries from 1 August to 2 September 2005 by the National Oceanic and Atmospheric Administration (NOAA), using dock-mounted traps placed at the Center for Coastal Fisheries and Habitat Research (CCFHR) on Pivers Island near Beaufort, North Carolina. Young-of-the-year gag were collected using 1 m × 2 m frame neuston nets (1–2 mm mesh) from Breach Inlet, a barrier island inlet located just north of Charleston, South Carolina, between Sullivan's Island and the Isle of Palms. These postlarvae were collected by SCDNR on a weekly basis from 22 March to 6 June 1985. Offshore adult Gulf gag were collected in 2004–2006 from two regions of the eastern Gulf of Mexico representing the northwestern (above Tampa Bay or 27.5°N latitude) and southwestern (below Tampa Bay or 27.5°N latitude) coasts of Florida, respectively.

Tissue samples were preserved in a sarcosyl–urea solution (1% sarcosyl, 8 mol·L^{-1} urea, 20 nmol·L^{-1} sodium phosphate, 1 mmol·L^{-1} EDTA, pH 6.8) before extraction. A SPRINTPrep magnetic bead procedure (Agencourt, Inc., Beverly, Massachusetts) was used to isolate genomic DNA.

Genotyping

A suite of 11 microsatellite loci developed for several grouper species including gag (Chapman et al. 1999), black grouper (*Mycteroperca bonaci* (Poey); Zatcoff et al. 2002), and Hawaiian grouper (*Epinephelus quernus* Seale; Rivera et al. 2003) were genotyped (for further information on microsatellite loci including locus names and sequences, see Supplemental Table S1, available online from the NRC Data Depository[2]). These loci were chosen because they were polymorphic, demonstrated clear and consistent amplification, and had been used successfully for *M. microlepis* in previous studies (Chapman et al. 1999; Jue 2006). For the offshore Atlantic adult and juvenile gag and the North Carolina juveniles (Pivers Island samples), eight of these 11 loci (GAG023, GAG038, GAG045, MBO029, MBO048, MBO086, CA2, CA6) were assayed with fluorescently labeled forward primers (IDTDNA, Applied Biosystems (ABI), Foster City, California) at Florida State University (FSU) in Tallahassee, Florida (P.I. Dr. Don Levitan). These eight loci were genotyped on an ABI 3130xl Genetic Analyzer with capillary electrophoresis. ABI Genemapper Software version 4.0 was used to read and determine final scores for the resulting data. Accuracy of scores was assessed by adherence of sample peaks to a predetermined locus-specific binning system in Genemapper, and a subset of 95 samples was analyzed by a second human reader to confirm score consistency. PCR amplifications for these eight

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5275. For more information on obtaining material, refer to http://cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

Table 1. Number of genotyped samples from each site and year class.

	Site					
Year class	EFL	SC	NC	NWFL	SWFL	Total
2005*	—	—	30	—	—	30
2002	2	—	4	—	—	
2001	4	43	82	—	—	129
2000	7	49	69	—	—	125
1999	13	32	10	—	—	55
1998	11	—	—	—	—	11
1997	2	—	—	—	—	2
1995	1	—	—	—	—	1
1994	3	—	—	—	—	3
1993	7	—	—	—	—	7
Unknown	1	—	—	211	131	343
1985†	—	158	—	—	—	158
Total	51	282	195	211	131	870

Note: Sites: EFL, eastern coast of Florida; SC, South Carolina; NC, North Carolina; NWFL, northwestern coast of Florida; SWFL, southwestern coast of Florida)

*NC young-of-the-year juveniles (Pivers Island).

†SC young-of-the-year postlarvae (Breach Inlet).

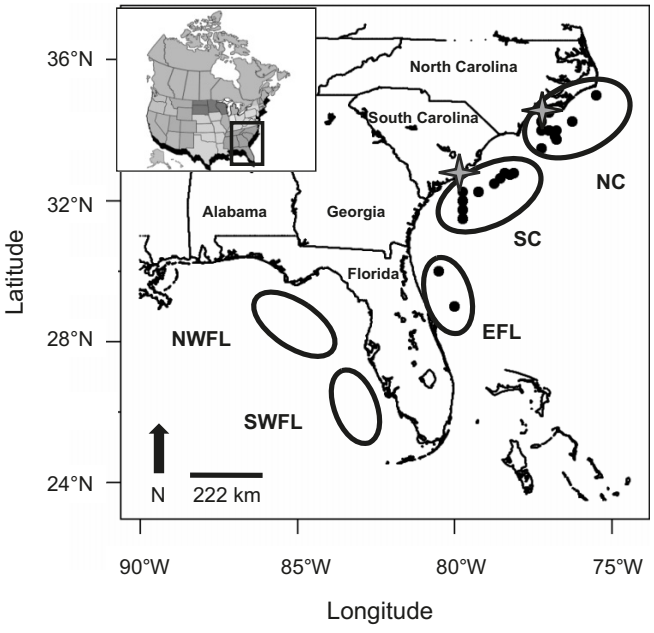


Fig. 1. Map of the southeastern coast of the United States (Atlantic) and the eastern Gulf of Mexico. General collecting locations for offshore adult and juvenile gag are indicated by ellypses. NC, North Carolina; SC, South Carolina; EFL, eastern coast of Florida; SWFL, southwestern coast of Florida; NWFL, northwestern coast of Florida. Coordinates for the majority of the Atlantic samples were provided, and individual collecting sites are indicated by solid circles. Estuarine sampling sites for North Carolina juvenile gag (Pivers Island) and South Carolina postlarvae (Breach Inlet) are indicated by shaded stars.

loci contained sample DNA (~2.5 ng·μL^{-1}), water, 5× Colorless GoTaq Reaction Buffer (1×, pH 8.5), $MgCl_2$ (2 mmol·L^{-1}), BSA (0.5 μg·μL^{-1}), dNTPs (0.2 mmol·L^{-1}), forward and reverse primer (0.3 μmol·L^{-1} each), and Promega GoTaq DNA polymerase (0.03 U·μL^{-1}) for a total reaction volume of 20 μL. For the Gulf offshore adults (NWFL and SWFL), all 11 loci were genotyped on the ABI system using the protocols described above. Genotyping for the remaining Atlantic samples was performed on an eight-capillary Beckman Coulter CEQ-8000 Genetic Analyzer (CEQ) at College of Charleston's (CofC) Molecular Core Facility. This includes the offshore adults and juveniles and the 2005 Pivers Island juveniles at three loci (GAG007, GAG010, MB088) and all of the 1985 South Carolina postlarvae (Breach Inlet samples) at six loci (GAG007, GAG023, GAG038, GAG045, MBO029, MBO048). Only these six loci could be amplified with any consistency for the 1985 SC postlarvae, possibly due to quality of the sample DNA after 20 years of storage. Loci genotyped on the CEQ were assayed with fluorescently labeled forward primers (SIGMA-Proligo, The Woodlands, Texas). Data were visualized and hand-scored using CEQ 8000 Fragment Analysis Software version 8.0, and a subset of samples for each locus was confirmed by a second human reader. PCR amplifications for the CEQ analyzed loci contained DNA (~2.5 ng·μL^{-1}), water, 10× AccuBuffer (1×, pH 8.3), BSA (0.5 μg·μL^{-1}), dNTPs (0.8 mmol·L^{-1}), forward and reverse primer (0.3 μmol·L^{-1} each), and BIOLINE Accuzyme DNA polymerase (0.013 U·μL^{-1}) for a final reaction volume of 20 μL (for locus-specific PCR protocols, see Supplemental Table S2[2]).

Because results from genotyping can differ between locations, machines, and reagents, the consistency of genotyping for each locus was tested on a subset of samples analyzed on both the ABI and CEQ systems: 30 samples at three loci (GAG007, GAG010, MB088) and 10 samples at five other loci (GAG023, GAG038, GAG045, MBO029, MBO048). It was noted that samples genotyped on the CEQ always scored 1–4 base pairs higher (depending on the locus) than the same samples genotyped on the ABI system. This discrepancy between systems was consistent among samples and was corrected before the analysis.

Rates of genotyping errors were not directly estimated, but it can be assumed that their effects on the results of this study were negligible for several reasons. No locus system-

atically deviated from Hardy–Weinberg equilibrium or was in linkage disequilibrium with other loci. Many suggested protocols were followed to minimize genotype errors (see Bonin et al. 2004), including amplifying negative controls, discarding samples with problems of amplification or repeatability, replicating amplifications for a subset of samples, cross-reading data with multiple eyes, and checking all data by hand. Moreover, Bonin et al. (2004) suggest that genotyping errors are more problematic for individual-based analyses (e.g., kinship, relatedness) than for the population-structure questions, which are the primary focus of this study.

Statistical analysis

Tests for Hardy–Weinberg equilibrium (HWE), linkage disequilibrium, and null alleles were performed for all loci within each group (site or year class) and for all loci across the entire data set. Tests for deviations from HWE were conducted using exact tests performed with Markov chain randomization in the program Arlequin (version 3.11; Excoffier et al. 2005). Chains had 200 000 steps with a 10 000 step burn-in. Tests for linkage equilibrium between all microsatellite pairs were executed in Arlequin using 20 000 permutations. Tests for the presence of null alleles and large-allele drop-out were performed with the program MICROCHECKER (version 2.2.3; van Oosterhout et al. 2004).

Spatial, temporal, and regional level comparisons were performed using analysis of molecular variation (AMOVA) procedures (Excoffier et al. 1992), pairwise Φ_{ST} estimation (Weir and Cockerham 1984), and a series of Markov chain Monte Carlo exact tests (Raymond and Rousset 1995) within Arlequin. The significance level for the first two tests was determined by 20 000 permutations. The number of steps in the Markov chain for the exact tests was 200 000, with a burn-in of 10 000 steps, as recommended by Arlequin. Significance levels (α value) for all analyses were adjusted using a sequential Bonferroni correction (Rice 1989). For the spatial analysis, adults and juveniles from North Carolina (33.5°N–34.5°N latitude, n = 165), South Carolina (31.5°N–32.8°N latitude, n = 124), and eastern Florida (28°N–30°N latitude, n = 51) were compared with one another and with two Gulf of Mexico locations (northwestern Florida, above 27.5°N latitude, n = 211, and southwestern Florida, below 27.5°N latitude, n = 131) at 11 loci. For the temporal analysis within the Atlantic, fish from the 2001 year class (n = 129), the 2000 year class (n = 125), and the 1999 year class (n = 55) were compared with one another and with the 2005 North Carolina young-of-the-year (Pivers Island samples, n = 30) with all 11 microsatellite loci, and with the 1985 South Carolina young-of-year postlarvae (Breach Inlet samples, n = 158) with 6 microsatellite loci. In addition, the 1985 samples were collected on a weekly basis, allowing postlarvae from two different time points of the 1985 larval ingress period into Breach Inlet (23 April and 9–11 May) to be compared to assess for within year class variation (for the 1985 sampling strategy, see Supplemental Figure S1[2]).

The relative influence of mutation and genetic drift rate on genetic differentiation was assessed by a comparison of R_{ST} values with F_{ST} values using the software SPAGeDi and following Hardy (2003). There was no significant difference between the two measures (data not shown), indicating that mutation does not have an overwhelming effect on levels of differentiation.

To contrast genetic diversity between Atlantic sites and the Atlantic and the Gulf of Mexico, standard diversity estimates were generated using Arlequin (for spatial and temporal diversity indices including the sample size, number of alleles, allelic size range, observed and expected heterozygosity, and probability of deviation from Hardy–Weinberg equilibrium for each locus, see Supplemental Tables S3–S5[2]), and a coalescent-based estimate of theta (Θ) was generated using MIGRATE (version 2.3; Beerli and Felsenstein 2001).

Allele frequencies of the microsatellites appeared to follow the expectations of a stepwise mutation pattern (for locus-specific allele frequencies, see Supplemental Figs. S2 and S3[2]), so a Brownian stepwise mutational model was utilized for all runs. To ensure that estimates of Θ for the Atlantic would be comparable with previous estimates of these parameters in the Gulf of Mexico, MIGRATE runs were modeled according to the methodology of Jue (2006): Θ had a uniform prior distribution over the interval {0, 50.0}, the simulation incorporated a Bayesian approach, runs were composed of 10 independent chains sampling 10 000 genealogies using a multiple Markov static heating scheme, and loci mutational rates were scaled by the number of alleles per locus across the entire sample set; the only exception was that initial runs (10 replicate runs of 20 random individuals) were done by site rather than by year of samples. Bayesian posterior distributions of all runs were examined for stability. As in Jue (2006), when spatial and temporal differentiation proved to be negligible, final estimates for Θ and its credible intervals (2.5%–97.5%) for the entire Atlantic were found by taking the average median scores of Θ and the average credible intervals across all loci for 10 replicate runs of 40 randomly selected individuals (500 "bins" posterior distribution) from the combined Atlantic data set. Replicate runs using independent starting values were performed to ensure stability of the results, and median values were examined as these will give the most stable and reliable estimates of the true value of Θ with this method (Beerli 2006).

Once the average median Θ values and credible intervals (2.5%–97.5%) were obtained, long-term N_e and its credible intervals were calculated for each Atlantic site and for the entire Atlantic (all sites and all year classes) using $\Theta = 4N_e\mu$. For the same 11 microsatellite loci, Jue (2006) estimated a mutation rate of $\mu = 2 \times 10^{-4}$ using the methodology of Turner et al. (2002). To conservatively reduce bias in the final estimates of long-term effective population size, the author utilized half of this value ($\mu = 1 \times 10^{-4}$) to calculate the long-term N_e for the Gulf (Jue 2006). Here, both mutation rates were used to estimate long-term N_e in the Atlantic.

Calculated in this manner, Θ is a broad-based, long-term measure of diversity generated from the mutation-drift equilibrium acting across hundreds if not thousands of generations and is therefore influenced by historical population dynamics. Despite this limitation of the coalescent approach, using Θ estimates from MIGRATE is likely conservative with regard to underestimation of N_e when populations are

recently subdivided and is preferable to other methods when N_e is large because of the low statistical power of other methods (e.g., temporal F_{ST}; Turner et al. 2002).

Gene flow simulations

To estimate the degree to which migration and time since divergence impact genetic differentiation, coalescent-based simulations were generated using SIMCOAL (Excoffier et al. 2000). These simulations utilized the average effective population size estimated from MIGRATE-derived Θ and the fastest mutation rate (~16 500 diploid individuals), but doubling the estimate of N_e did not change our overall conclusions (data not shown). An ancestral population was allowed to diverge into two equally sized populations of 16 500 each between 500 and 500 000 generations ago. The range in the number of generations took into account uncertainties in the generation time of gag (3–10 years; calculations drawn from SCDNR unpublished data and Morris et al. 2000), the indeterminate timing of the split between the Gulf and the Atlantic, which likely occurred during the Pleistocene epoch (11 500 – 1.8 million years ago; Avise 1992), and any possible bias in the underestimation of N_e in the results. Starting immediately after divergence and continuing until the present, the two populations were then allowed to disperse at a rate (i.e., N_m) of between zero and 50 individuals per generation, tracking 11 microsatellite loci for each individual. One-hundred simulation replicates under each combination of parameters were run. At the end of each simulation replicate, Rmetasim (Strand 2002; http://cran.r-project.org) sampled 300 individuals per population, approximating the number of genotyped fish samples, and calculated the average Weir and Cockerham's Φ_{ST}. The mean Φ_{ST} across replicates and its 2.5% and 97.5% quantiles were then visualized. These simulations should be considered as a first-order approximation of gag evolution, given that gag demography (particularly overlapping generations and unequal sex ratio) violates the underlying assumptions of the coalescent approach implemented in SIMCOAL. A simultaneous estimate of gene flow and migration using MIGRATE was attempted but the estimates were unreliable because likelihood surfaces were extremely flat (data not shown).

Results

Statistical analysis

When samples were pooled, all loci met Hardy–Weinberg expectations. Site and year-class level tests for the Atlantic and the western coast of Florida (eastern Gulf of Mexico) showed departures from HWE in one instance (MBO88 locus in the Florida population) after Bonferroni correction. MICROCHECKER detected the presence of null alleles for three loci (GAG023, MBO48, and MBO086); however, the signal was not consistent across sites or year classes, suggesting that this may be a sampling artifact. No loci were found to be affected by linkage disequilibrium or large-allele drop-out after Bonferroni correction.

AMOVA procedures revealed no genetic differentiation at any spatial or temporal scale within the Atlantic (all $F_{ST} < 0.005$, all $p > 0.05$) or any spatial differentiation between the Gulf and Atlantic groups ($F_{ST} = -0.0002$, $p = 1.0$) (for complete spatial and temporal AMOVA results, see Supplemental Tables S6–S8[2]). For all AMOVAs, virtually all of the total variance was explained by within-group variation (97%–100%) rather than by among-group variation. An analysis of pairwise Φ_{ST} between locations and year classes yielded low, insignificant values before and after Bonferroni corrections (Table 2). Similarly, exact tests on allele frequencies also yielded no significant differences ($p > 0.05$; results not shown).

Significant differences in genetic diversity between sets of locations and year classes were not detected (see Supplemental Tables S3–S5[2]). Observed and expected heterozygosity averaged ~0.73–0.79 for each location and year class in the Atlantic and Gulf, and theta (Θ) estimates did not differ between sites in the Atlantic or between the Atlantic and the Gulf (Fig. 2). The average median Θ value and average credible intervals (2.5%–97.5%) for each site were as follows: EFL = 12.07 (8.21–16.04), SC = 12.82 (8.95–17.37), and NC = 13.83 (9.86–17.83). The Θ value and credible intervals (2.5%–97.5%) for the entire Atlantic (all sites combined) was 14.51 (9.81–18.76). Using $\Theta = 4N_e\mu$, the long-term N_e and its credible intervals for the entire Atlantic was 18 138 (12 263 – 23 450) at $\mu = 2 \times 10^{-4}$ and 36 275 (24 525 – 46 900) at $\mu = 1 \times 10^{-4}$ (see Materials and methods). The estimates of Θ did not differ significantly from the Gulf values (Fig. 2), as was originally reported in Jue (2006): $\Theta = 12.39$ (9.1–15.24) yields $N_e = 15\,488$ (11 375 – 19 050) at $\mu = 2 \times 10^{-4}$ or 30 975 (22 750 – 38 100) at $\mu = 1 \times 10^{-4}$.

Gene flow simulations

The coalescent-based simulations provide an estimate of the lowest amount of migration that could maintain genetic homogeneity between regions under nonequilibrium conditions. Assuming that gag have a diploid effective population size of ~16 500 (the average N_e when assuming fastest mutation rates; see above), the expected level of genetic differentiation between the Gulf and the Atlantic should be low but detectable when migration ceases and time of divergence is between 5000 to 500 000 generations (Fig. 3). Increases in migration rate yields declines in Φ_{ST}, regardless of the time since divergence, but the confidence intervals do not include $\Phi_{ST} \sim 0.001$ until migration rates reach 20–30 individuals per generation or more. Therefore, assuming that the microsatellite sampling in this study could detect levels of $\Theta_{ST} = 0.001$ (e.g., Rose et al. 2006; see Discussion), the gag genetic homogeneity between Atlantic and Gulf regions could be maintained by as few as 20–30 migrants per generation. The upper estimate of migrants per generation cannot be assessed using these simulations and could theoretically be hundreds to thousands of individuals.

Discussion

This analysis of 11 microsatellite loci from 870 gag from the northwestern Atlantic and eastern Gulf of Mexico among four year classes (1984, 1999, 2000, and 2001) indicates genetic homogeneity across broad temporal (~20 years) and spatial (~2000 km) scales. In all cases, the majority of total genetic variation could be found within, rather than between, locations or year classes (97%–100%). These conclusions do

Table 2. Φ_{ST} values for pairwise spatial (site) and temporal (year-class) comparisons.

(*a*) Spatial comparisons.

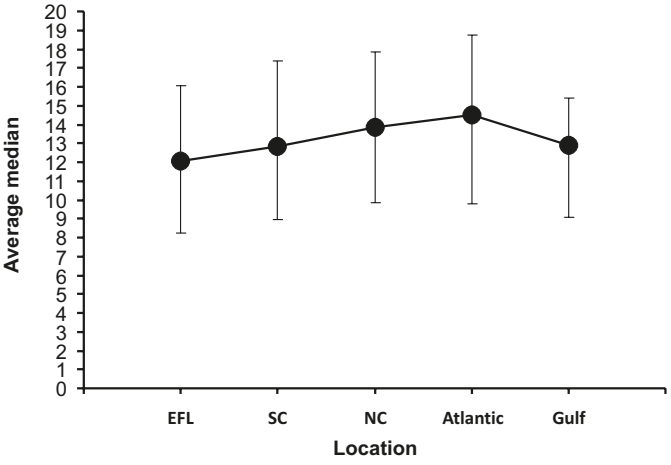
Site	EFL (51)	SC (124)	NC (165)	NWFL (211)	SWFL (131)
EFL	0				
SC	–0.0009	0			
NC	–0.0002	–0.0005	0		
NWFL	–0.0003	–0.0003	–0.0007	0	
SWFL	–0.0003	0.0002	0.0001	0.0006	0

(*b*) Temporal comparisons.

	11 Loci				6 Loci				
Year class	1999 (55)	2000 (125)	2001 (129)	2005 (30)	1985 (158)	1999 (55)	2000 (125)	2001 (129)	2005 (30)
1985					0				
1999	0				–0.0007	0			
2000	–0.0005	0			–0.0003	–0.0004	0		
2001	0.0003	0.0008	0		–0.0006	0.0004	0.0006	0	
2005	0.0006	0.0026	0.0035	0	–0.0001	0.0003	0.0032	0.0004	0

Note: The total number of individuals is given in parentheses following the site or year class; both 6 loci (including the 1985 SC postlarvae) and 11 loci (excluding the 1985 SC postlarvae) results are shown for the temporal tests. Sites: EFL, eastern coast of Florida; SC, South Carolina; NC, North Carolina; NWFL, northwestern coast of Florida; SWFL, southwestern coast of Florida. No comparisons were statistically significant.

Fig. 2. Average median theta (Θ) values and average credible intervals (2.5% and 97.5%) as estimated by MIGRATE (Beerli and Felsenstein 2001) for each Atlantic site and the Atlantic and eastern Gulf of Mexico (Gulf) as a whole. Theta (Θ) estimates did not differ significantly between sites in the Atlantic or between the Atlantic and the Gulf of Mexico. EFL, eastern coast of Florida; SC, South Carolina; NC, North Carolina.



not confirm Chapman et al. (1999), the only other population genetic treatment of gag from both Gulf of Mexico and Atlantic sites. Using a subset of the microsatellite loci in this study (GAG010, GAG023, GAG038) and a smaller number of sample locations and genotypes, Chapman et al. (1999) report patchy genetic structure among populations and significant departures from Hardy–Weinberg equilibrium due to inflated inbreeding coefficients (F_{IS} = 0.02 to 0.44; mean F_{IS} = 0.27) at two of three loci. In contrast, this study found uniformly homogenous genetic structure, no consistent deviations from HWE, and low, nonsignificant levels of F_{IS} (~0.01) within and among locations. It is likely that the causes of these discrepancies are methodological, as the present study used modern genotyping methods (automated capillary-based genotyping versus gel scoring), a greater sample size (870 versus 246), and a greater number of genetic loci (11 vs. 3). The large-scale homogeneity found in this study does, however, mirror a similar conclusion of Jue (2006), which also reported no temporal or spatial genetic differentiation within the eastern Gulf of Mexico using these same 11 microsatellites. In addition, no significant difference in genetic diversity (as measured by theta and heterozygosity) was detected between the northwestern Atlantic and the eastern Gulf of Mexico in this study (see Supplemental Tables S3–S5[2]), suggesting that the effective population sizes are similar among regions. These data imply then that gag stocks from the northwestern Atlantic and eastern Gulf of Mexico belong to a single genetically panmictic population.

However, the lack of genetic differentiation between the eastern Gulf of Mexico and the Atlantic may be the consequence of (*i*) the low statistical power of our data to resolve genetic structure, (*ii*) migration between regions, (*iii*) nonequilibrium between gene flow and drift since populations diverged, or some combination of these three explanations. Low F_{ST} values are particularly common for marine fishes (Ward et al. 1994; Waples 1998) and elevate concerns about the statistical power necessary to infer the degree of gene flow among marine populations. A first-approximation power analysis was generated using the program POWSIM (Ryman and Palm 2006) and indicated that there was a 99.5% chance of detecting a significant difference at an F_{ST} of 0.001 with 11 loci and a sample size of 300 per region. Coupled with the consistency of the values across loci, cohorts, and statistical tests, we have confidence that the absolute levels of differentiation obtained in this study (F_{ST} < 0.001) are biologically real.

It is possible that low levels of genetic differentiation are maintained by effective long-distance migration of gag. Gag larvae have a relatively long planktonic existence (mean 43 days; Keener et al. 1988), and surface-drogue evidence indicates that surface currents can travel from the Florida Keys to the eastern coast of Florida within 45 days (Hare and Walsh 2007). In addition, substantial numbers of adult

Fig. 3. Predicted level of genetic differentiation (Weir and Cockerham's Φ_{ST}) for 11 microsatellite loci with varying numbers of migrants per generation (N_m) after an ancestral population (diploid N_e = 16 500) splits into two subpopulations (N_e = 16 500 each). *t*, number of generations since divergence. Error bars represent the 95% confidence interval of 100 replicate runs under each combination of parameters (varying migrations rates and generation times). Simulations show that even after as many as 500 000 generations, migration rates (N_m) as low as 20–30 individuals per generation yield Φ_{ST} values near 0 (error bars extend to 0).

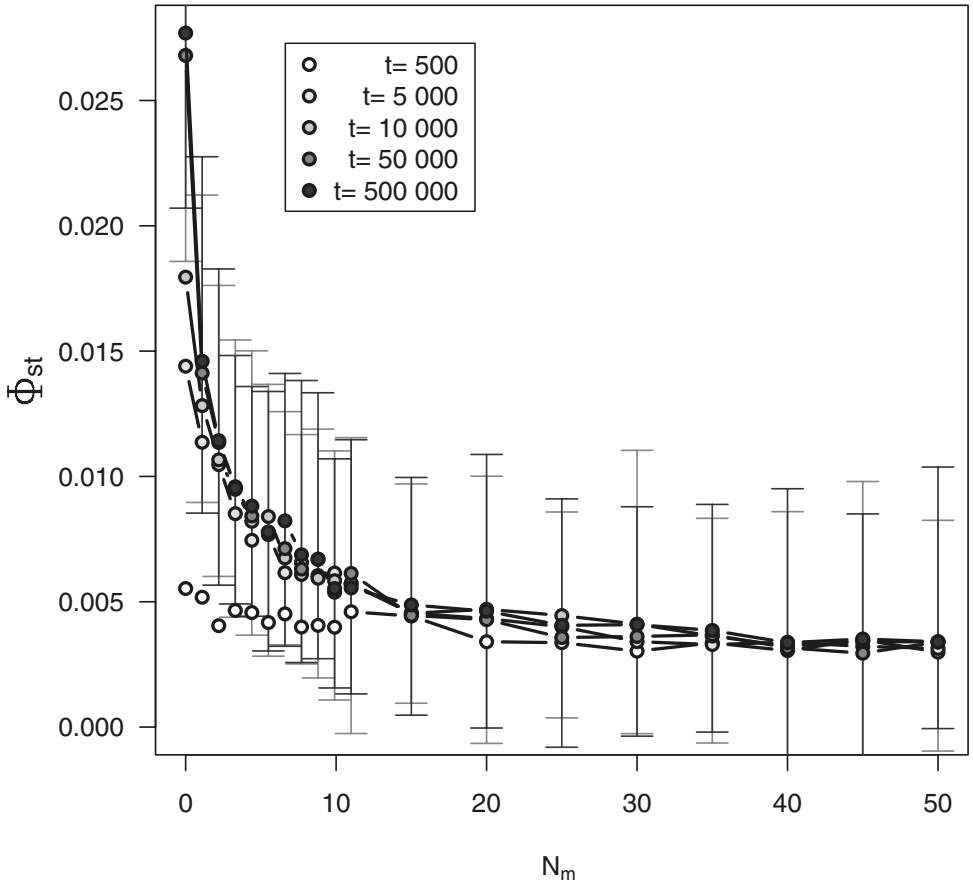


Table 3. Studies of marine fishes that have compared the Gulf of Mexico with the Atlantic.

Fish	Loci	F_{ST}	Reference
Vermillion snapper	Msats (7)	0.001 (ns)	Bagley et al. 1999
Red grouper	Msats (4)	<0.000 (ns)	Zatcoff et al. 2004
Scamp	Msats (6)	<0.000 (ns)	Zatcoff et al. 2004
Striped mullet	mtDNA (1)	<0.000 (ns)	Rocha-Olivares et al. 2000
Red snapper	mtDNA (1)	<0.000 (ns)	Garber et al. 2004
Red porgy	Msats (6)	0.000 (ns)	Ball et al. 2007

Note: Msats, microsatellites; mtDNA, mitochondrial control region. The number of loci used is given in parentheses; ns, nonsignificant.

and subadult gag are known to move among reefs within the Gulf (Heinisch and Fable 1999; Lindberg et al. 2006; Southeast Data, Assessment, and Review (SEDAR) 2006*b*) and the Atlantic (McGovern et al. 2005), and a small proportion of these are moving between regions. McGovern et al. (2005) tagged 3876 gag largely from North carolina and South Carolina and recovered 435 (11.2%) tagged individuals. Of these individuals, five gag moved into the eastern Gulf of Mexico, or almost 1.15% of recovered individuals. If this rate approximated the average number of adult or subadult individuals that moved between regions (i.e., migration rate *m*), then with a census size (N_C) of 1–3 million (following Jue 2006), approximately 12 000 to 35 000 adult or juvenile individuals move from the Atlantic to the Gulf per generation. There are several assumptions underlying this estimate, including bias in recapture (McGovern et al. 2005), but it seems reasonable to assume that hundreds to thousands of gag are able to move among regions per generation. To affect the population genetics, however, at least some of these migrants must be reproducing. A tentative estimate of adult gene flow can be generated by calculating the effective size of this migrant pool, given the tagging-based estimate of migration (1.15%). Assuming an N_e/N_C ratio of 10^{-2} (Jue 2006), the effective number of migrants

(or N_em) based on the McGovern et al. tagging data is between 120 to 350. Acknowledging that one should be cautious in using F_{ST} to estimate N_m (Whitlock and McCauley 1999), it is interesting to note that this estimate is consistent with the genetic data presented in this study: a simplistic analysis of the tagging-based migrant pool yields F_{ST} of 0.0007 to 0.0021, a level of F_{ST} that is at or below the limit of detectable signal using our genetic sampling.

The third possible explanation for the lack of genetic differentiation between the Gulf of Mexico and the Atlantic is that these regions are not at an evolutionary equilibrium between genetic drift and gene flow. When a subdivided population has not had enough time since divergence to differentiate via lineage sorting, genetic homogeneity between sampling locations would be an artifact of past connectivity rather than present isolation and one could mistakenly infer larger migration rates than the true rates (Whitlock and McCauley 1999). Such nonequilibrium conditions are especially likely among large marine populations (Grosberg and Cunningham 2001). Computer-based simulations were utilized to assess the degree to which lineage sorting (which for a given N_e is determined by the time since divergence), gene flow or both can be responsible for neutral genetic homogeneity between locations. Such analyses are emerging as powerful tools in analyzing empirical population genetic data (e.g., Strand and Niehaus 2007; Selkoe et al. 2008) in large part because they allow alternative evolutionary hypotheses to be evaluated quantitatively. Here, the effect of time since divergence and effective migration rates on Φ_{ST} was modeled using the coalescent simulator in SIMCOAL. The results suggest that even after a conservatively long time since divergence (500 000 generations or ~1.5–5 million years for gag), effective migration rates as low as 20 to 30 individuals per generation yield levels of genetic differentiation that were measured (i.e., $\Phi_{ST} < 0.001$). Our simulations and other coalescent analyses (data not shown) indicate that providing a more precise estimate of genetic differentiation is not possible given our genetic and population sampling. This suggests that genetic homogeneity between Gulf and Atlantic gag results from either broad dispersal (hundreds to thousands of individuals per generation) or dispersal rates as low as approximately 20–30 per generation when populations are not in genetic equilibrium.

Our study highlights an important aspect of computer-based simulations, which is to estimate the lower bound of dispersal between regions when populations reveal genetic homogeneity. Simulations could be more broadly used to provide lower bounds of the number of migrants per generation among a suite of marine species. This includes a number of commercially important fish species that reveal no Gulf–Atlantic genetic separation (Table 3) as we have seen in gag: red snapper (*Lutjanus champechanus*; Garber et al. 2004), vermillion snapper (*Rhomboplites aurorubens*; Bagley et al. 1999), red porgy (*Pagrus pagrus*; Ball et al. 2007), red grouper (*Epinephelus morio*; Zatcoff et al. 2004), scamp (*Mycteroperca phenax*; Zatcoff et al. 2004), and striped mullet (*Mugil cephalus*; Rocha-Olivares et al. 2000). Similar to gag, these fish have large census sizes (hundreds of thousands) and show low, nonsignificant levels of differentiation between regions. Because simulations provide quantification of gene flow and time since divergence, the approach dramatically improves on the qualitative assessment that has been pursued by previous studies. For example, authors have suggested only general guidelines (e.g., $1/m$ generations to reach equilibrium; Bagley et al. 1999; for theory, see Slatkin 1995) to assess the degree by which nonequilibrium is important in driving population genetics. The advantage of simulations is their ability to quantify the synergistic effects among population size, time since divergence, and migration rate in predicting levels of genetic divergence, while providing quantitative measures of migration that can be compared with empirical data.

The data presented in this study suggest at least two implications for fisheries management. First, there is no evidence that overfishing has had demonstrable consequences on the neutral population genetics of gag over the period of time examined. The South Atlantic Fisheries Management Council (SAFMC) and the Gulf of Mexico Fisheries Management Council (GOMFMC) consider gag to be overfished (i.e., the current fishing mortality rate is above that which maintains a maximum sustainable yield; SEDAR 2006*a*, 2006*b*). Overfishing has led to female-biased sex ratios in these regions as gag are protogynous hermaphrodites that become male when large (Coleman et al. 1996; McGovern et al. 1998; Harris and Collins 2000). Shifts in sex ratios, in addition to the lowered census sizes, will have detrimental long-term effects on gag populations by lowering effective sizes (or N_e) and, as a consequence, increasing rates of genetic drift and inbreeding. The results from this study do not reveal clear genetic effects of fishing pressure only to the extent that there is no significant signal of inbreeding; however, we caution that the relatively long generation time of gag (3–10 years) will delay the appearance of fishing-related losses in genetic diversity.

Second, the data presented in the study address the long-standing strategy of managing gag fisheries in the United States as two separate stock units, with the Atlantic stock unit (North Carolina to Key West) managed by the South Atlantic Fishery Management Council and the Gulf of Mexico stock unit managed by the Gulf of Mexico Fishery Management Council (Key West to Mexico) (SEDAR 2006*a*, 2006*b*). Current management schemes take into account the greater population size and the larger fishing industry in the Gulf of Mexico when compared with the Atlantic (Koenig et al. 2000; SEDAR 2006*a*, 2006*b*), yet there is a need to understand effective migration rates between the zones. These genetic data indicate low divergence between the Atlantic and the Gulf of Mexico, which implies substantial connectivity between these regions and would support their combination into a single genetically homogenous management unit. However, because of general concerns with statistical power and nonequilibrium in large marine populations, many authors have now cautioned against using panmixia (i.e., genetic homogeneity) as the sole standard by which management units are determined for marine organisms (Taylor and Dizon 1996; Taylor and Dizon 1999; Palsbøll et al. 2007). Simulations were used to conclude that the rates of dispersal between the eastern Gulf of Mexico and Atlantic regions could be as low as approximately 20–30 per generation or as high as hundreds to thousands per generation, and distinguishing these possibilities will likely re-

quire greater sampling of both individuals and the gag genome. Given the imprecision, this study can neither support nor refute current management schemes that independently regulate gag in the Gulf and the Atlantic.

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