Marine Biology (2004) 144: 769–777
DOI 10.1007/s00227-003-1236-z

RESEARCH ARTICLE

M. S. Zatcoff · A. O. Ball · G. R. Sedberry

Population genetic analysis of red grouper, *Epinephelus morio*, and scamp, *Mycteroperca phenax*, from the southeastern U.S. Atlantic and Gulf of Mexico

Received: 10 April 2003 / Accepted: 26 September 2003 / Published online: 14 November 2003
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Abstract The genetic population structure of red grouper, *Epinephelus morio* (Valenciennes), and scamp, *Mycteroperca phenax* Jordan and Swain, from the southeastern U.S. Atlantic coast and the Gulf of Mexico was examined using nuclear microsatellite DNA markers in order to test the null hypothesis of panmixia throughout this range. Physical and biological data indicate that relatively isolated populations of these fish exist. Genetic variation was assessed at four microsatellite loci in red grouper and six loci in scamp. The fish were collected on different dates between 1991 and 2001. The microsatellite loci were highly polymorphic, with an average expected heterozygosity of 0.75 in red grouper and 0.68 in scamp. Heterozygote deficiencies (significant deviations from Hardy–Weinberg equilibrium, HWE) were found at two of four loci in all red grouper samples except the eastern Gulf of Mexico, and for all red grouper combined. In contrast, all loci conformed to HWE in the separate scamp samples. Minimal genetic differences distinguished southeastern U.S. Atlantic or Mexican red grouper from other localities, and no indication of genetic differentiation was observed in scamp. This large-scale genetic homogeneity may be attributed to ongoing gene flow and/or historical contact between present-day populations. For management purposes, genetic homogeneity does not necessarily imply a single stock. Because larval dispersal may be sufficient to homogenize gene frequencies but not to replenish depleted stocks, other data must be considered in the management of these species.

Electronic Supplementary Material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00227-003-1236-z.

Communicated by J.P. Grassle, New Brunswick

M. S. Zatcoff
Grice Marine Laboratory,
College of Charleston,
205 Fort Johnson Road,
Charleston, SC 29412, USA

A. O. Ball (✉) · G. R. Sedberry
South Carolina Department of Natural Resources,
Marine Resources Research Institute,
217 Fort Johnson Road, P.O. Box 12559,
Charleston, SC 29412–2559, USA
E-mail: balla@mrd.dnr.state.sc.us
Fax: +1-843-7628737

Present address: M. S. Zatcoff
625 West Maple Street,
San Diego, CA 92103, USA

Introduction

Red grouper, *Epinephelus morio*, and scamp, *Mycteroperca phenax*, are members of the family Serranidae from the warm temperate and tropical waters of the western Atlantic. *E. morio* is found over hard bottom and coral reefs from North Carolina to southern Brazil, including Bermuda and the Gulf of Mexico, with concentrations over the broad continental shelf areas off western Florida and the Yucatan Peninsula (Heemstra and Randall 1993). *M. phenax* is found on rocky reefs from 30 to 90 m off the eastern coast of the USA (Harris et al. 2002), over *Oculina varicosa* coral reefs off eastern Florida (Gilmore and Jones 1992), and over high-relief rocky bottoms in the eastern Gulf of Mexico (Bullock and Smith 1991). Scamp has a more limited range and reaches only from North Carolina through the Gulf of Mexico to the southern Caribbean Sea (Heemstra and Randall 1993). Both are primarily continental shelf species.

E. morio and *M. phenax* are managed by the Gulf of Mexico, South Atlantic, and Caribbean Fishery Management Councils (NMFS 2002a). In the United States, red grouper catches in the Gulf of Mexico have recently declined, and the species is considered overfished (Schirripa et al. 1999; NMFS 2002b). Although scamp is not considered overfished (Manooch et al. 1998; NMFS 2002a), there have been declines in the proportion of males and a trend toward decreasing size, suggesting that these stocks may be at risk (Coleman et al. 1996; Harris et al. 2002).

Like all groupers, *E. morio* and *M. phenax* are vulnerable to overexploitation due to their life-history characteristics, being slow growing, late maturing and long-lived. In addition, both species are protogynous, whereby individuals begin life as females and change to males at a later age (Heemstra and Randall 1993; Musick 1999; Coleman et al. 2000; Sadovy 2001). Red grouper males and females co-occur year round and do not aggregate to spawn, but form small polygynous spawning groups dispersed over broad areas (Coleman et al. 1996). Despite heavy fishing for decades in the Gulf of Mexico, Coleman et al. (1996) found neither a size- nor a sex-ratio change in red grouper from the west coast of Florida over a period of 25–30 years. In contrast to red grouper, many groupers, including scamp, form presumed spawning aggregations (Gilmore and Jones 1992; Heemstra and Randall 1993; Coleman et al. 1996; Eklund et al. 2000). Targeting of spawning groups can eliminate a large portion of reproductively active fish in the total population, disrupt the social mechanisms necessary for sexual transition to take place, and further skew sex ratios by removing males from the population (Gilmore and Jones 1992; Coleman et al. 1996). Skewed sex ratios, in turn, can lead to reduced effective population sizes and local inbreeding, as evidenced by genetic heterogeneity and significant heterozygote deficiencies (Chapman et al. 1999).

E. morio and *M. phenax* are distributed discontinuously throughout their ranges, but it is not known whether geographically separate assemblages represent reproductively isolated populations. Despite their potential for dispersal over great distances (Lindeman et al. 2000), the stock structure of most grouper species is not well understood. Previous genetic studies using the entire mitochondrial DNA (mtDNA) molecule found no population subdivision in red grouper from the Gulf of Mexico (Richardson and Gold 1997), or in red and black grouper from the northwestern Atlantic (Zatcoff 2001; Chapman et al. 2002). These results have been attributed in part to low mtDNA genetic variation in these species. In this study, we used microsatellite genetic markers, which have often revealed population structure where allozymes and mtDNA have not (Shaklee and Bentzen 1998).

Analysis of allele frequency variation in microsatellite DNA was used to test the null hypothesis that no significant genetic differences exist among *E. morio* and *M. phenax* sampled from several locations in the western North Atlantic. We expected less differentiation among local populations in red grouper, as the species does not aggregate to spawn, has large population sizes which would slow genetic drift, and has exhibited stable sex ratios over the past 20 years (Coleman et al. 1996). Somewhat greater genetic differentiation among local populations and evidence of inbreeding (manifested as heterozygote deficiencies) was considered more likely in scamp, which does aggregate to spawn, has smaller population sizes, and has experienced sex-ratio changes over the past two decades (Coleman et al. 1996).

Materials and methods

Samples of red grouper, *Epinephelus morio* (Valenciennes), were collected along the U.S. Atlantic coast and in the Gulf of Mexico via trap collection, port sampling at docks, or from fish distributors (Zatcoff 2001; Fig. 1; Table 1). For this species, collection dates spanned the years 1991–1999. Tissues were stored in 1% *n*-lauryl-sarcosine, 8 M urea, 20 mM sodium phosphate, and 1 mM EDTA, pH 6.8. Total nucleic acids were extracted using a standard phenol extraction protocol or a silica bead–DNA binding method (Ausubel 1995). Samples of scamp, *Mycteroperca phenax* Jordan and Swain, were collected in similar fashion from South Carolina to Panama City, Florida, from 1996 to 2001 (Fig. 1; Table 1). Details of the collections, including precise dates and length-frequency data, are available from the authors.

Microsatellite primers developed from gag, *M. microlepis* (Chapman et al. 1999), and black grouper, *M. bonaci*, were assayed in *E. morio* and *M. phenax* as described by Zatcoff et al. (2002) (Table 2; Appendix 1). For the loci *Gag23* and *Gag45*, approximately one-half of the red grouper specimens were assayed using [γ^{32}P]-labeled forward primers (Zatcoff et al. 2002). All other red grouper loci and specimens, as well as all scamp specimens, were assayed using fluorescently labeled primers (Operon Technologies). Samples were analyzed on an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, Calif.). Data were read using ABI Genescan software and analyzed using ABI Genotyper Software version 2.1. See Appendix 1 for details.

Ranges in allele size, number of alleles, and frequency of alleles were recorded for each locus. The expected and observed numbers of homozygotes and heterozygotes were calculated using GENEPOP v. 3.1 (Raymond and Rousset 1995), as implemented for online access by E. Morgan (http://wbiomed.curtin.edu.au/genepop/). Conformation of observed genotypes to Hardy–Weinberg equilibrium (HWE) was tested (Guo and Thompson 1992; Rousset and Raymond 1995) using the standard probability test in GENEPOP. A sequential Bonferroni correction was applied to account for multiple comparisons (Rice 1989).

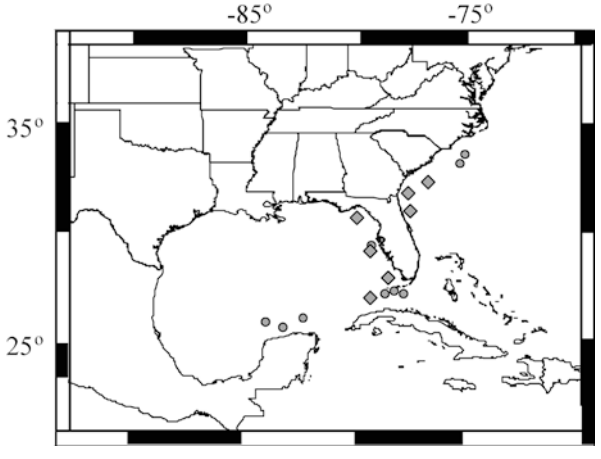

Fig. 1 *Epinephelus morio*, *Mycteroperca phenax*. Map of collection sites for red grouper (*circles*) and scamp (*diamonds*), see also Table 1. The geographic range for *E. morio* extends south to Brazil, and that for *M. phenax* into the Caribbean Sea, but sampling for both was limited to the Gulf of Mexico and eastern United States

Table 1 *Epinephelus morio*, *Mycteroperca phenax*. Collection data for fish used in this study. Location is listed as either the landing site (approximate locations) or the collection site (latitude and longitude)

Area (abbreviation)	Location	Collection dates	No. of individuals
E. morio			
North and South Carolina (NSC)	33°N; 77°W	Apr–Jul 1997	40
	Wrightsville Beach, N.C.	Jan–Mar 1998	90
Florida Keys (FLK)	24°N; 82°W	Feb 1998–Dec 1999	109
Eastern Gulf of Mexico (EGOM)	Florida Middle Grounds	Jan 1999	20
	Panama City, Fla.	Jul–Dec 1999	20
	Apalachicola, Fla.	May 1999	11
Mexico (MEX)	Celestun	Nov 1991	34
	San Felipe	Nov 1991	19
	Progresso or Merida	Jun 1999	68
M. phenax			
Southeast U.S. Atlantic coast (SE)			
South Carolina (SC)	32°N; 79°W	May 1996–Aug 2000	58
Georgia (GA)	31°N; 80°W	May–Jul 2000	17
Florida east coast (FLE)	Daytona Beach	Jul, Dec 2000	26
Florida Keys (FLK)	24°N; 82°W	Nov 1998–Aug 2000	47
Eastern Gulf of Mexico (EGOM)			
Florida southwest coast (FLSW)	25°N; 82°W–25°N; 84°W	Aug 2000–Feb 2001	52
Florida west coast (FLW)	27°N; 83°W–29°N; 85°W	Mar 1999–Jan 2001	86
Florida Panhandle (FLNW)	Panama City	Jul–Oct 1999	35

All pairs of populations at each locus were tested for linkage disequilibrium and then for population differentiation. The expected frequency of null alleles was calculated according to Brookfield (1996) using Micro-Checker (available from http://www.microchecker.hull.ac.uk/), and *E. morio* genotypes at *Gag23* and *Mbo48* were adjusted for null alleles and again tested for population differentiation. The significance of population differentiation was also assessed using the permutation methods implemented in FSTAT, which do not assume HWE within samples (Goudet 1995).

Analysis of variance using analogues of F_{ST} (Wright 1951; Weir and Cockerham 1984) and R_{ST} (Slatkin 1995) was carried out using the AMOVA function in Arlequin ver. 2.001 (Michalakis and Excoffier 1996; Schneider et al. 2000), including a hierarchical analysis of the *M. phenax* populations. The significance of these estimates was assessed by 10,000 permutations. Isolation by distance was tested as the significance of the correlation of $F_{ST}/(1-F_{ST})$ to ln(distance) using ISOLDE implemented in GENEPOP. Nei's standard genetic distance D (Nei 1972) and $(\delta\mu)^2$ (Goldstein et al. 1995) were calculated and bootstrapped over loci using Microsat v. 1.5d (E. Minch; http://hpgl.stanford.edu/projects/microsat/). Because the analyses indicated a high level of genetic homogeneity among locations, the results of the distance analyses are available as Electronic Supplementary Material (Appendices 2, 3).

Results

Genetic data

Four and six microsatellite loci were successfully amplified in *Epinephelus morio* and *Mycteroperca phenax*, respectively (Table 2). A broad range of allele sizes and gene diversities was observed at these loci. For *E. morio*, the number of alleles per locus varied from 8 (*Mbo66*) to 27 (*Gag45*), and average gene diversity (H_e) for all samples ranged from 0.540 at *Mbo66* to 0.928 at *Gag45* (Fig. 2; Table 3). For *M. phenax*, the number of

Table 2 *Epinephelus morio* (Emo), *Mycteroperca phenax* (Mph). Microsatellite primer designation, repeat type, GenBank accession number, sequence, and species (bp range of amplified products) in which loci were amplified

Locus	Cloned repeat	GenBank accession no.	Primer sequence (5′–3′)	Species (bp)
*Gag23*F	$(GT)_{25}$	AF183143	GCATTTGTGTTAGGATGACACT	Emo (89–139), Mph (94–150
*Gag23*R			CACATGGACAGGATTGAGGA	
*Gag45*F[a]	$(GT)_{14}$	AF184959	CCTCACGACGAGTCCAGGAG	Emo (81–137), Mph (118–130)
*Gag45*R[a]			GTTTGCCTTAACGGATGTCTTTCT	
*Mbo48*F	$(GACA)_{11}$	AF325164	CAACGTTGTCATAATCTGAGCAT	Emo (98–151), Mph (106–122)
*Mbo48*R			CGTGGATGATGTTAACTTTGGTG	
*Mbo66*F	$(GT)_8$	AF325163	CGCATGTTTGTAAGAACAGGAAG	Emo (106–128), Mph (104–124)
*Mbo66*R			GCTTCACTCTTGGGTTGTTGG	
*Mbo29*F	$(GT)_{14}$	AF325162	GAGCACGCACACTGAGCAAC	Mph (144–208)
*Mbo29*R			TGCCAGTAAGGCAAAGTGGTC	
*Mbo88*F	$(GT)_{12}$	AF325165	TGAACACCTTCACAATACTTTTCG	Mph (90–138)
*Mbo88*R			TTGAGTATACCAGCAGTTGAACATC	

[a]Primer redesigned from the original (Chapman et al. 1999)

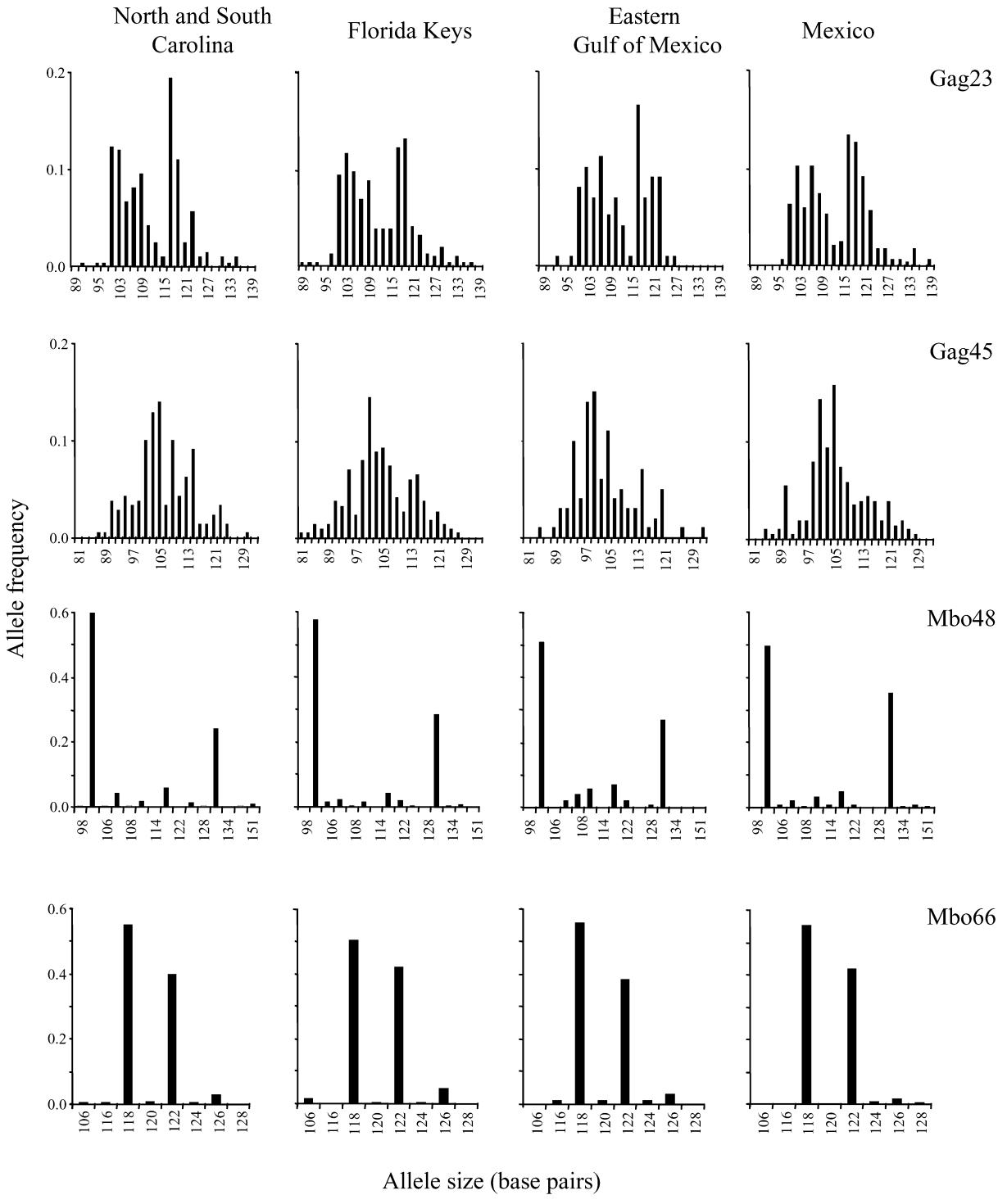

Fig. 2 *Epinephelus morio*. Allele frequency-distribution histograms

alleles per locus varied from 5 (*Mbo48*) to 29 (*Mbo29*), and average gene diversity ranged from 0.264 at *Gag45* to 0.936 at *Mbo29* (Fig. 3; Table 4). For both species, gene diversity was comparable among locations (not shown, Kruskal–Wallis test). Four loci were assayed in both species (*Gag23*, *Gag45*, *Mbo48*, and *Mbo66*), and, while heterozygosities were similar at three loci, at *Gag45*, $H_e = 0.928$ in *E. morio* and $H_e = 0.264$ in *M. phenax*.

For *E. morio*, genotypic proportions in 6 of 16 exact tests deviated from HWE (at loci *Gag23* and *Mbo48* only) after correcting for multiple tests (Table 3). The correlation of alleles within individuals within populations (F_{IS}) at these loci was correspondingly large. For

Table 3 *Epinephelus morio*. Genetic variability statistics for each sample of red grouper and for all combined (*N* sample size; *a* number of alleles; H_o observed heterozygosity; H_e expected heterozygosity; *P(HW)* probability of deviation from Hardy–Weinberg proportions, * significant at $P<0.05$ following a sequential Bonferroni correction; *F* F_{IS} for individual samples and F_{IT} for combined samples)

	Gag23	*Gag45*	*Mbo48*	*Mbo66*
North and South Carolina				
N	106	104	110	110
a	20	21	12	7
H_o	0.745	0.923	0.473	0.591
H_e	0.900	0.923	0.584	0.539
P(HW)	0.000*	0.082	0.008*	0.482
F	0.172	0.000	0.191	−0.097
Florida Keys				
N	107	107	108	106
a	23	24	11	6
H_o	0.804	0.907	0.444	0.604
H_e	0.920	0.933	0.582	0.565
P(HW)	0.002*	0.184	0.013*	0.628
F	0.126	0.029	0.238	−0.069
Eastern Gulf of Mexico				
N	49	50	50	51
a	16	20	8	7
H_o	0.612	0.860	0.560	0.627
H_e	0.916	0.924	0.663	0.545
P(HW)	0.000*	0.645	0.101	0.202
F	0.334	0.070	0.156	−0.152
Mexico				
N	116	101	111	120
a	20	23	12	5
H_o	0.750	0.950	0.505	0.508
H_e	0.920	0.922	0.630	0.521
P(HW)	0.000*	0.324	0.042	0.902
F	0.185	−0.031	0.199	0.025
E. morio				
N	378	362	379	387
a	25	27	15	8
H_o	0.746	0.917	0.485	0.574
H_e	0.914	0.928	0.609	0.540
P(HW)	0.000*	0.296	0.000*	0.619
F	0.184	0.011	0.203	−0.062

M. phenax, no single locus in the separate samples deviated from HWE, and only *Mbo88* in all individuals combined showed a significant deviation from HWE (Table 4). No genotypic linkage disequilibrium was evident in either species (analysis not shown).

Population differentiation

Pairwise tests for genetic differentiation revealed no significant heterogeneity of allele frequency distributions ($P>0.05$ after correction for multiple tests) among samples for either species. Both methods of randomization used, Markov chain and permutations, were consistent in demonstrating a lack of significant population differentiation for pairwise comparisons. There was also no indication of population differentiation with an *E. morio* data set corrected for the presence of null alleles. The lack of genetic differentiation among samples was corroborated by F_{ST} and R_{ST} estimates not significantly different from 0 (Table 5), and there was no indication of isolation by distance (not shown).

Bootstrapped estimates of genetic distance measures likewise supported the lack of genetic structure among each of the *E. morio* and *M. phenax* samples. With red grouper, Nei's *D* gave larger values for comparisons involving the Carolina sample, while $(\delta\mu)^2$ values suggested that Mexico differed (Appendix 2). Support for the slight differentiation of either the Carolinas or Mexico was weakened by this inconsistency and by the fact that the standard error of these estimates was as large as the values themselves. We observed no discernible pattern in the pairwise distance measures for scamp, which indicated only small amounts of random heterogeneity (Appendix 3).

Discussion

This study on *Epinephelus morio* and *Mycteroperca phenax* provided an opportunity to test the genetic structure in two related species with somewhat different breeding structures, the former without spawning aggregations and the latter with them, and to consider any effects of overfishing. The data indicated no significant population differentiation in either species, either local or broad-scale. There was no evidence of heterozygote deficiencies in scamp, and we could not reasonably attribute the heterozygote deficiencies in red grouper to inbreeding. None of the data indicated that overfishing had reduced populations enough that local inbreeding or local differentiation would occur (contrast with *Mycteroperca microlepis*, Chapman et al. 1999). In red grouper, the significant departures from HWE were found within loci and across most samples, rather than within samples and across loci, consistent with null alleles and possibly resulting from the use of primers from non-source species.

The apparent genetic homogeneity, especially for *E. morio*, should be viewed in light of the technical limitations of this study. Despite extensive efforts, we were not able to obtain *E. morio* samples from the far extent of their range in Brazil, nor *M. phenax* from the Caribbean. Another limitation in the red grouper analysis was that we relied on four microsatellite loci, and two with heterozygote deficiencies. Although the variance for F_{ST}, R_{ST}, and distance measures decreases with increasing number of loci (e.g. Ruzzante 1998), the sample sizes for red grouper were large: >100 in three samples and >50 in the fourth. In addition, no locus-specific test measuring allele frequency differences was significant. A number of analyses were performed to test for the effect of null alleles on determination of population structure, and in all cases we could not disprove the null hypothesis of genetically homogeneous populations. Despite these drawbacks, our data indicate that

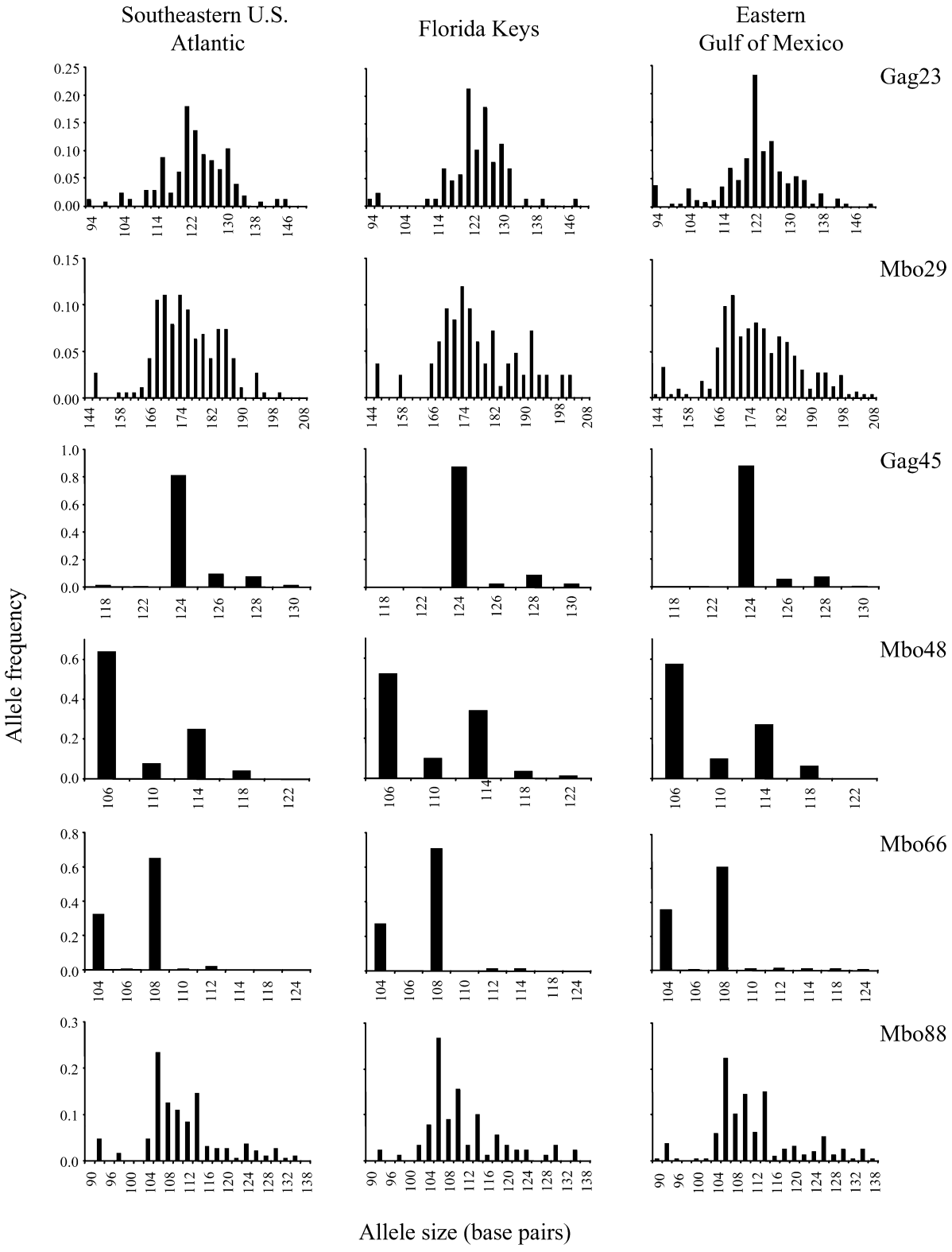

Fig. 3 *Mycteroperca phenax*. Allele frequency-distribution histograms. Allele frequencies for scamp were placed in three groups instead of seven locations (see Table 1)

the major concentrations of red grouper along the southeastern United States and in the Gulf of Mexico from the Yucatan peninsula to western Florida showed no differentiation, and that scamp from their primary target areas of fishing in the United States, the eastern coast of the USA and western Florida, were likewise genetically homogeneous.

For *E. morio*, *M. phenax*, and other reef fishes, various factors could lead to their population structures.

Table 4 *Mycteroperca phenax*. Genetic variability statistics for each sample of scamp and for all combined. Abbreviations as in Table 3

	Gag23	*Gag45*	*Mbo48*	*Mbo66*	*Mbo29*	*Mbo88*
South Carolina						
N	52	58	58	58	54	54
a	19	6	4	5	18	15
H_o	0.846	0.362	0.448	0.310	0.926	0.868
H_e	0.909	0.346	0.506	0.472	0.924	0.887
P(HW)	0.172	0.779	0.239	0.029	0.282	0.080
F	0.069	−0.046	0.115	0.344	−0.002	0.022
Georgia						
N	17	17	17	17	17	17
a	12	3	3	3	13	12
H_o	0.824	0.118	0.412	0.471	0.941	0.882
H_e	0.866	0.219	0.469	0.508	0.923	0.904
P(HW)	0.311	0.172	0.717	0.560	0.518	0.765
F	0.051	0.471	0.125	0.076	−0.020	0.024
Florida east coast						
N	22	26	26	26	24	26
a	14	4	4	3	17	14
H_o	0.955	0.346	0.577	0.423	0.833	0.808
H_e	0.926	0.384	0.594	0.486	0.930	0.884
P(HW)	0.783	0.228	0.618	0.789	0.236	0.025
F	−0.032	0.100	0.030	0.132	0.106	0.088
Florida Keys						
N	45	46	46	46	42	45
a	16	4	5	4	20	17
H_o	0.800	0.261	0.522	0.413	0.952	0.844
H_e	0.890	0.238	0.610	0.431	0.944	0.881
P(HW)	0.426	1	0.246	0.859	0.968	0.251
F	0.102	−0.009	−0.098	0.146	0.043	0.042
Florida southwest coast						
N	52	52	51	52	48	52
a	19	4	4	4	20	19
H_o	0.865	0.231	0.490	0.538	0.938	0.923
H_e	0.906	0.213	0.574	0.492	0.935	0.888
P(HW)	0.663	1	0.534	0.824	0.509	0.838
F	0.046	−0.086	0.147	−0.095	−0.003	−0.039
Florida west coast						
N	83	86	85	86	85	76
a	20	3	4	6	26	18
H_o	0.892	0.209	0.576	0.512	0.965	0.855
H_e	0.896	0.223	0.599	0.514	0.942	0.891
P(HW)	0.780	0.418	0.500	0.607	0.997	0.361
F	0.005	0.062	0.037	0.005	−0.024	0.041
Florida Panhandle						
N	32	34	34	34	33	34
a	17	3	4	5	21	14
H_o	0.906	0.294	0.559	0.441	1	0.912
H_e	0.904	0.265	0.596	0.520	0.944	0.876
P(HW)	0.274	1	0.234	0.033	0.294	0.516
F	−0.003	−0.111	0.063	0.154	−0.060	−0.041
> *M. phenax*						
N	303	319	317	319	303	304
a	27	6	5	8	29	23
H_o	0.868	0.263	0.521	0.448	0.944	0.871
H_e	0.904	0.264	0.572	0.487	0.936	0.885
P(HW)	0.161	0.632	0.339	0.087	0.981	0.009
F	0.040	0.003	0.091	0.080	−0.009	0.015

Table 5 *Epinephelus morio*, *Mycteroperca phenax*. Variance measures calculated for all samples for all loci combined. For *M. phenax*, the three groups for the hierarchical AMOVA, selected on the basis of potential physical oceanographic separation, represented the southeast U.S. coast, Florida Keys, and eastern Gulf of Mexico (see Table 1) (*numbers in parentheses* probability that the calculated values were significantly different from 0; F_{SC}, R_{SC} variation among populations within groups; F_{CT}, R_{CT} variation among groups; F_{ST}, R_{ST} variation among populations); n/a not applicable

	E. morio	*M. phenax*
F_{SC}	n/a	−0.00235 (0.86)
F_{CT}	n/a	0.00217 (0.06)
F_{ST}	−0.00069 (0.79)	−0.00017 (0.72)
R_{SC}	n/a	−0.00640 (0.76)
R_{CT}	n/a	0.00538 (0.09)
R_{ST}	0.00527 (0.08)	−0.00099 (0.74)

Early studies of the Caribbean currents by Molinari et al. (1980) concluded that loops and meanders may result in longer residence time for fish larvae within the Caribbean, and recent studies suggest that local retention and recruitment may be common for reef fishes with pelagic larvae (e.g. Jones et al. 1999; Swearer et al. 1999). Red grouper adults and juveniles are generally sedentary, inhabiting reefs and ledges over continental shelves; tagging data indicated little movement (Moe 1969; Brulé and Déniel 1996; Schirripa et al. 1999). Lack of suitable habitat between established populations may also prevent mixing, since red grouper may be unlikely to cross the deep water between western Florida and Yucatan coasts (Rezak et al. 1985) and are rarely found between Long Bay, South Carolina, and southeastern Florida, although there is no obvious explanation for this (P. Harris, personal communication). Any combination of entraining currents, active larval retention, and disjunct distributions could lead to significant population differentiation, but we saw no evidence of this. In fact, many of the population studies of reef fishes from the western Atlantic show little or no differentiation across broad ranges, including vermilion snapper, *Rhomboplites aurorubens* (Bagley et al. 1999), greater amberjack, *Seriola dumerili*, red snapper, *Lutjanus campechanus*, and red grouper (Gold and Richardson 1998).

There are a number of explanations for these observations of genetic homogeneity. Occasional dispersal by the relatively swift currents of the Caribbean and Gulf of Mexico could carry larvae over broad distances within the reported 30- to 50-day time frame of grouper larval duration (Lindeman et al. 2000). This dispersal need not be extensive, since as little as one migrant per generation can effectively homogenize populations, as assayed by molecular markers (Nei 1987). Another likely explanation is that recent historical contact continues to dominate the genetic population structure. Pleistocene glacial cycles may have induced mixing among grouper populations as lower sea levels and water temperatures pushed warm-adapted species southward (CLIMAP 1976; Petuch 1997). Given that genetic differentiation resulting from mutation or drift requires time on a scale of

N_e generations (Hauser and Ward 1998), not enough time may have passed since populations were last in contact for genetic differences to be detected today.

This study was designed to examine the possible influence of the presence or absence of spawning aggregations and overfishing on the genetics of *E. morio* and *M. phenax*. In fact, for both species, we saw similarly high levels of diversity, no conclusive evidence of biologically significant heterozygote deficiencies, and no convincing evidence of either local or large-scale population differentiation. The data suggest that the populations have not been so disturbed by overfishing that genetic impacts are evident, with the following caveats: there still may be impacts on quantitative traits that have a genetic component (Law 2000), and long-lived species may be slow to exhibit a response to population bottlenecks (Nei et al. 1975; Hauser et al. 2002). Furthermore, self-recruiting populations may indeed exist, and continued monitoring of any genetic changes resulting from fishing pressure is useful to ensure the health of these species in the future.

Acknowledgements We acknowledge R.W. Chapman for valuable consultations regarding genetic analyses and sample collections; and B. Luckhurst, T. Brulé, C. Koenig, the South Carolina Department of Natural Resources' MARMAP program, C. Veach, D. DeMaria, P. Morales, and J. Bradley for sample collections. C. Woodley and P. Rosel provided laboratory space and computer time, and P. Harris provided helpful information about grouper life history and fishery management. This research was supported in part by the South Carolina Governor's Cup Series, the Slocum-Lunz Foundation, and MARFIN grant no. NA87FF0423, R.W. Chapman, P.I. This is contribution 525 of the South Carolina Marine Resources Center, P.O. Box 12559, Charleston, SC 29422, FISHTEC contribution FT03-01, and contribution no. 243 of the Grice Marine Laboratory, College of Charleston, Charleston, South Carolina. These experiments complied with the current laws of the United States.

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