Bull. Natl. Res. Inst. Aquacult., Suppl. 1 : 79−87 (1999)

POPULATION STRUCTURE OF TWO SPECIES TARGETED FOR MARINE STOCK ENHANCEMENT IN THE GULF OF MEXICO

John R. Gold and Linda R. Richardson

Center for Biosystematics and Biodiversity, Texas A&M University
College Station, Texas 77843-2258 USA
Voice : 409-847-8778
Fax : 409-845-4096
Internet : goldfish@tamu.edu

ABSTRACT

Enhancement of overfished stocks of harvested marine species through supplementation with hatchery-raised individuals is a viable option in the Gulf of Mexico (Gulf), where virtually all U. S. Gulf Coast states have active programs supplementing wild stocks or are carrying out research designed to that end. Two targeted species are red drum *Sciaenops ocellatus* (Sciaenidae) and red snapper *Lutjanus campechanus* (Lutjanidae). Both support important fisheries and are overfished. Life-history observations and mark-recapture experiments are consistent with the hypothesis that red drum comprise a single population (stock) in the Gulf. Red snapper, alternatively, appear to be sedentary, non-migratory, and typically associated with specific substrates or structures. We employed molecular genetic tools to assess population structure across the northern Gulf in both species. Analysis of mitochondrial (mt) DNA genotypes of red drum revealed a significant isolation-by-distance effect, where female migration is inversely related to distance from the estuary or bay of natal origin. Trajectories of correlograms in spatial autocorrelation analysis suggest an upper limit to geographic neighborhood size of roughly 700-750 km. Enhancement of red drum should thus be based on the premise that supplementation of a given bay or estuary will impact geographically proximal bays or estuaries more than geographically distant ones. Analysis of mtDNA and microsatellite DNA loci of red snapper indicated that red snapper from the northern and western Gulf comprise a single population. This finding is not consistent with expectations based on life history and adult movement. Our studies demonstrate that population structure is not necessarily concordant with the perceived biology of species. Analysis of population structure should be examined thoroughly prior to implementation of enhancement programs for marine species.

INTRODUCTION

Knowledge of the population structure of economically important marine fish species is critical to several issues pertinent to management of a fishery, including stock enhancement. Identification of subpopulations (stocks) provides biologically meaningful geographic boundaries for assessing a number of parameters, including genetic diversity. The latter is important because subpopulations (stocks) may possess novel genetic, physiological, behavioral, and/or other characters that promote distinct differences in life-history traits such as growth rates, fecundity, abundance, and disease resistance (Stepien 1995). In addition, subpopulations subjected to environmental or other stress (e. g., overexploitation) that reduce effective population size may be prone to "mutational meltdown" where slightly deleterious, recessive mutations accumulate and erode fitness (Lynch *et al.* 1995). Knowledge of stock structure also is important for conservation and management of a fishery because assessment and allocation decisions can be tailored to the unique needs of both resource and resource users within a subregion. Finally, knowledge of stock structure is critical to stock enhancement or supportive breeding (*sensu* Ryman and Laikre 1991) relative to defining geographic boundaries for monitoring post-supplementation effects on genetic effective population size and/or assessing supplementation (enhancement) success (Blankenship and Leber 1995).

Over the past decade, studies in our laboratory have been directed in large part towards assessing population structure of economically important marine fish species in U.S. waters of the Gulf of Mexico (hereafter, northern

Gulf). We have employed genetic markers, including allozymes (Gold *et al.* 1994), mitochondrial (mt)DNA (Richardson and Gold 1997, Gold and Richardson 1998, Gold *et al.* 1997a, b, 1999), and microsatellite DNA loci (Broughton and Gold 1997, Heist and Gold 1999), to test genetic homogeneity and quantify gene flow among geographic localities for several species. In a general way, population structure occurs when gene flow is restricted for any of a number of reasons, including oceanographic parameters (e.g., current patterns), diffusion effects, behavioral characteristics, natural selection, or past history (Palumbi 1994).

In this paper, we synopsize several years work on population structure of two species of marine fishes in the northern Gulf for which supportive breeding or supplementation programs are either operational or in the planning stage. The first is the red drum *Sciaenops ocellatus*, a member of the family Sciaenidae. Red drum is an important recreational species in bays and estuaries of the northern Gulf and it is managed intensively by various coastal states (Pattillo *et al.* 1997). The species has considerable name recognition in the restaurant trade (viz., "blackened" redfish), and at one time, supported a viable commercial fishery as well (Pattillo *et al.* 1997). Hatchery technology (husbandry) of red drum is well developed, and the life cycle is effectively "closed" (McCarty 1990). Stock enhancement programs are being considered by several Gulf Coast states, and a long-standing program exists in the state of Texas where hatchery-raised red drum fingerlings have been stocked in various bays and estuaries since the 1970s (McEachron *et al.* 1995). The second species is the red snapper *Lutjanus campechanus*, a member of the family Lutjanidae. Red snapper is arguably the most important recreational and commercial marine fish species in offshore waters of the northern Gulf, and is managed intensively by the U.S. federal government (GMFMC 1989, 1991). Red snapper also have considerable name recognition in Gulf Coast states, both among fishermen and as a food item in restaurants. Hatchery (husbandry) technology for red snapper is in its infancy, and no stock enhancement programs are yet operational. However, a major stock-enhancement effort for red snapper in the northern Gulf is currently in the planning stage (Pruder and Hawkins in press).

Consideration of the life history and other aspects of the two species yields different predictions regarding population structure (gene flow) in the northern Gulf. Red drum spawn near passes and inlets to bays and estuaries, and the pelagic eggs and larvae move into nursery areas on incoming tides (Matlock 1987). Juveniles remain in bays and estuaries for three to four years, and then move offshore, typically forming large schools (Matlock 1987). Mark-recapture studies have demonstrated little to no movement of juveniles between bays and estuaries; adults, however, can migrate extensively across the northern Gulf (Matlock 1987, Matlock and Weaver 1979). Because adults appear capable of spawning throughout their >30 year life-span (Murphy and Taylor 1990), the potential for gene flow throughout the northern Gulf appears high, leading to the prediction that little to no population (stock) structure in red drum should exist in the northern Gulf.

Red snapper, alternatively, spawn offshore on the continental shelf, where eggs and larvae remain pelagic for approximately 30 days (Leis 1987). After settlement, post-larvae are generally sedentary and are associated with low or high relief bottoms (Bradley and Bryan 1975). Mark-recapture and sonic-tracking experiments (Fable 1980, Szedlmayer 1997, Bradley and Bryan 1975) indicate there is little to no movement of adults. Based primarily on the latter, the potential for gene flow among red snapper in the northern Gulf would appear to be limited, leading to the prediction that population (stock) structure of red snapper could exist in the northern Gulf.

The primary genetic markers employed in our studies of population structure have been restriction-enzyme sites in mitochondrial (mt)DNA. Use of mtDNA in studies of population structure is well documented in a number of vertebrate species, including several fishes (Ovenden 1990, Avise 1992). Major benefits of using mtDNA markers, relative to analogous, nuclear-encoded sequences, include (i) a lower genetic effective population size because of genetic haploidy and maternal inheritance (Birkey et al. 1983), and (ii) a higher rate of nucleotide substitution (Brown 1983). Because of the former, mtDNA is expected to be four times more sensitive in detecting population structure, should it exist; because of the latter, mtDNA is capable, in theory, of detecting population structure that has occurred in recent evolutionary time.

MATERIALS AND METHODS

Localities in the northern Gulf where fish have been obtained, along with sample sizes at each locality, are given in Figures 1 and 2. Information regarding methods, dates of collection, tissue removal, and preservation are given in the primary papers: red drum (Gold *et al.* 1993, 1999), and red snapper (Gold *et al.* 1997b). Red drum were sampled as juveniles from bays and estuaries; adult red snapper were sampled in offshore waters. The total number of fish assayed from the northern Gulf was 1,371 for red drum and 707 for red snapper. Samples of red drum from four consecutive cohorts (year classes) were obtained at several localities (listed in Gold *et al.* 1999).

Assay of mtDNA restriction sites followed methods outlined in Gold and Richardson (1991). Specific restriction enzymes and mtDNA probes used to digest whole mtDNA molecules are given in Gold *et al.* (1999) for red drum and Gold *et al.* (1997b) for red snapper. A total of 104 red drum and 93 red snapper restriction sites,

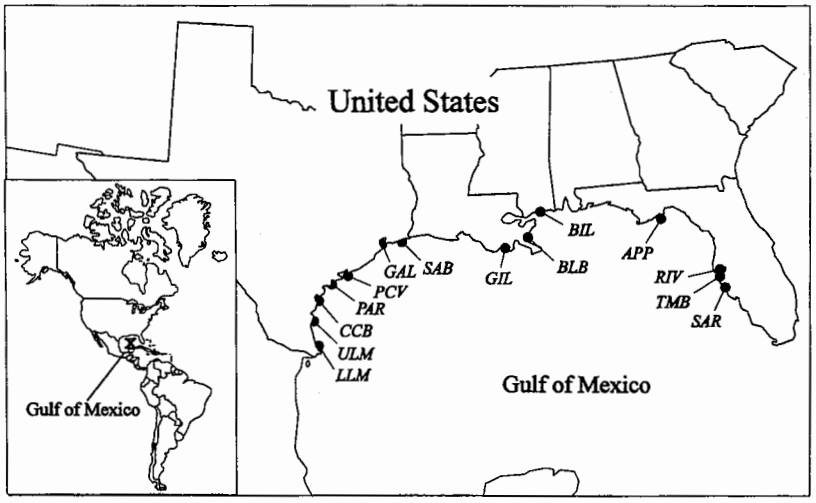

Figure 1. Localities in the Gulf of Mexico where samples of red drum were obtained. Acronyms are as follows (sample sizes in parentheses): *LLM*-Lower Laguna Madre, Texas (147); *ULM* - Upper Laguna Madre, Texas (94); *CCB* - Corpus Christi Bay, Texas (104); *PAR* - Redfish Bay, Texas (38); *PCV* - Pass Cavallo, Texas (90); *GAL* - Galveston Bay, Texas (117); *SAB* - Sabine Pass, Texas (106); *GIL* - Grand Isle, Louisiana (121); *BLB* - Black Bay, Louisiana (20); *BIL* - Biloxi Bay, Mississippi (117); *APP* - Apalachicola Bay, Florida (154); *RIV* - Riviera Bay, Florida (69); *TMB* - Tampa Bay, Florida (83); and *SAR* - Sarasota Bay, Florida (111).

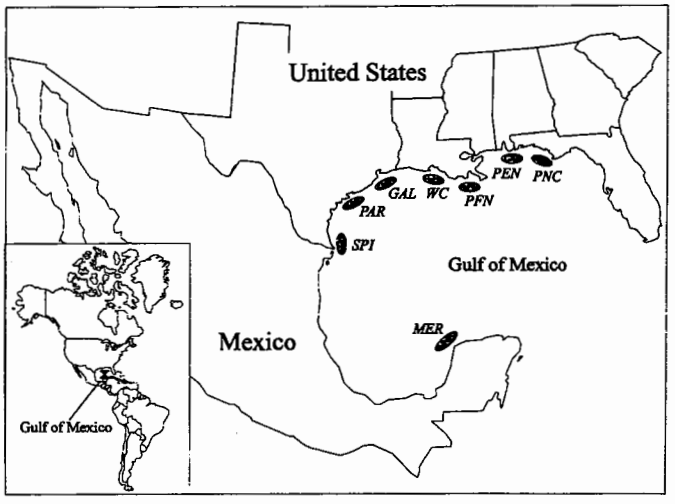

Figure 2. Localities in the Gulf of Mexico where offshore samples of red snapper were obtained. Acronyms are as follows (sample sizes in parentheses): *MER* - Merida, Mexico (44); *SPI* - South Padre Island, Texas (95); *PAR* - Port Aransas, Texas (112); *GAL* - Galveston, Texas (97); *WC* - West Cameron, Louisiana (54); *PFN* - Port Fourchon, Louisiana (86); *DIL* - Dauphin Island, Alabama (103); *PEN* - Pensacola, Florida (25); and *PNC* - Panama City, Florida (91).

distributed essentially randomly in each mtDNA genome (Figure 3), were assayed. The number of unique mtDNA haplotypes identified was 145 for red drum and 92 for red snapper. Statistical approaches, including molecular analysis of variance (Excoffier *et al.* 1992) and spatial autocorrelation analysis (Wartenberg 1989), used to examine the spatial distribution of mtDNA haplotypes and their frequencies are given in Gold *et al.* (1999) for red drum and Gold *et al.* (1997b) for red snapper.

RESULTS AND DISCUSSION

Red drum: Prior to examining the distribution of mtDNA

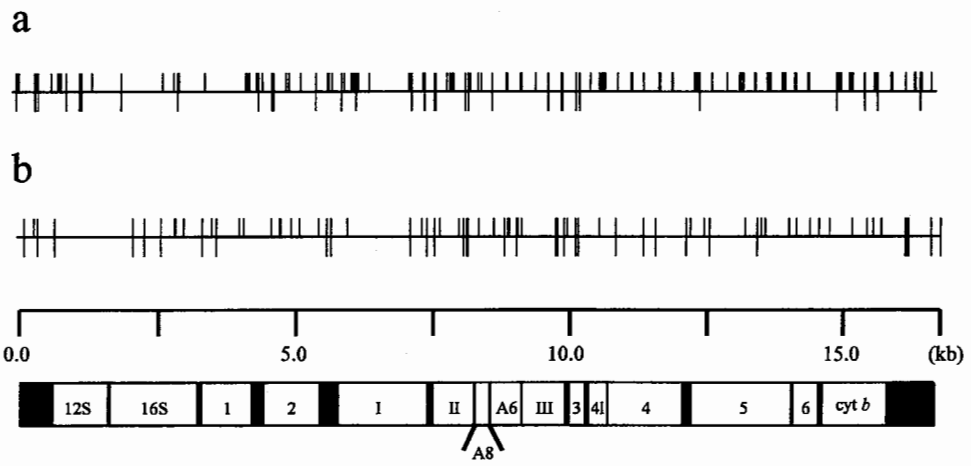

Figure 3. Restriction-enzyme maps for mtDNA of red drum (a) and red snapper (b). Restriction sites that extend below the line were found in all individuals surveyed. Maps are oriented to the human mtDNA gene map: clear boxes refer to the small and large rRNA genes (12S and 16S), NADH dehydrogenase subunits (1-6, 4L), cytochrome oxidase subunits (I-III), ATPase subunits (A6, A8), and cytochrome *b* (b); transfer RNA genes and spacers appear as black areas and the D-loop or control region is shaded.

haplotypes across localities, we first tested homogeneity of mtDNA haplotype frequencies across year classes within localities. All tests of temporal homogeneity (i.e., homogeneity of mtDNA haplotype frequencies between or among year classes at individual localities) were non-significant, permitting pooling of temporal samples at each locality. Analysis of molecular variation (AMOVA) revealed significant spatial heterogeneity (Table 1) both among localities ($P=0.014$) and among regions ($P=0.008$). In the latter, localities were pooled into three regions (East, Central, and West Gulf), permitting a test of homogeneity among localities within regions. This test was non-significant ($P=0.135$), indicating the absence of significant genetic divergence in mtDNA haplotype frequencies among proximate geographic localities. These results were corroborated by tests of homogeneity of mtDNA frequencies: significant spatial heterogeneity ($P<0.001$) was found both among localities and among regions (i.e., East, Central, and West Gulf); whereas tests between adjacent localities in different regions were non-significant (Gold *et al.* 1999). Taken together, these results indicate that significant genetic divergence exists among red drum in the Gulf, and moreover, that the degree of genetic divergence between localities increases over geographic distance. A final point to note is that over 99% of the variance in mtDNA haplotypes frequencies was distributed within sampling localities.

Spatial autocorrelation analysis (SAAP) of mtDNA haplotypes was used to examine further whether genetic divergence in red drum in the Gulf was largely a function of geographic distance between individual bays and estuaries. SAAP runs employed mtDNA haplotypes that occurred in seven or more individuals (27 haplotypes total). Seventeen significant ($P<0.05$) Moran's I values were generated when equal distances between distance classes were used. Six of these (five positive) occurred in the first two distance classes, while 11 (nine negative) occurred in the last two distance classes. Four of the positive values in the first distance class, and four of the negative values in the last distance class, were highly significant ($P<0.01$). Identical results were obtained in SAAP runs that used equal numbers of pairwise comparisons in each distance class. These results demonstrate a significant isolation-by-distance effect (Sokal and Oden 1978), where frequencies of mtDNA haplotypes are positively correlated in geographically proximate localities and negatively correlated in geographically distant ones. This is exemplified by correlograms of mtDNA haplotypes exhibiting significant Moran's I values (Figure 4). In both cases (i.e., equal distances between distance classes and equal frequencies per distance class), there was a fairly regular decline from significant, positive autocorrelation at 300 km between localities to negative autocorrelation at 1000 to 1500 km between localities (Figure 4). The geographic distance where no correlation exists is between 700-750 km, suggesting perhaps an upper geographic limit to gene flow from natal bays or estuaries (Gold *et al.* 1999).

Our finding of significant genetic heterogeneity among spatial samples of red drum is counter to the prediction, based on mark-recapture data and life history of adults, that little to no stock structure in red drum should exist in the northern Gulf. It also differs from our previous studies of red drum from the northern Gulf where no significant differences were found in frequencies of both mtDNA haplotypes and alleles at several allozyme loci (Gold *et al.* 1993, 1994). We did, however, identify a weak isolation-

Table 1. Analysis of molecular variance (AMOVA) among mitochondrial DNA haplotypes of red drum (*Sciaenops ocellatus*) from the Gulf of Mexico.

Variance component	Observed partition		Φ_{ST} values	P[a]
	Variance	% total		
Gulf of Mexico				
Among localities	0.00100	0.21	0.002	0.014
Within localities	0.47443	99.79	–	–
East vs Central vs West Gulf[b]				
Among regions	0.00072	0.15	0.002	0.008
Among localities within regions	0.00052	0.11	0.001	0.135
Within localities	0.47443	99.74	–	–

[a]Probability of more extreme variance component by chance (1,000 permutations)
[b]East Gulf localities (Sarasota Bay, Tampa Bay, Riviera Bay, Apalachicola Bay); Central Gulf localities (Biloxi Bay, Black Bay, Grand Isle): West Gulf localities (Sabine Pass, Galveston Bay, Pass Cavallo, Redfish Bay, Corpus Christi Bay, Upper Laguna Madre, Lower Laguna Madre)

by-distance effect in the distribution of mtDNA haplotypes (Gold *et al.* 1993). The difference between this and our previous studies is the almost twofold difference in the number of fish sampled from individual localities, from an average of 50-60 fish per locality to nearly 100. This increase in sample size appears to have amplified the isolation-by-distance effect to the point where the differences in mtDNA haplotype frequencies between samples from geographically distant localities have become statistically significant. What this demonstrates is that the significant genetic heterogeneity observed among red drum in the northern Gulf does not necessarily identify discrete subpopulations or stocks with definable and discrete geographic boundaries but rather the existence of genetic units defined by geographic distance from a bay or estuary of natal origin. This "stock" concept differs from that typically encountered in most studies of stock structure in marine fishes where subpopulations (stocks) are assumed to occupy areas defined by geographic boundaries. In the case of red drum, we suggest the upper limit to geographic boundaries from natal bays or estuaries could be approximately 700-750 km.

Two final points are as follows. First, we have hypothesized elsewhere (Gold *et al.* 1999) that the isolation-by-distance effect observed in red drum is likely a function of behavior and could stem from either natal site philopatry (homing) or limited offshore movement relative to a natal bay or estuary. Genetic data alone do not discern between these alternatives, and it is necessary to point out that in red drum, the hypothesis is restricted to females, as the effect is based on the maternally inherited mtDNA molecule. The second point is that the degree of genetic divergence among red drum in the northern Gulf, although significant, is small. The Φ_{ST} values of 0.002, obtained from AMOVA (Table 1), differed significantly from zero but are consistent with relatively high levels of genetic

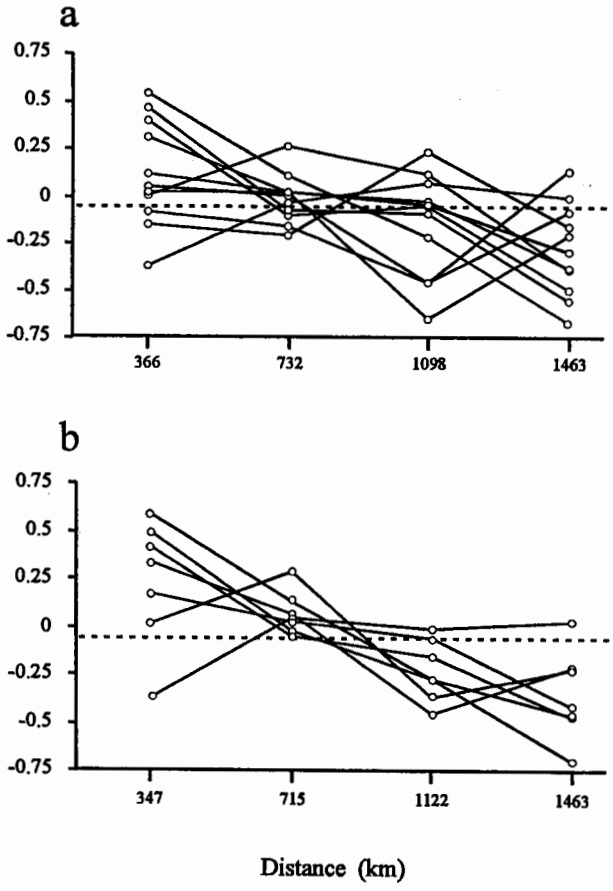

Figure 4. Spatial autocorrelation correlograms of mtDNA haplotypes of red drum with a significant Moran's I value in one or more distance classes. Abscissa: distance classes, with equal frequencies per distance class (a) and equal distances between distance classes (b). Ordinate: Moran's I values. Dashed line is Moran's I value expected when no correlation exists.

effective migration throughout the northern Gulf. Using the island model of Wright (1943), and substituting Φ_{ST} for F_{ST}, Gold *et al.* (1999) estimated the number of genetic effective migrants (N_em) to be approximately 250 individuals per generation. This dramatically underscores the principle noted by Wright (1969) that significant genetic divergence can occur in the face of high gene flow and points to the use of caution in employing N_em estimates as an index of population subdivision.

Red snapper: Results of our analysis of mtDNA variation among samples of red snapper from the Gulf are reported fully in Gold *et al.* (1997b) and Gold and Richardson (1998). Briefly, analysis of the distribution of mtDNA haplotypes both between or among samples of red snapper taken at different years from the same localities, and among localities from northern Florida to the Yucatan Peninsula in Mexico, revealed little evidence of temporal or spatial population structure. Analyses included tests of homogeneity in mtDNA haplotype frequency, estimates of Θ (the F_{ST} analog of Weir and Cockerham 1984), and *V* tests (DeSalle *et al.* 1987) of the spatial distribution of individual haplotypes found in four or more individuals. We also constructed a minimum-parsimony network (Figure 5.) by connecting composite haplotypes based on single restriction-site gains or losses. The most common haplotype (*a*), occurring in >48% of all individuals surveyed, formed a central "hub" that was connected by single restriction-site differences to other "hub" haplotypes (*b-e*), occurring in frequencies of 13%, 5.5%, 4.1%, and 2.4%, respectively. None of the other "hub" haplotypes or their derivative haplotype groupings were restricted geographically, i.e., each occurred occurring at multiple localities across the northern Gulf. The same pattern, i.e., absence of geographic cohesion or restriction of a haplotype or haplotype grouping, also was observed for haplotypes occurring in 2% or fewer of the individuals assayed (Table 2). Our finding that both haplotype groups and rare haplotypes do not exhibit spatial partitioning is consistent with results of homogeneity testing in demonstrating the absence of detectable population structure in red snapper from the northern Gulf.

We also carried out spatial autocorrelation analysis of mtDNA haplotypes occurring in nine or more individuals (10 haplotypes total). Three significant ($P<0.05$) Moran's I values (all negative) were generated in SAAP runs when equal distances between distance classes were used: two occurred in the penultimate distance class and one occurred in the last distance class (Gold *et al.* 1997b). Nearly identical results were obtained in SAAP runs that used equal numbers of pairwise comparisons in each distance class. Overall, mean Moran's I values were negative in all distance classes and did not differ significantly ($P > 0.05$) from expected values of I in the absence of autocorrelation (Gold *et al.* 1997b).

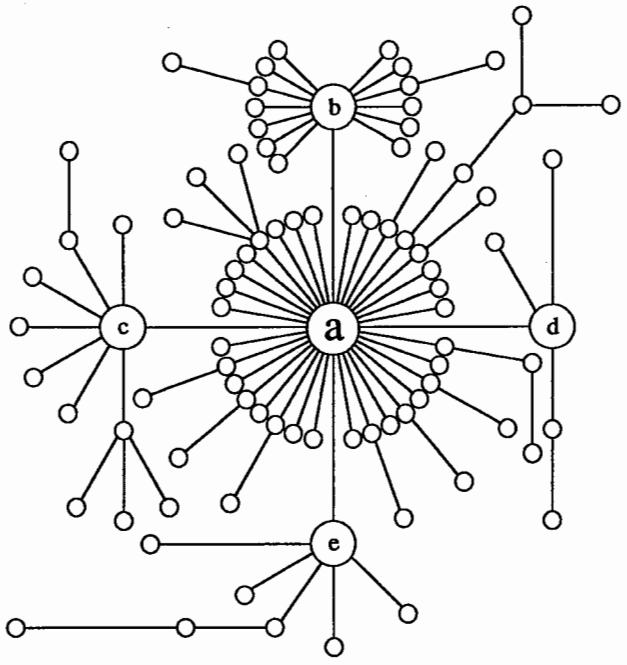

Figure 5. Minimum-length parsimony network of mtDNA haplotypes of red snapper sampled from the Gulf of Mexico. Except for "hub" haplotypes (*b-e*), branch lengths between haplotypes are proportional to the number of (inferred) restriction-site changes. "Hub" mtDNA haplotypes b-e differ by one restriction-site difference from the most common (*a*) haplotype.

Heist and Gold (1999) recently examined the distribution of alleles at five, nuclear-encoded microsatellite DNA loci among four of the samples of red snapper examined previously for variation in mtDNA. Briefly, microsatellites are short stretches of nuclear DNA composed of di-, tri-, and tetranucleotide arrays that are distributed throughout chromosomes and embedded in unique DNA sequences (Weber 1990, Wright 1993). Because variants (new alleles) at microsatellite loci are believed to arise more rapidly than variants in mtDNA or nuclear sequences encoding proteins, microsatellites have the potential of being more effective in detecting population structure than either mtDNA or allozymes. This has been demonstrated recently in both Atlantic cod (Bentzen *et al.* 1996, Ruzzante *et al.* 1997) and Atlantic salmon (McConnell *et al.* 1997).

We examined a total of 194 individuals from the four geographic localities: Panama City (Florida), Dauphin Island (Alabama), Galveston (Texas), and Merida (Mexico). Allele distributions at each locus were similar; each locus possessed one or two common alleles and a number of low-frequency alleles (data not shown). Genotype frequencies at each locus, whether considered by sample or pooled across samples, did not differ significantly ($P>0.05$) from expected genotype proportions based on Hardy Weinberg equilibrium. Tests of (spatial)

Table 2. Geographic distribution of low frequency mitochondrial (mt)DNA haplotypes in red snapper (*Lutjanus campechanus*) from the Gulf of Mexico.

Locality	MtDNA haplotype number													
	4	6	7	13	14	15	17	19	27	29	30	38	55	68
Merida, Mexico	1	–	2	1	–	–	–	–	1	–	1	–	–	–
South Padre Island, TX	–	3	1	3	1	2	–	2	3	–	1	1	–	–
Port Aransas, TX	1	–	1	3	2	1	–	1	2	2	–	2	4	1
Galveston, TX	–	4	1	–	–	1	1	–	4	1	2	–	–	1
West Cameron, LA	3	–	–	1	–	–	2	–	1	1	–	–	–	2
Port Fourchon, LA	–	–	–	–	–	1	5	1	1	1	2	1	–	–
Dauphin Island, AL	1	–	2	3	–	1	3	–	1	–	–	–	1	–
Pensacola, FL	1	1	1	1	1	–	–	–	–	–	–	–	–	–
Panama City, FL	3	1	3	1	1	1	6	1	–	1	–	1	1	1

homogeneity of allele frequencies at each of the five loci, and tests of whether estimated Θ (after Weir and Cockerham 1984) and R_{ST} (another analog of Wright's F_{ST}, after Slatkin 1995) values differed from zero, were uniformly non-significant (Table 3). Estimates of Θ and R_{ST} between pairs of samples at each locus (and over all loci) also did not differ significantly from zero, nor did pairwise values of either Θ or R_{ST} vary with geographic distance between localities (data not shown).

Patterns of genetic variation observed to date in red snapper are consistent with the hypothesis of a single population (stock) in the northern Gulf. Frequencies of both common and rare mtDNA haplotypes and of alleles at five microsatellite loci appear randomly distributed across the northern Gulf and there appears to be no phylogeographic structure to mtDNA haplotypes or haplotype groupings (lineages). Existence of a single, Gulf-wide population of red snapper is counter to the prediction, based largely on mark-recapture and sonic-tracking experiments involving adults (Bradley and Bryan 1975, Fable 1980, Szedlmayer 1997), that population structure could exist because of limited potential for gene flow. Assuming that adults are sedentary, the simplest hypothesis (based on the observed genetic data) is that gene flow occurs via hydrodynamic transport of pelagic eggs and larvae (Goodyear 1992).

There are, however, caveats to the hypothesis of a single red snapper population in the northern Gulf. The first is that one cannot prove a null hypothesis. Red snapper could be subdivided in the northern Gulf yet have the same parametric allele frequencies for the loci thus far examined. A second caveat is that under a hydrodynamic-transport hypothesis one would expect more extensive movement of pelagic eggs and larvae between geographically contiguous localities than between geographically distant ones. Surface current patterns in the northern Gulf are not particularly strong and often go in reverse directions (Shulman and Bermingham 1995). This would be expected to generate a "stepping-stone" pattern of egg and larval transport, that in turn should produce an isolation-by-distance effect where correlations in mtDNA haplotype frequencies vary from positive values between geographically proximal localities to negative values between geographically distant localities. Spatial autocorrelation analysis of red snapper mtDNA, however, revealed no overall correlation (positive or negative) among haplotypes based on geographic distance between localities. In addition, the relationship between genetic distance and geographic distance at microsatellite loci was non-significant. The absence of patterns indicating an isolation-by-distance effect in red snapper from the northern Gulf remains puzzling. Finally, one of the assumptions underlying use of genetic data to assess population structure is that populations are at genetic equilibrium with respect to genetic drift and migration. We have hypothesized elsewhere (Gold *et al.* 1997b) that red snapper in the northern Gulf may represent immigrant subpopulations that expanded northward following the last glacial retreat. Waters on the reduced continental shelf in the northern Gulf were much cooler during the Pleistocene (Rezak *et al.* 1985), and may not have provided suitable habitat for red snapper. Very possibly, there has been insufficient time for present-day red snapper subpopulations to reach the genetic equilibrium.

CONCLUSIONS

Our genetic studies to date on red drum and red snapper in the Gulf of Mexico (Gulf) demonstrate the following. First, predictions regarding population structure that are based on approaches such as mark-recapture (tagging) or are inferred from life history may be misleading. Red drum form large schools in offshore waters of the Gulf and

Table 3. Tests of spatial homogeneity of five microsatellite loci among samples of red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Sample localities were Panama City (Florida), Dauphin Island (Alabama), Galveston (Texas), and Merida (Mexico).

Locus	[a]P_{RB}	Θ	[b]P	R_{ST}	[b]P
Lca-20	0.32	− 0.001	0.87	− 0.007	0.76
Lca-22	0.21	− 0.003	0.77	− 0.009	0.90
Lca-43	0.08	0.012	0.08	0.008	0.26
Lca-64	0.28	0.001	0.27	0.008	0.12
Lca-91	0.43	0.004	0.22	− 0.014	0.85

[a] P_{RB} is probability based on the randomization procedure (1,000 replicates) of Roff and Bentzen (1989)
[b] P is probability of the null hypothesis that Θ or $R_{ST}=0$, based on random permutation (1,000 trials per comparison).

are known to move considerable distances yet gene flow appears to be related inversely to the bay or estuary of natal origin. Red snapper, alternatively, exhibit sedentary behavior as adults yet gene flow appears to be unrestrained across the northern Gulf. Thus, contrary to prediction, there is limited gene flow in red drum but not red snapper. Programs for stock enhancement for the two species accordingly should be designed differently. Enhancement of red drum should be based on the premise that supplementation of a given bay or estuary will impact geographically proximal bays or estuaries more than geographically distant ones. Second, stock structure *per se* does not necessarily take the form of discrete, geographic units where gene flow is abruptly limited. Gene flow among red drum in the northern Gulf appears to be limited not by geography but by other factors that are perhaps behavioral in nature. Finally, there are constraints regarding inferences about population structure that are based on genetic studies. These constraints largely relate to whether a population is, or subpopulations are, in genetic equilibrium and whether historical or contemporaneous genetic signatures have been uncovered. At present, the issue of stock structure of red snapper in the northern Gulf may be constrained by this problem.

ACKNOWLEDGMENTS

Individuals and management agencies providing assistance in obtaining samples of red drum and red snapper are acknowledged in the original papers. Work was supported by the Texas Sea Grant College Program (Grant NA89AA-D-SG139), the Marfin Program of the U.S. Department of Commerce (Grant NA90AA-H-MF107), the Coastal Fisheries Branch of the Texas Parks and Wildlife Department, the Advanced Research Program (ARP) of the State of Texas (Project 999902-103), and the Texas Agricultural Experiment Station (Project H-6703). Funds for travel to Japan to present this paper were provided by the Texas Sea Grant College Program. Views expressed in the paper are those of the authors and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration or any of its subagencies. This paper is No. 32 in the series "Genetic Studies in Marine Fishes" and Contribution No. 86 of the Center for Biosystematics and Biodiversity at Texas A & M University.

LITERATURE CITED

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63 : 62-76.

Bentzen, P., C. T. Taggart, D. E. Ruzzante, and D. Cook. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 53 : 2706-2721.

Birkey, Jr., C. W., T. Maruyama, and P. Fuerst. 1983. Mitochondrial DNAs and phylogenetic relationships, pp. 107-137. *In* : S. K. Dutta (ed.), DNA systematics. CRC Press, Boca Raton, Florida.

Blankenship, H. L. and K. M. Leber. 1995. A responsible approach to marine stock enhancement. Am. Fish. Soc. Symp. 15: 167-175.

Bradley, E. and C. E. Bryan. 1975. Life history and fishery of the red snapper, *Lutjanus campechanus*, in the northwestern Gulf of Mexico. Proc. Gulf Caribb. Fish. Inst. 27 : 77-106.

Broughton, R. E. and J. R. Gold. 1997. Microsatellite variation in northern bluefin tuna. Mol. Mar. Biol. Biotechnol. 6 : 308-314.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA, pp. 62-88. *In* : M. Nei and R. K. Koehn (eds.), Evolution of genes and proteins. Sinauer, Sunderland, Massachusetts.

DeSalle, R., A. Templeton, I. Mori, S. Pletscher, and J. S. Johnston. 1987. Temporal and spatial heterogeneity of mtDNA polymorphisms in natural populations of *Drosophila mercatorum*. Genetics 116 : 215-233.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes : application to human mitochondrial DNA restriction data. Genetics 131 : 479-491.

Fable, W. A., Jr. 1980. Tagging studies of red snapper (*Lutjanus campechanus*) and vermilion snapper (*Rhomboplites aurorubens*) off the south Texas coast. Contrib. Mar. Sci. 23 : 115-121.

GMFMC (Gulf of Mexico Fishery Management Council). 1989.

Amendment number 1 to the reef fish fishery management plan. GMFMC, Tampa, Florida.

GMFMC (Gulf of Mexico Fishery Management Council). 1991. Amendment 3 to the reef fishery management plan for the reef fish resources of the Gulf of Mexico. GMFMC, Tampa, Florida.

Gold, J. R. and L. R. Richardson. 1991. Genetic studies of marine fishes. IV. An analysis of population structure in the red drum (*Sciaenops ocellatus*) using mitochondrial DNA. Fish. Res. 12 : 213-241.

Gold, J. R. and L. R. Richardson. 1998. Mitochondrial DNA diversification and population structure in fishes from the Gulf of Mexico and western Atlantic. J. Hered. 89 : 404-414.

Gold, J. R., L. R. Richardson, C. Furman, and T. L. King. 1993. Mitochondrial DNA differentiation and population structure in red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. Mar. Biol. 116 : 175-185.

Gold, J. R., T. L. King, L. R. Richardson, D. A. Bohlmeyer, and G. C. Matlock. 1994. Allozyme differentiation within and between red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. J. Fish. Biol. 44 : 567-590.

Gold, J. R., Á. Ý. Kristmundsdóttir, and L. R. Richardson. 1997a. Genetic structure of king mackerel, *Scomberomorus cavalla*, in the Gulf of Mexico. Mar. Biol. 129 : 221-232.

Gold, J. R., F. Sun, and L. R. Richardson. 1997b. Population structure of red snapper from the Gulf of Mexico as inferred from analysis of mitochondrial DNA. Trans. Am. Fish. Soc. 126 : 386-396.

Gold, J. R., L. R. Richardson, and T. F. Turner. 1999. Temporal stability and spatial divergence of mitochondrial DNA haplotype frequencies in red drum (*Sciaenops ocellatus*) from coastal regions of the western Atlantic Ocean and Gulf of Mexico. Mar. Biol. 133 : 593-602.

Goodyear, C. P. 1992. Red snapper in U. S. waters of the Gulf of Mexico. Natl. Mar. Fish. Serv., Miami Lab. CRD 89/90-05. Miami, Florida.

Heist, E. J. and J. R. Gold. 1999. Microsatellite DNA variation in sandbar sharks (*Carcharhinus plumbeus*) from the Gulf of Mexico and mid-Atlantic Bight. Copeia 1999 : 182-186.

Leis, J. M. 1987. Review of the early life history of tropical groupers (Serranidae) and snappers (Lutjanidae), pp. 189-237. *In* : J. J. Polovina and S. Ralston (eds.), Tropical groupers and snappers : biology and fisheries management. Westview Press, Boulder, Colorado.

Lynch, M., J. Conery, and R. Bürger. 1995. Mutation meltdown and the extinction of small populations. Am. Nat. 146 : 489-518.

Matlock, G. C. 1987. The life history of the red drum, pp. 1-47. *In* : G. W. Chamberlain, R. J. Miget, and M. G. Haby (eds.), Manual on red drum aquaculture. Texas Agricul. Ext. Serv. and Sea Grant College Prog., Texas A&M Univ, College Station, Texas.

Matlock, G. C. and J. E. Weaver. 1979. Fish tagging in Texas bays during November 1975 - September 1976. Texas Parks Wildl. Dept., Manage. Data Ser. 1 : 1-136.

McConnell, S. K. J., D. E. Ruzzante, P. T. O'Reilly, L. Hamilton, and J. M. Wright. 1997. Microsatellite loci reveal highly significant genetic differentiation among Atlantic salmon (*Salmo salar* L.) stocks from the east coast of Canada. Mol. Ecol. 6 : 1075-1089.

McEachron, L. W., C. E. McCarty, and R. R. Vega. 1995. Beneficial uses of marine fish hatcheries : enhancement of red drum in Texas coastal waters. Am. Fish. Soc. Symp. 15 : 161-166.

McCarty, C. E. 1990. Design and operation of a photoperiod/temperature spawning system for red drum. Texas A&M Univ., Sea Grant College Prog., TAMU-SG-90-603 : 44-45.

Murphy, M. D. and R. G. Taylor. 1990. Reproduction, growth, and mortality of red drum, *Sciaenops ocellatus*, in Florida. U. S. Fish. Bull. 88 : 531-542.

Ovenden, J. R. 1990. Mitochondrial DNA and marine stock assessment : a review. Aust. J. Mar. Freshwater Res. 41 : 835-853.

Palumbi, S. R. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annu.* Rev. Ecol. Syst. 25 : 547-572.

Pattillo, M. E., T. E. Czapla, D. M. Nelson, and M. E. Monaco. 1997. Distribution and abundance of fishes and invertebrates in Gulf of Mexico estuaries, Volume II. Species life history summaries. ELMR Rep. No. 11. NOAA/NOS Strategic Environ. Assessments Div., Silver Spring, Maryland.

Pruder, G. D. and W. E. Hawkins. In press. An integrated stock enhancement programe : USGMMSEP. Bull. Nat. Res.. Inst. Aquacult. (this volume)

Rezak, R., T. J. Bright, and D. W. McGrail. 1985. Reefs and banks of the northwestern Gulf of Mexico : their geological, biological, and physical dynamics. Wiley, New York.

Richardson, L. R. and J. R. Gold. 1997. Mitochondrial DNA diversity and population structure in red grouper, *Epinephelus morio*, from the Gulf of Mexico. U. S. Fish. Bull. 95 : 174-179.

Roff, D. A. and P. Bentzen. 1989. The statistical analysis of mitochondrial polymorphisms : chi-square and the problem of small samples. Mol. Biol. Evol. 6 : 539-545.

Ruzzante, D. E., C. T. Taggart, D. Cook, and S. Goddard. 1997. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland : microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. 53 : 634-645.

Ryman, N. and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. Conserv. Biol. 3 : 325-329.

Shulman, M. J. and E. Bermingham. 1995. Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49 : 897-910.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139 : 457-462.

Sokal, R. R. and N. L. Oden. 1978. Spatial autocorrelation in biology. 2. Some biological implications and four applications of evolutionary and ecological interest. Biol. J. Linn. Soc. 10 : 229-249.

Stepien, C. 1995. Population genetic divergence and geographic patterns from DNA sequences : examples from marine and freshwater fishes. Am. Fish. Soc. Symp. 17 : 263-287.

Szedlmayer, S. T. 1997. Ultrasonic telemetry of red snapper, *Lutjanus campechanus*, at artificial reef sites in the northeast Gulf of Mexico. Copeia 1997 : 846-850.

Wartenberg, D. 1989. SAAP : A spatial autocorrelation analysis program. Dept. Environ. Commun. Medicine, Robert Wood Johnson Med. School, Piscataway, New Jersey.

Weber, J. L. 1990. Informativeness of human $(dC\ dA)_n$ $(dG\ dT)_n$ polymorphisms. Genomics 7 : 524-530.

Weir, B. S. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38 : 1358-1370.

Wright, J. M. 1993. DNA fingerprinting in fishes, pp. 57-91. *In* : T. Mommsen and P. W. Hochachka (eds.), The biochemistry and molecular biology of fishes. Elsevier Press, Amsterdam, The Netherlands.

Wright, S. 1943. Isolation by distance. Genetics 28 : 114-138.

Wright, S. 1969. Evolution and genetics of populations. Vol. 2. The theory of gene frequencies. Univ. Chicago Press, Chicago, Illinois.