

# Preliminary genetic population structure of southern flounder, *Paralichthys lethostigma*, along the Atlantic Coast and Gulf of Mexico

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Southern flounder, *Paralichthys lethostigma*, inhabit coastal waters from Albemarle Sound, North Carolina to the Baja Laguna Madre del Sur in northern Mexico, but they are apparently absent from southern Florida (Ginsburg, 1952). This species inhabits coastal bays, sounds, and lagoons from spring to fall and migrates offshore to spawn in late fall and winter (Stokes, 1977). Valuable sport and commercial fisheries for southern flounder exist in both the northern Gulf of Mexico (Warren et al.<sup>1</sup>; Robinson et al.<sup>2</sup>) and the western North Atlantic (Monaghan<sup>3</sup>).

Declines in southern flounder absolute abundance in some regions (e.g. Texas during the 1980s; Fuls and McEachron<sup>4</sup>) have prompted some management agencies to institute re-

strictions on recreational and commercial fisheries including reductions in bag limits and minimum size. Should these measures fail to recover this fishery, other measures may be considered by managers, including further restrictions on harvest, or artificial propagation and stocking, or both. Implementation of such enhancement programs requires that genetic surveys be conducted to determine genetic variability and stock structure of managed fish populations (King et al., 1995). Failure to understand underlying genetic structure prior to implementing stocking programs places the genetic resources of target species at risk (Allendorf et al., 1987) and may result in the reduction or loss of among-population variability and changes in within-population genetic characteristics. Ge-

netic analyses of population structure may also provide insight into management options that do not require stocking (Nelson and Soulé, 1987).

The objective of our study was to characterize population structure of southern flounder in coastal regions of the northern Gulf of Mexico and northwestern Atlantic Ocean and to test the null hypothesis of no genetic differentiation within the region surveyed. If this hypothesis was rejected, a number of processes would operate to structure southern flounder population(s). Genetic differentiation in some nearshore organisms in the northern Gulf of Mexico (e.g. *Sciaenops ocellatus*; Gold and Richardson, 1999) has been explained as isolation by distance (Wright, 1943). This model describes a population structured by isolation caused by limited individual migration potential in relation to the size of the species' distribution (Kimura and Weis, 1964). The hypothesis of isolation by distance is supported when geographic distance and genetic distance are positively correlated. Alternatively, differentiation may arise as an adaptive response to localized environmental conditions (King and Zimmerman, 1993) or from the operation of physical barriers, such

<sup>1</sup> Warren, T. A., L. M. Green, and K. W. Spiller. 1994. Trends in finfish landings of sportboat anglers in Texas marine waters May 1974–May 1992, 259 p. Manage. Data Ser. 109, Texas Parks and Wildlife (TPW), Coastal Fish. Div., Austin, TX 78744.

<sup>2</sup> Robinson, L., P. Campbell, and L. Butler. 1995. Trends in Texas commercial fishery landings, 1972–1994, 133 p. Manage. Data Ser. 117, Texas Parks and Wildlife (TPW), Coastal Fish. Div., Austin, TX 78744.

<sup>3</sup> Monaghan, J. P., Jr. 1996. Migration of paralicthid flounders tagged in North Carolina. Study 2 in Life history aspects of selected marine recreational fishes in North Carolina, 44 p. Completion Rep. Grant F-43, Segments 1–5, North Carolina Division of Marine Fisheries, 3441 Arendell Street, Morehead City, NC 28557.

<sup>4</sup> Fuls, B., and L. W. McEachron. 1997. Trends in relative abundance and size of selected finfishes and shellfishes along the Texas coast: November 1975–December 1995, 108 p. Manage. Data Ser. 137, Texas Parks and Wildlife (TPW), Coastal Fish. Div., Austin, TX 78744.

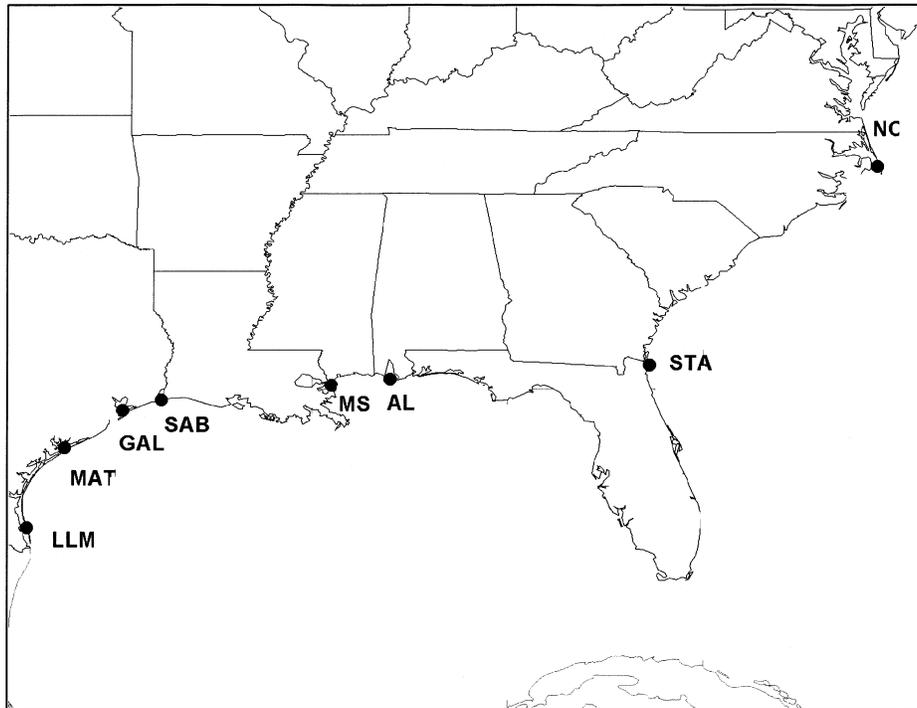


Figure 1

Collection sites for southern flounder in North American waters. LLM = Lower Laguna Madre; MAT = Matagorda Bay; GAL = Galveston Bay; SAB = Sabine Lake; MS = Mississippi; AL = Alabama; STA = St. Augustine, Florida; NC = North Carolina.

as current patterns (King et al., 1994). Support for competing models comes from examination of specific patterns observed in structured populations. Such patterns, if observed, may have important management implications.

## Materials and methods

Southern flounder were collected during the summers of 1996 and 1997 by rod and reel, flounder gigs, or Texas Parks and Wildlife (TPW) gill nets in four Texas bays (Sabine Lake, Galveston Bay, Matagorda Bay, and lower Laguna Madre), by pound nets in Core Sound, North Carolina, from gill nets in estuarine waters near Biloxi, Mississippi, and from commercial fish houses in Dauphin Island, Alabama, and St. Augustine, Florida (Fig. 1). Southern flounder from commercial fish houses were reported by the house operator to be caught locally. A majority of individuals collected were adult and were not reliably assignable to year classes. Samples were screened by using isoelectric focusing (IEF) of sarcoplasmic proteins (methods of Ward et al., 1995) to insure that individuals belonging to other *Paralichthys* species were not included in our analyses (necessary because of accidental inclusion of congeners both in samples obtained from commercial sources and in samples of juvenile flounder obtained during routine resource sampling in Texas).

Skeletal muscle, liver, heart, and kidney tissues were excised from fresh or frozen fish. Sample preparation and

electrophoretic techniques and conditions followed those of King and Pate (1992). Gel and electrode buffers used were tris-borate-EDTA, pH 8.0 (Selander et al., 1971), tris-citrate, pH 8.0 (Selander et al., 1971), lithium hydroxide, pH 8.0 (Selander et al., 1971), borate buffer, pH 9.0 (modified from Sackler, 1966), and Poulik's discontinuous system, pH 8.7 (Selander et al., 1971). Histochemical methods were primarily taken from Manchenko (1994). Genetic nomenclature followed the recommendations of Shaklee et al. (1990).

BIOSYS-1 (Swofford and Selander, 1981) was used to generate an allele frequency table, to estimate the proportion of loci heterozygous ( $H$ ) in the average individual, the proportion of polymorphic loci in individuals from each bay population, and genetic divergence using the  $F$ -statistics of Wright (1978). Exact tests calculated by GENEPOP (v. 3.1; Raymond and Rousset, 1995) were used to test for conformance of genotypic frequencies at each locus within a sample to Hardy-Weinberg expectations, genotypic linkage disequilibrium, and allelic and genotypic heterogeneity. Pairwise differences between samples were tested by using the genic differentiation randomization test in GENEPOP. Results were combined over loci with Fisher's method (Sokal and Rohlf, 1994). Differences between each pair of populations were summarized by using the chord distance of Cavalli-Sforza and Edwards (1967). An unrooted phylogenetic tree was fitted to the chord distance matrix by using the neighbor-joining (NJ) algorithm. TreeView (Page, 1996) was used to visualize

the tree. The strength of support for each node in the tree was tested by bootstrapping over loci with NJBPOP (Cornuet et al., 1999). To further quantify spatial heterogeneity, the fixation index ( $F_{ST}$ ) was calculated for each locus to provide measures of interpopulation differentiation and estimates of reduction in heterozygosity of a subpopulation due to population subdivision. A  $\chi^2$  test was used to test the null hypothesis,  $F_{ST}=0$  (Workman and Niswander, 1970). All  $\chi^2$  probability values from tests for conformance to Hardy-Weinberg expectations, heterogeneity, linkage disequilibrium, and  $F_{ST}$  were adjusted for multiple simultaneous tablewide tests by using sequential Bonferroni adjustments to minimize type-I statistical inference errors (Rice, 1989).

Partitioning of variance components among geographic regions and within samples was accomplished by using a hierarchical analysis of molecular variance (AMOVA, Cockerham, 1969, 1973) with the package ARLEQUIN (version 2, Schneider et al., 1999). Sample sites were nested into regional groupings for separate analyses (Atlantic versus Gulf of Mexico, and Atlantic combined with eastern Gulf sites versus western Gulf). A phenogram was generated from the chord distance matrix with the neighbor-joining (N-J) algorithm. The N-J phenogram, with bootstrap estimates (as percentage of 10,000 replications) obtained by resampling loci within samples, was generated with NJBPOP (Cornuet, et al., 1999). The significance of the relationship between genetic (i.e. chord) and geographic (bay to bay shoreline distance) distance matrices was determined by sampling the randomization distribution generated from 1000 replications with the MX-COMP (matrix comparison) routine in NTSYS-PC 2.0 by Rohlf (1997) to allow a Mantel test (Mantel, 1967). An assignment test (WHICHRUN 4.1, Banks and Eichert, 2000) tested the ability to discriminate population of origin based on an individual's multilocus genotypic profile.

Clinal trends in heterozygosity and allele frequency were examined by using nonparametric correlation analyses (SAS Institute, 1989). Significance of Spearman's correlation coefficients was determined as the probability a correlation differed from zero. Probabilities less than 0.05 were considered statistically significant (Snedecor and Cochran, 1980).

## Results

Misidentifications detected by IEF resulted in a reduced sample size in some samples especially from Alabama and Florida. Examination of 46 enzymes and structural protein systems in southern flounder produced scorable phenotypes for 68 putative gene loci. Two dimeric esterase loci (*ESTD-1\** and *ESTD-2\**, 3.1.1.. [IUBMBNC, 1992]) a tripeptide aminopeptidase locus (*PEPB-2\**, 3.4.13.), glycerol-3-phosphate dehydrogenase (*G3PDH\**, 1.1.1.8), two glucose-6-phosphate isomerase loci (*GPI-A\** and *GPI-B\**, 5.3.1.9), phosphoglucosyltransferase (*PGM\**, 5.4.2.2) and two glucose-6-phosphate dehydrogenase loci (*G6PDH-1\** and *G6PDH-2\**, 1.1.1.49) were resolved, scored as variable, and included in analyses. The remaining 59 loci were mono-

morphic or could not be scored consistently and were omitted. All polymorphic loci had the same common allele across all localities (Table 1). *ESTD-1\**, *ESTD-2\**, *GPI-B\**, and *G6PD-1\** each expressed an allele unique to a single locality. The percentage of polymorphic loci ( $P_{0.99}$ ) averaged 7.5% and ranged from 4.41% to 10.29% (Table 1). Mean individual heterozygosity ranged from 0.03 (SE=0.02) in North Carolina and Florida to 0.12 (SE=0.12) in Matagorda Bay. Statistically significant clinal relationships were found in the Gulf of Mexico between the frequency of the common allele of the *G6PDH-2\** locus and degrees west longitude ( $r_s=-0.829$ ,  $P<0.05$ ) and degrees north latitude ( $r_s=0.829$ ,  $P<0.05$ ). Further differentiation of populations in the western Gulf of Mexico was observed at the *G3PDH\** locus. The *G3PDH\*1* allele occurred at a frequency of 12.0% in the Laguna Madre, declined to a frequency of 1.4% in Galveston Bay, and was absent in all collections east of Galveston Bay. Four loci had alleles confined to a single locality in this same region of Gulf of Mexico (Table 1), including *G6PDH-1\*2* which was limited to the Laguna Madre. Samples were in Hardy-Weinberg equilibrium at the nine allozyme loci surveyed for each of the 72 possible comparisons of variable loci for each locality, except *ESTD-2\** in Galveston Bay. No statistically significant genotypic linkage disequilibrium was observed between any loci for any population.

Tests for homogeneity of allelic frequencies at variable loci across all localities were statistically significant at six of the nine loci: *ESTD-2\** ( $P<0.01$ ), *G3PDH\** ( $P<0.01$ ), *GPI-A\** ( $P=0.01$ ), *GPI-B\** ( $P<0.01$ ), *PGM\** ( $P<0.01$ ), and *G6PDH\*2* ( $P<0.01$ ). Tests for homogeneity of genotypic frequencies across all localities were significant at four loci: *G3PDH\** ( $P<0.01$ ), *GPI-B\** ( $P<0.01$ ), *PGM\** ( $P<0.01$ ), and *G6PDH-2\** ( $P<0.01$ ).  $F_{ST}$  averaged 0.088 and varied from 0.181 for *G6PDH-2\** to 0.001 for *G6PDH-1\**. All estimates of  $F_{ST}$  were found to be statistically different from 0, except for loci *G6PDH-1\**, *PEPB-2\**, and *GPI-A\**.

Regional differentiation among southern flounder was demonstrated (Fig. 2) by using chord distance and N-J clustering. The greatest discontinuity was between all samples from Galveston Bay eastward and a cluster composed of Matagorda Bay and the Laguna Madre. No apparent differentiation exists between Atlantic Coast populations and populations collected from the eastern Gulf of Mexico. This pattern is supported by the outcome of the assignment test (Table 2). Percentage correct assignment ranged from 0% for Florida to 78% for Matagorda Bay. However, when assignments between groups identified in the cluster analysis were examined, the overall correct percentage increased to 81%. The assignment test supports the interpretation of the cluster analysis, suggesting within-region differentiation was minimal and not geographically consistent, but between-region differences were considerable. A different pattern was discerned by using AMOVA (Table 3). The majority of the variance (>99%) was distributed within samples and the among-sites-within-coasts component was nonsignificant. Comparison of Atlantic versus Gulf of Mexico sample sites was statistically significant ( $P=0.04$ ) and comparison of western Gulf of Mexico sample sites with those from the east-

Table 1

Allele frequencies, sample sizes (*n*), percentage of polymorphic loci per population, and mean heterozygosity per locus. LM = Laguna Madre, Texas; MAT = Matagorda Bay, Texas; GAL = Galveston Bay, Texas; SAB = Sabine Lake, Texas; MIS = off Biloxi, Mississippi; ALA = Mobile Bay, Alabama; STA = off St. Augustine, Florida; NC = Core Sound, North Carolina.

Locus	Allele	LM	MAT	GAL	SAB	MIS	ALA	STA	NC
<i>ESTD-1</i> <sup>1,3</sup>	*1	0.972	0.918	0.889	0.917	0.885	1.000	1.000	0.959
	*2	0.028	0.082	0.111	0.083	0.115	0.000	0.000	0.027
	*3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
	<i>n</i>	54	61	36	24	13	21	20	37
<i>ESTD-2</i> <sup>1,3</sup>	*1	0.009	0.000	0.014	0.000	0.000	0.000	0.000	0.054
	*2	0.981	1.000	0.917	1.000	1.000	1.000	1.000	0.946
	*3	0.009	0.000	0.069	0.000	0.000	0.000	0.000	0.000
	<i>n</i>	54	61	36	24	13	21	20	37
<i>PEPB-2</i> <sup>1,3</sup>	*1	0.074	0.016	0.014	0.000	0.038	0.024	0.025	0.000
	*2	0.926	0.984	0.986	0.958	0.962	0.976	0.975	1.000
	*3	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000
	<i>n</i>	54	61	36	24	13	21	20	37
<i>G3PDH</i> <sup>2,3</sup>	*1	0.120	0.098	0.014	0.000	0.000	0.000	0.000	0.000
	*2	0.778	0.779	0.972	0.848	0.808	0.833	0.875	0.946
	*3	0.102	0.123	0.014	0.152	0.192	0.167	0.125	0.054
	<i>n</i>	54	61	36	23	13	21	20	37
<i>GPI-A</i> <sup>1,3</sup>	*1	1.000	0.992	1.000	1.000	0.962	0.929	0.975	1.000
	*2	0.000	0.008	0.000	0.000	0.038	0.071	0.025	0.000
	<i>n</i>	54	61	36	24	13	21	20	36
<i>GPI-B</i> <sup>1,4</sup>	*1	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000
	*2	1.000	1.000	0.986	0.875	1.000	1.000	1.000	1.000
	*3	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000
	<i>n</i>	54	61	36	24	13	21	20	37
<i>PGM</i> <sup>1,4</sup>	*1	0.009	0.008	0.086	0.000	0.000	0.000	0.000	0.000
	*2	0.991	0.992	0.829	1.000	1.000	1.000	1.000	1.000
	*3	0.000	0.000	0.086	0.000	0.000	0.000	0.000	0.000
	<i>n</i>	54	61	35	24	13	21	20	37
<i>G6PDH-1</i> <sup>1,3</sup>	*1	0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*2	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	<i>n</i>	54	61	36	24	13	21	20	37
<i>G6PDH-2</i> <sup>1,3</sup>	*1	0.000	0.000	0.114	0.000	0.000	0.095	0.025	0.000
	*2	0.639	0.648	0.871	0.896	0.962	0.881	0.975	1.000
	*3	0.361	0.352	0.014	0.104	0.038	0.024	0.000	0.000
	<i>n</i>	36	61	35	24	13	21	20	37
% polymorphic loci		10.29	8.82	10.29	7.35	7.35	5.88	5.88	4.41
Heterozygosity		0.10	0.12	0.09	0.08	0.08	0.09	0.03	0.03
Direct count (SE)		(0.05)	(0.06)	(0.04)	(0.03)	(0.03)	(0.04)	(0.02)	(0.02)

<sup>1</sup> Buffer = TVB (Tris-borate-EDTA, pH 8.0).

<sup>2</sup> Buffer = borate 9.

<sup>3</sup> Tissue = muscle.

<sup>4</sup> Tissue = liver.

ern Gulf and the Atlantic coast approached significance ( $P=0.07$ ). The results of chi-square tests for population differentiation (Table 4) allowed a pairwise examination of differences among the eight sampling sites. Eastern sample sites were not significantly different ( $P>0.01$ ) from

their nearest geographic neighbors. Western sites, from Sabine Lake to the lower Laguna Madre, were statistically different from at least one neighbor, suggesting more pronounced genetic differentiation in the western Gulf of Mexico. Among Gulf of Mexico samples, no sta-

Table 2

Assignment test outcomes. Values indicate frequency of assignment of individuals from a collection locality (rows) to the locality to which it is most similar (columns). Numbers on the diagonal indicate correct assignments. The "East" category combines collections from North Carolina to Sabine Lake, Texas, and the "West" category combines Texas sites from Galveston Bay to Laguna Madre. NC = Core Sound, North Carolina; STA = off St. Augustine, Florida; ALA = Mobile Bay, Alabama; MIS = off Biloxi, Mississippi; SAB = Sabine Lake, Texas; GAL = Galveston Bay, Texas; MAT = Matagorda Bay, Texas; LM = Laguna Madre, Texas.

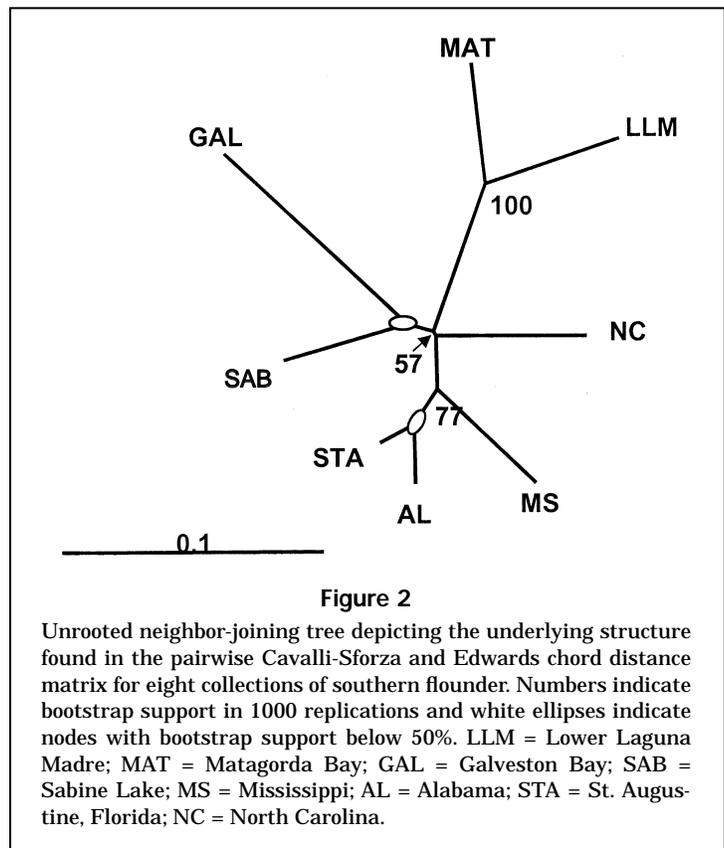
From	Assigned to							
	NC	STA	ALA	MIS	SAB	GAL	MAT	LM
NC	29	0	4	0	2	0	0	0
STA	13	0	5	1	0	1	0	0
ALA	8	0	6	3	0	0	0	0
MIS	4	1	0	4	3	1	0	0
SAB	15	1	4	2	10	5	0	0
GAL	9	0	2	1	2	22	1	0
MAT	8	0	6	6	1	0	14	25
LM	7	0	4	0	0	1	3	19
East			158					1
West			33					61

tistically significant correlations were observed between chord distances and geographic distances ( $r=0.416$ ,  $t=1.549$ ,  $P=0.06$ ).

## Discussion

Genetic differentiation was not extensive over most of the range of *P. lethostigma* examined in this study. Samples collected from Core Sound in North Carolina to Sabine Lake on the upper Texas coast were genetically similar. However, a discontinuity in allele frequencies was identified on the Texas coast between Galveston and Matagorda Bays. In addition, statistically significant clines in allele frequencies at the *G6PDH-2\** locus and in average individual heterozygosity were observed across the Gulf of Mexico. These observations do not suggest the occurrence of independent stocks of southern flounder in the Gulf of Mexico but do support the hypothesis that genetic structuring is present. Southern flounder samples collected off St. Augustine, FL, and off North Carolina cluster with samples in the Gulf of Mexico from Galveston Bay, Texas, eastward, despite a modern-era distributional gap that encompasses the southern reaches of Florida from the Loxahatchee River on the Atlantic Coast to the Caloosatchee Estuary on the Gulf Coast (NOAA<sup>5</sup>). This apparent gap may not represent an effective barrier to gene flow or may be of such recent origin that differentiation has been minimal. It is also possible that differences existed that were undetected by techniques used in our study.

Significant correlation between genetic and geographic distance was not found, lending no support to application of the isolation by distance model (Wright, 1943) to pop-



<sup>5</sup> NOAA. 1985. Gulf of Mexico coastal and ocean zones strategic assessment: data atlas, 188 p. Strat. Assess. Branch, Ocean Assess. Div., Off. Oceanography Mar. Assess., Nat. Ocean Serv. and the Southeast Fish. Center, NMFS, NOAA, U.S. Dep. Commer., 75 Virginia Beach Dr, Miami, FL 33149.



In our study, allele frequency discontinuities and clinal variation in genetic characters were identified among southern flounder inhabiting the western portion of the species' range. Short-term goals (e.g. supplementing exploited populations) that fail to account for this structuring could result in management programs that undermine the long-term resource management objective: maintaining the evolutionary potential of this species. The results of this survey suggest that southern flounder populations should be considered potentially distinct pending further resolution of population differentiation and clinal variation. Stocking efforts, especially those involving interbay transfers, should be undertaken only after careful consideration of all pertinent information, and be contingent upon careful studies of genetic variation at the appropriate local level. Observed discontinuities and clinal variations in allele frequencies may indicate adaptation to localized conditions and should be incorporated into comprehensive management strategies developed for southern flounder.

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