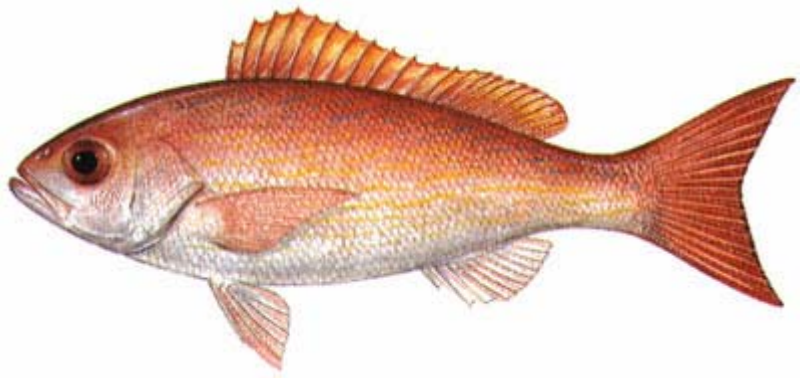
GENETIC STOCK STRUCTURE OF VERMILION SNAPPER (*RHOMBOPLITES AURORUBENS*) IN THE GULF OF MEXICO AND SOUTHEASTERN UNITED STATES



Report to the Florida Fish and Wildlife Conservation Commission

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EXECUTIVE SUMMARY

- Management of the vermilion snapper *Rhomboplites aurorubens* is the responsibility of the regional fishery management councils. Currently, the species is managed as a single unit stock in the Gulf of Mexico.

- Vermilion snapper growth rates vary spatially in the Gulf of Mexico and may be slower in the eastern Gulf than in the northern and western Gulf.

- Although prior genetic studies found no genetic differences among southern Atlantic and northeastern Gulf vermilion snapper, sample coverage in the western Gulf was either absent or not sufficient to test the hypothesis that fishery stocks within the Gulf are regionally structured.

- In this study, 618 vermilion snapper were assayed using 12 microsatellite DNA markers. Regional sample groups included the southeastern Atlantic (n=98), eastern Gulf (n=122), northern Gulf (n=250), and western Gulf (n=148).

- Global tests for heterogeneity in allele frequencies showed significant differences between the Atlantic regional group and each of the three Gulf regional sample groups, respectively, but not among Gulf regional sample groups.

- All other analyses (AMOVA, FCA, and individual-based Bayesian assignment procedures) were consistent with the null hypothesis that vermilion snapper comprise a single, randomly mating stock over the sampled range.

- A significant pattern of 'isolation by distance', indicative of geographically restricted dispersal, was observed over the entire (Atlantic and Gulf) range of samples (Z=1859, p=0.008). When the analysis was limited to Gulf of Mexico samples, this pattern became less clear and the Mantel coefficient (Z=564) was non-significant (p=0.062).

- Overall, there was no genetic evidence for the existence of discrete stocks within the Gulf of Mexico. Evidence for subdivision between southern Atlantic and Gulf of Mexico vermilion snapper stocks was limited to minor differences in allele frequencies at several loci; other evidence was suggestive of connectivity via recent or ongoing gene flow between the Atlantic and Gulf regions.

- Results do not support alteration of current management practices as they pertain to stock delineation.

INTRODUCTION

By definition, the dynamics of spatially structured fishery stocks are *independent.* Therefore, the manner in which stocks are spatially structured directly effects how they will respond to exploitation (Hilborn and Walters 1992). Moreover, growth rates, age structures, egg production and recruitment levels, biomasses, mortalities, etc. commonly differ among discrete stocks. Therefore, delineating unit stocks is usually the first order of business in the fishery assessment process.

The vermilion snapper (*Rhomboplites aurorubens*) supports substantial commercial and recreational fisheries in Florida and throughout its range within the U.S. EEZ. Under section 303 of the Magnuson-Stevens Act, regional fishery councils are required to assess the condition of vermilion stocks. In 2003, NOAA fisheries reported to the Gulf Council that vermilion snapper was overfished in the Gulf of Mexico (Turner 2003). In the past, fishery managers have treated this species as a single unit stock throughout the Gulf of Mexico. However, growth rates of vermilion snapper vary spatially and are apparently slower in the eastern Gulf (and southeastern Atlantic) than in the northern and western Gulf (Grimes, 1978; Potts et al., 1998; Schirripa, 1998 and references therein; Hood and Johnson, 1999). In some cases, regional differences in growth are associated with inherent genetic differences among distinct stocks (e.g., Tringali and Bert 1996).

The hypothesis that genetically distinct regional stocks exist within the Gulf of Mexico is still in need of robust testing. In a previous study, Bagley et al. (1999) examined approximately 500 vermilion snapper specimens from four Atlantic locations (ranging from Morehead City, North Carolina to St. Augustine, Florida) and one Gulf location (Orange Beach, Alabama) using seven variable microsatellite DNA loci. Whereas they found no evidence of stock subdivision within the range of their sampling, they did not analyze samples west of the Mississippi River. Similarly, Schwartz and Bert (2003) examined mtDNA control region sequence in 120 vermilion snapper collected from the eastern Gulf of Mexico and southern Atlantic waters, finding no significant spatial differences in haplotype distributions. Unfortunately, they were only able to obtain four specimens from the western Gulf.

The goal of this study was to produce genetic-based stock-identification data for use by regional management authorities, including the 2005 SEDAR9 Review Panel. Specific analytical objectives were as follows:

- Determine spatial patterns of neutral genetic variation and levels of gene flow within and among vermilion snapper populations in southeastern Atlantic and Gulf of Mexico waters.

- Identify potential regional assessment units in Gulf of Mexico and southeastern Atlantic waters based on the observed partitioning of genetic heterogeneity and individual-based dispersal analyses.

To accomplish these objectives, we assayed 618 vermilion snapper specimens obtained from various locations in the southeastern Atlantic, eastern-Gulf, northern-Gulf, and western-Gulf regions using 12 polymorphic microsatellite DNA markers. Thus, the geographic coverage of sampling, sample sizes, and number and nature of markers employed allowed the most rigorous testing to date of the hypothesis of intra-Gulf substructure.

METHODS

Collection locations (sampling points) and respective sample sizes are depicted in Figure 1. The nine sampling points were partitioned into four regional groups: southeastern Atlantic (n=98), eastern Gulf (n=122), northern Gulf (n=250), and western Gulf (n=148) locations. Study material included fin clips and somatic tissues, stored frozen or in 95% ethanol prior to use. Genomic DNA was isolated using the PUREGENE ® DNA Purification Kit (Gentra Systems, Inc., Minneapolis, MN) in accordance with the manufacturer's directions. Final DNA volumes were adjusted to 85 μl with sterile dH_20.

Genotyping assay procedures: Table 1 lists information for the 12 microsatellite markers employed herein. PCR amplifications were conducted in 25 μl reactions using 2.5 units of HotStar Taq DNA polymerase (Qiagen Inc., Valencia, CA) and 1-2 μl of genomic DNA; component concentrations included 1× Qiagen PCR buffer, 2.5 mM MgCl2, 200 μM of each dNTP, and 1.0 μM total of forward and reverse PCR primers. Forward primers were labeled with the fluorescing dyes 6-FAM, HEX, TET (Invitrogen/Life Technologies, San Diego, CA), or NED (Applied Biosystems, Foster City, CA). Four reaction profiles, based on variable annealing temperatures (Table 1) were used for multiplex (grouped) assays: 94°C for 15 min, 32 × (94 °C for 35 s, [52, 56, 58, or 62 °C] for 35 s, 72 °C for 35 s), and 72 °C for 15 min. One μl of the undiluted PCR product was mixed with 12.5 μl Hi-Di™ formamide (Applied Biosystems) and 0.25 μl of 500 base-pair ROX size standard, denatured at 95°C for 4 min, and immediately chilled on ice. Fragments were processed on an ABI 310 genetic analyzer and sized using Genescan software (Applied Biosystems). Negative-control PCR reactions were performed for all grouped assays. Electropherograms were each scored by two readers independently; one attempt was usually made to resolve disputed or unscorable results via re-assay.

Statistical analysis: Throughout, 'sample' refers to a group of individuals collected from one of the nine sampling points identified in Figure 1. Genetic diversity within each sample was assessed using standard measures, including allelic richness (Petit et al. 1998), which accounts for differences due to disparate sample sizes (Leberg 2002). Unbiased estimates of heterozygosity were computed for each locus and averaged over all loci. We evaluated conformance to Hardy-Weinberg equilibrium (HWE) genotypic proportions using the permutation test implemented in GENETIX (version 4.02, Belkhir et al. 2000). To ensure independent segregation of loci, linkage (genotypic) disequilibrium exact tests were conducted (GENEPOP v.3.4; Raymond and Rousset 1995); associated probabilities were assessed using Guo and Thompson's (1992) Markov-chain method (500 batches, 5000 iterations per batch). For all pairwise estimates, a critical significance threshold of 5% was maintained using a sequential Bonferroni adjustment for multiple tests (Rice 1989) to avoid type-I errors.

To examine spatial structure among samples, locus by locus and global tests for differences in allele (genic) frequencies among samples were performed using GENEPOP; significance was determined using a Markov chain of 500 batches with 5000 iterations per batch. The fixation index θ (Weir and Cockerham 1984) was computed using GENETIX. Hierarchical analyses of molecular variance (AMOVA; Excoffier et al. 1992) were conducted using ARLEQUIN v.2.0. Significance of the partitioning of molecular variance was assessed by comparison to a permuted null distribution. Additional AMOVA analyses were performed to determine which loci contributed most to the observed variance.

The extent of sample and regional differentiation was further investigated by performing an individual-based Factorial Correspondence Analysis (FCA) using GENETIX. Each nuclear allele was treated as an independent variable (She et al. 1987). Contingency table entries were based on allelic states; individuals having 2, 1, or 0 copies of a given

allele received scores of 2, 1, or 0, respectively for that allele. Groups of individuals were depicted as a cloud of points in K-dimensional hyperspace R^k (projected on a factor map) where K is the number of modalities (alleles over all loci). Factor axes were given as the orthogonal directions of R^k, along which the inertia (variance) was greatest. Eigenvalues were ordered such that the largest (first axis) explains the most general pattern or structure contained in the data. Individuals having incomplete multilocus genotypes were excluded from FCA analyses.

PARTITION (Dawson and Belkhir 2001) was used to test for the existence of regional substructure. The underlying individual-based genetic model in PARTITION assumes that individuals belong to some number of separate (panmictic) source populations. Whereas these populations are assumed to be in HWE and linkage equilibrium, the number of source populations (k) represented in the total dataset and their allelic compositions are treated as parameters whose values are unknown. PARTITION yields a Bayesian estimate for the posterior distribution of the number of panmictic units represented in a dataset. It also computes a 'Bayes factor' (B_k), from which support can be gauged for the existence of a single genetic stock against the alternate hypothesis of more than one genetic stock. When $B_k > 1$, the evidence favours the hypothesis that $k = 1$. Here, parameter settings included 10,000 observations of the Markov chain with 10 iterations between successive observations. The maximum number of possible source populations and priors for θ_B (allelic diversity, *see* Dawson and Belkhir 2001) and k were set at 4 (and 2), 1, and 1, respectively.

Finally, to test for an isolation-by-distance pattern (IBD) of gene flow, the Mantel correlation coefficient (Z) was estimated using GENETIX for pairwise matrices of geographic distance and genetic distance [$D_{CE}/(1-D_{CE})$, where D_{CE} was the Cavalli-Sforza/Edwards chord distance]; significances of correlations were assessed from 5000 random permutations of the matrix.

RESULTS

Summary statistics for the genetic data by locus and by sample, including standard measures diversity, appear in Table 2. Mean values of gene diversity, allelic richness, and heterozygosity were similar across samples. Upon sequential Bonferroni correction, the null hypothesis of HWE was not rejected at any locus in any sample with the exception of locus *Prs*229, which deviated significantly from HWE in samples NG1 and WG3. In both cases, a deficiency in the observed number of heterozygous genotypes accounted for the significant test result. Sample F_{IS} values, which measure the extent of departure from HWE proportions, were positive and generally higher for *Prs*229 than for other loci. Because it was confined to one marker, it is not likely that the observed single-locus disequilibrium resulted from undetected population structure within samples or from temporal effects. Rather, it was more likely caused by a technical artifact of genotype screening (i.e., null allelism). Accordingly, data from this locus were not considered in subsequent analyses. The global value over all samples of F_{IS} (0.0203) did not differ significantly from zero. The null hypothesis in tests of linkage disequilibrium could not be rejected at the table-wide error rate for any locus pair in any sample or over pooled samples (Table 3), indicating that the 11 tested loci segregate independently in vermilion snapper.

In exact tests for allele frequency heterogeneity, no significant differences were observed at the table-wide error rate in any sample pair at any locus or globally (over all loci). When samples were pooled into regional groups, there were no significant differences in locus-by-locus comparisons. However, significant differences at the table-wide error rate occurred in the global test between the Atlantic group and each of the three Gulf groups,

respectively (Table 4). Thus, the overall allelic composition differed somewhat between Atlantic and Gulf vermilion snapper but not among Gulf vermilion snapper. This finding guided subsequent hierarchical testing – i.e., the partitioning of molecular variation was examined not only by sample within and among regional groups, but also by regional group within and among the Atlantic and Gulf.

The fixation index, which is based on allele frequency variance under an infinite alleles model (Weir and Cockerham 1984), should yield a value of zero (or its statistical equivalent) when no detectable genetic structure is present and be greater than zero when mating is non-random. Here, the computed value of θ (0.00046) over all samples, which was two orders of magnitude lower than F_{IS}, did not differ significantly from zero. The computed value of θ was 0.00078 when specimens were grouped by region and 0.00167 when specimens were grouped by Atlantic and Gulf origin. Components of the AMOVA are given in Table 5. In the AMOVA, the majority of variation in the markers (>99.95%) was distributed within samples. No portion of the variation was attributable to regional groupings or to Atlantic/Gulf groupings. In addition to the regional grouping indicated in Figure 1, other regional groupings were examined (e.g., grouping sampling point EG1 with the northern Gulf samples NG1 and NG2). The effect on the above analyses was, in all cases, negligible.

Two forms of individual-based analyses were employed – factorial correspondence analysis and Bayesian population assignment. There was no evidence of dimensional structure among vermilion snapper genotype clouds in the FCA factor map; the principal (first) axis accounted for only 2.25% of the total variance. Dimensional genotype ordination was not associated with sample (factor map not shown) or regional group (Fig. 2) membership. For the Bayesian analysis, the modal log-likelihood of the posterior distribution of k (at k = 1) was 0.9739 (Fig. 3); the calculated Bayes factor was much greater than one (B_k = 111.894). Thus, parameter estimates in the Bayesian analysis indicated that the genetic evidence was consistent with the null hypothesis that study specimens comprised a randomly drawn sample from a single, panmictic population.

Despite the lack of detectable regional structure among vermilion snapper genotypes, a clear pattern of isolation-by-distance gene flow emerged from the Mantel testing. When all samples were included in matrices (Fig. 4A), the Mantel coefficient was high (Z = 1859) and the correlation was significant (p=0.008). When the analysis was limited to Gulf of Mexico samples (Fig. 4B), a similar pattern was observed but it became less clear and the Mantel coefficient (Z = 564) was no longer significant (p=0.062), in part, due to the reduced number of data points. As is typical for marine fish (in our experience), there was considerable variance in genetic distance coupled with little evidence for IBD among samples occurring within 500 *km* of each other.

CONCLUSIONS

Data analyses indicated that vermilion snapper are highly interconnected by gene flow on a regional basis. Despite minor parametric differences in allele frequency between Atlantic and Gulf specimens, the apparent connectivity extended over the entire sampled range. Assuming dynamic equilibrium between migration and genetic drift, population genetic theory suggests that the exchange of a few migrant individuals each generation can prevent genetic differentiation (Slatkin 1985). The magnitude of θ estimates in this study indicate that the per-generation effective number of migrants (N_em) is, minimally, 550 individuals among samples, 320 among regions, and 150 between Atlantic and Gulf waters. Because in the relationship between θ and the pseudoparameter N_em is asymptotic (Waples 1998) and because the 'genetically effective' number of migrants is usually a small fraction of the actual

number of migrants (Hedgecock 1994), the actual numbers of dispersers each generation are potentially much higher, perhaps by orders of magnitude.

For reef fish in particular, the processes of adult movement and larval transport are important determinants of stock structure (Schulman and Bermingham 1995). Capture-recapture data for reef-fish species are fairly limited. If anything, there appears to be a general trend for site-fidelity among the adults recovered (Beaumariage 1969). However, reef fish, including vermilion snapper, typically spawn around offshore reefs and produce larvae in the open ocean. These larvae can be transported over hundreds of kilometers in the 20-50 days prior to settlement (Brothers et al. 1983, Keener et al. 1988, Jones 1991, Coleman et al. 1996). Therefore, depending on the episodic, extrinsic/physical properties affecting larval dispersal (e.g., Mahmoudi 1985), there may be broad inter-regional, inter-jurisdictional stock connectivity in reef fish or various degrees of recruitment subsidization among non-local sources. Ichthyofaunal collections indicate that vermilion snapper larvae occur in mid- to outer-shelf waters (Powles 1977). Thus, the capacity for long-distance transport would be seemingly high. The clear pattern of isolation by distance observed over all samples shows that comparatively more genetic flux (individual dispersal via adult movement or larval transport) occurs on the scale of hundreds rather than thousands of kilometers.

Overall, results of this study are consistent with the working hypothesis for management that there is a single stock of vermilion snapper in the Gulf of Mexico. Because the Gulf of Mexico Fishery Management Council currently considers vermilion snapper as a single assessment unit in the Gulf, no changes are recommended at this time.

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Table 1. Characterization of the 12 dinucleotide microsatellite DNA loci used to assess genetic structure in *Rhomboplites aurorubens*. N_a = the number of different alleles observed in this study; the numbers in parentheses refer to the observed number of alleles in 192 *Lutjanus campechanus* specimens [a]; nr = not reported.

Locus	Primer Sequence 5´ to 3´ (label)	Repeat Motif	N_a	Allele sizes
*Prs*260 [a,d]	F:GGTAAAATGCTCCCTTCCT(HEX)	$(TG)_4AGTGCA(TG)_2$	3 (5)	100-110
	R:GTGGTAGTGGGTGAAATTCT	$TA(TG)_6$		
*Prs*229 [a,d]	F:CACATTGAACCGTTTAACCC(FAM)	$(CA)_8$	8 (8)	119-135
	R:GAAATGATGACCCAGCACAG			
*Prs*291 [a,e]	F:TAAACCCAAGGAAACGCTCAT(HEX)	$(AT)_{12}$	12 (nr)	106-132
	R:GCCGAGGGGTGAGTGAGGA			
*Prs*305 [a,e]	F:CTGCAATTAAGCCAACTGTCAA(FAM)	$(CA)_{16}$	8 (nr)	125-163
	R:TGAGAGGACGCAACAATACAAC			
*Ra*3 [b,e]	F:CAAACTGCAGTGACCTACT(HEX)	$(CA)_2CG(CA)_{27}(TA)_2(CA)_6$	29	126-188
	R:ATCTGTGTTACCCGGAGT			
*Lca*43 [c,e]	F:ACTGAAATGCTGCTCTCCTT(TET)	$G(TG)_8GGGAC(GT)_5ATGG$ $TGTTTAA(GT)_2AGACGGTG$ $AG(TG)_3TT(TG)_4$	19 (8)	197-235
	R:CACTGTTTACTTCTTCTGTT			
*Lca*20 [c,d]	F:CAACCCTCTGGCTAGTGTCA(FAM)	$(CA)_9$	23 (5)	207-255
	R:ATCCTGAAGCCCTGGTTTAC			
*Lca*22 [c,d]	F:TCCACAGGCTTTCACTCTTTCAG(HEX)	$(CA)_{18}$	21 (14)	217-279
	R:TGCTCTTTTCTTTCCGTCATTCC			
*Ra*12 [b,f]	F:AGATGTCGTCCCACAAACGGA(TET)	$(CA)_{10}$	7	246-258
	R:GCATGAATCTGACAGCCTCCCA			
*Ra*7 [b,f]	F:GGAGGGGATGGCTGACTGAT(HEX)	$(CA)_9TACAA(CA)_3CG(CA)_2$ $A(CA)_7ACACG(CA)_2TACAA$ $(CA)_{10}$	15	172-200
	R:CATTGAATGGTGGCCAAGGA			
*Prs*240 [a,g]	F:CAAGAGGGTGATGAATGA(TET)	$(CA)_{21}$	20 (20)	195-237
	R:AATGAAATACCCACTGCT			
*Prs*328 [a,g]	F:AGGTCATTGTGGTGGGTGTAT(HEX)	$(TG)_9$	8 (5)	199-219
	R:TTACCGTCACTTCCAGAACAG			

[a] Source: Gold et al. (2001).
[b] Source: Bagley et al. (1999).
[c] Source: Heist and Gold (2000).
[d] Annealing temperature: 58°C.
[e] Annealing temperature: 56°C.
[f] Annealing temperature: 62°C.
[g] Annealing temperature: 52°C.

Table 2. Summary statistics for microsatellite variation in *Rhomboplites aurorubens*. For each locus, *n* = sample size. Allele designations (represented by fragment size) appear in italics and sample allele frequencies appear to the right of each allele designation. Standard measures of variability (described in text) appear below sample frequencies. Asterisks indicate table-wide statistical significance.

Locus/sample size/allele/ diversity measure	AT2	AT1	EG2	EG1	NG2	NG1	WG3	WG2	WG1
*Prs*260									
(n)	28	30	31	62	42	143	45	28	52
100	0	0	0	0	0	0	0.011	0	0
108	0.589	0.6	0.581	0.581	0.5	0.521	0.567	0.518	0.558
110	0.411	0.4	0.419	0.419	0.5	0.479	0.422	0.482	0.442
Gene diversity	0.493	0.487	0.496	0.491	0.506	0.501	0.507	0.508	0.497
Allelic richness	2	2	2	2	2	2	2.444	2	2
F_{IS}	0.059	-0.094	0.089	0.081	0.06	0.205	0.167	-0.055	-0.16
Obs. heterozygosity	13	16	14	28	20	57	19	15	30
Exp. heterozygosity	13.8	14.6	15.3	30.4	21.2	71.6	22.8	14.2	25.9
*Prs*229									
(n)	20	22	30	62	42	128	46	23	50
119	0.125	0.136	0.15	0.129	0.131	0.086	0.087	0.174	0.04
121	0.025	0.023	0.017	0.008	0.024	0.027	0.022	0.022	0.01
123	0	0	0.083	0.024	0.012	0.012	0.011	0.065	0.02
125	0.775	0.773	0.717	0.766	0.786	0.801	0.815	0.717	0.87
127	0.025	0.068	0.033	0.073	0.048	0.066	0.054	0.022	0.06
129	0	0	0	0	0	0.004	0	0	0
131	0.05	0	0	0	0	0.004	0	0	0
135	0	0	0	0	0	0	0.011	0	0
Gene diversity	0.391	0.391	0.464	0.394	0.367	0.348	0.33	0.463	0.241
Allelic richness	5	3.909	4.556	3.988	4.134	4.343	4.492	4.738	3.876
F_{IS}	0.104	0.302	0.066	0.223	0.092	0.326*	0.605*	0.343	0.335
Obs. heterozygosity	7	6	13	19	14	30	6	7	8
Exp. heterozygosity	7.8	8.5	13.9	24.4	15.4	44.5	15.1	10.6	12
*Prs*291									
(n)	34	52	48	69	43	188	45	40	50
106	0	0	0	0	0.023	0	0	0	0
108	0	0	0	0	0	0	0	0.013	0
110	0	0	0	0.014	0	0	0	0	0.01
114	0	0.01	0	0.007	0	0	0.022	0	0
116	0.059	0.029	0.01	0	0.023	0.019	0.011	0.038	0.02
118	0	0.01	0	0	0	0	0	0	0
120	0.721	0.817	0.875	0.783	0.744	0.801	0.867	0.713	0.8
122	0.044	0.038	0.052	0.072	0.058	0.08	0.033	0.088	0.02
124	0.118	0.067	0.042	0.065	0.116	0.066	0.044	0.125	0.09
126	0.044	0.019	0.021	0.051	0.035	0.032	0.022	0.025	0.05
128	0	0.01	0	0.007	0	0.003	0	0	0.01
132	0.015	0	0	0	0	0	0	0	0

Gene diversity	0.467	0.328	0.232	0.378	0.432	0.348	0.248	0.473	0.352
Allelic richness	5.432	5.381	3.906	4.922	5.245	4.317	4.576	5.127	5.005
F_{IS}	0.118	0.18	0.102	0.08	0.031	0.006	0.103	0.102	0.092
Obs. heterozygosity	14	17	10	24	18	65	10	17	16
Exp. heterozygosity	15.9	14	11.1	26	18.6	65.4	11.1	18.9	17.6
*Prs*305									
(n)	34	50	48	68	43	182	45	37	49
125	0	0	0.021	0.015	0	0.011	0.022	0	0
151	0.044	0.05	0.042	0.088	0.023	0.102	0.111	0.027	0.071
153	0.029	0.03	0.01	0	0	0.005	0	0	0
155	0	0	0	0.007	0	0.003	0	0	0
157	0.044	0.01	0.021	0.029	0.023	0.011	0.067	0.014	0.02
159	0.868	0.9	0.865	0.831	0.93	0.841	0.789	0.959	0.888
161	0.015	0.01	0.031	0.029	0.023	0.027	0.011	0	0.02
163	0	0	0.01	0	0	0	0	0	0
Gene diversity	0.246	0.188	0.252	0.302	0.135	0.283	0.364	0.08	0.208
Allelic richness	4.292	3.516	4.853	4.298	3.15	3.747	4.111	2.333	3.283
F_{IS}	-0.078	-0.063	0.173	-0.023	-0.037	0.009	0.024	0.324	-0.08
Obs. heterozygosity	9	10	10	21	6	51	16	2	11
Exp. heterozygosity	8.4	9.4	12.1	20.5	5.8	51.5	16.4	2.9	10.2
*Ra*3									
(n)	34	50	46	67	43	161	43	33	49
126	0.015	0	0.011	0	0	0	0	0	0
130	0	0	0	0	0	0	0.012	0	0
134	0.015	0	0	0.007	0	0.003	0	0.015	0
136	0	0.01	0	0	0	0	0	0	0
138	0.132	0.1	0.087	0.052	0.128	0.056	0.035	0.136	0.041
140	0.029	0.02	0.043	0.067	0.047	0.056	0.047	0.061	0.031
142	0.029	0.04	0.011	0.045	0.047	0.034	0.07	0.03	0.051
144	0.015	0.01	0.054	0.007	0.012	0.028	0.058	0.061	0.041
146	0	0	0	0.015	0.012	0.012	0	0	0.01
148	0	0.01	0	0	0	0.012	0	0	0.01
150	0.015	0.02	0.054	0.007	0.023	0.025	0.012	0.03	0.051
152	0.088	0.05	0.065	0.075	0.047	0.068	0.047	0	0.071
154	0.044	0.06	0.011	0.037	0.035	0.034	0.058	0.045	0.031
156	0.118	0.13	0.043	0.134	0.128	0.115	0.093	0.121	0.082
158	0.103	0.11	0.13	0.104	0.128	0.081	0.105	0.061	0.153
160	0.103	0.13	0.087	0.082	0.128	0.112	0.081	0.076	0.061
162	0.088	0.09	0.065	0.052	0.093	0.112	0.081	0.136	0.092
164	0.044	0.1	0.13	0.097	0.07	0.056	0.081	0.045	0.041
166	0.088	0.04	0.087	0.075	0.058	0.065	0.058	0.061	0.082
168	0.044	0.05	0.054	0.052	0.023	0.043	0.081	0.03	0.02
170	0.015	0	0.033	0.037	0.023	0.031	0.047	0.061	0.082
172	0.015	0.02	0.011	0.015	0	0.037	0	0.015	0.01
174	0	0	0.022	0	0	0.012	0.012	0.015	0.031
176	0	0	0	0.015	0	0.003	0	0	0.01
178	0	0.01	0	0	0	0.003	0	0	0
180	0	0	0	0.007	0	0	0	0	0
182	0	0	0	0.007	0	0	0.012	0	0
186	0	0	0	0.007	0	0	0	0	0
188	0	0	0	0	0	0	0.012	0	0
Gene diversity	0.93	0.922	0.931	0.932	0.919	0.931	0.941	0.93	0.935
Allelic richness	14.988	14.071	14.812	15.527	13.635	15.505	15.762	15.138	16.085

F_{IS}	0.115	-0.02	0.066	0.007	0.039	0.02	-0.013	-0.01	-0.004
Obs. heterozygosity	28	47	40	62	38	147	41	31	46
Exp. heterozygosity	31.6	46.1	42.8	62.5	39.5	150	40.5	30.7	45.8
*Lca*43									
(n)	22	39	45	66	43	139	41	34	24
197	0	0.026	0	0	0	0.007	0.012	0	0
201	0.045	0.077	0.078	0.053	0.023	0.032	0.024	0.074	0.083
203	0.023	0.026	0.011	0.015	0	0.025	0.012	0.059	0
205	0	0	0	0.015	0.023	0.014	0	0	0
207	0.023	0.051	0.022	0.015	0.035	0.011	0.012	0.015	0.021
209	0.455	0.423	0.356	0.379	0.372	0.356	0.366	0.412	0.333
211	0.023	0.038	0.011	0.053	0.012	0.072	0.037	0.015	0.125
213	0.159	0.141	0.156	0.144	0.186	0.205	0.22	0.162	0.25
215	0.182	0.077	0.167	0.106	0.128	0.108	0.159	0.074	0.042
217	0.023	0.051	0.089	0.03	0.058	0.047	0.098	0.044	0.021
219	0	0.013	0.044	0.045	0.023	0.025	0.024	0.044	0.063
221	0.023	0.051	0.033	0.045	0.058	0.018	0	0.044	0
223	0	0	0	0	0.012	0.018	0.012	0	0.021
225	0.023	0	0.011	0.008	0.035	0.007	0	0.029	0
227	0.023	0	0.011	0.076	0.023	0.029	0.024	0.015	0
229	0	0.026	0.011	0.008	0	0.018	0	0.015	0.021
231	0	0	0	0	0.012	0.004	0	0	0.021
233	0	0	0	0.008	0	0	0	0	0
235	0	0	0	0	0	0.004	0	0	0
Gene diversity	0.749	0.789	0.813	0.813	0.808	0.81	0.789	0.795	0.812
Allelic richness	10.357	10.523	9.639	10.831	10.887	10.926	9.04	10.947	10.138
F_{IS}	0.15	0.09	0.016	-0.081	-0.007	-0.021	0.011	0.113	-0.078
Obs. heterozygosity	14	28	36	58	35	115	32	24	21
Exp. heterozygosity	16.4	30.7	36.6	53.7	34.8	112.7	32.4	27	19.5
*Lca*20									
(n)	35	48	48	69	42	184	47	28	53
207	0.057	0.031	0.021	0.014	0	0.027	0.032	0	0.028
209	0.057	0.01	0.042	0.109	0.06	0.049	0.043	0.071	0.028
211	0.014	0.052	0.063	0.043	0.083	0.073	0.074	0.054	0.038
213	0.229	0.135	0.094	0.188	0.143	0.166	0.202	0.196	0.142
215	0.029	0.042	0.104	0.065	0.012	0.033	0.043	0	0.028
217	0.014	0.01	0.01	0.036	0.036	0.014	0	0	0.028
219	0.014	0.021	0.021	0.022	0	0.014	0.021	0.018	0.028
221	0.257	0.313	0.302	0.21	0.333	0.332	0.33	0.393	0.321
223	0.057	0.073	0.063	0.029	0.036	0.057	0.032	0.089	0.075
225	0.143	0.125	0.115	0.152	0.143	0.092	0.128	0.071	0.132
227	0.029	0.042	0.031	0.022	0.012	0.024	0	0	0.038
229	0.029	0.063	0	0.007	0	0.022	0.011	0.071	0.066
231	0.014	0.042	0.01	0.014	0.024	0.033	0	0	0.009
233	0.029	0.021	0.052	0.014	0.024	0.014	0.054	0	0
235	0	0.01	0.01	0.029	0.048	0.008	0.011	0	0.019
237	0.014	0	0.031	0	0	0.011	0.021	0.018	0
239	0.014	0	0.01	0.014	0.024	0.011	0	0.018	0.009
241	0	0.01	0.01	0.029	0	0.011	0	0	0
243	0	0	0	0	0.012	0.003	0	0	0
245	0	0	0	0	0	0.005	0	0	0.009
247	0	0	0	0	0	0.003	0	0	0
251	0	0	0	0	0.012	0	0	0	0
255	0	0	0.01	0	0	0	0	0	0

Gene diversity	0.859	0.859	0.87	0.88	0.841	0.84	0.829	0.796	0.85
Allelic richness	12.618	12.347	13.183	12.476	11.698	12.406	10.923	9.107	12.218
F_{IS}	-0.031	0.078	0.042	-0.005	0.037	-0.028	-0.001	0.102	-0.021
Obs. heterozygosity	31	38	40	61	34	159	39	20	46
Exp. heterozygosity	30.1	41.2	41.7	60.7	35.3	154.7	38.9	22.2	45.1
*Lca*22									
(n)	30	30	36	65	41	129	44	22	52
217	0	0	0	0	0	0.004	0	0	0
231	0	0	0	0	0	0	0.011	0	0
235	0	0	0	0	0	0	0.011	0	0
241	0.033	0	0	0.008	0	0	0	0	0
245	0	0.033	0.014	0.062	0	0.012	0.023	0	0.029
247	0.183	0.217	0.208	0.192	0.232	0.124	0.17	0.091	0.144
249	0.167	0.05	0.097	0.131	0.183	0.147	0.114	0.114	0.173
251	0.2	0.3	0.264	0.2	0.159	0.233	0.25	0.295	0.183
253	0.067	0.05	0.111	0.077	0.098	0.147	0.114	0.068	0.077
255	0.017	0.017	0.056	0.062	0.037	0.07	0.034	0.114	0.048
257	0.033	0.117	0.056	0.077	0.061	0.058	0.045	0.068	0.106
259	0.017	0.05	0.069	0.031	0.061	0.047	0.091	0	0.058
261	0.05	0.067	0.042	0.077	0.098	0.062	0.08	0.159	0.096
263	0	0.033	0.028	0.008	0.012	0.035	0.011	0.045	0.019
265	0.067	0	0	0	0.012	0.008	0	0.023	0.01
267	0.05	0.017	0	0.015	0.012	0.016	0.011	0.023	0.01
269	0.05	0	0.042	0.015	0.012	0.008	0.011	0	0.038
271	0.05	0.017	0.014	0.023	0.024	0.019	0	0	0.01
273	0.017	0.033	0	0.008	0	0.008	0.011	0	0
275	0	0	0	0.008	0	0.004	0.011	0	0
279	0	0	0	0.008	0	0	0	0	0
Gene diversity	0.893	0.85	0.862	0.886	0.869	0.874	0.873	0.859	0.889
Allelic richness	12.632	11.568	10.663	11.869	10.493	11.109	11.626	9.811	11.251
F_{IS}	0.066	0.176	0.066	0.062	-0.01	0.007	-0.068	-0.058	-0.038
Obs. heterozygosity	25	21	29	54	36	112	41	20	48
Exp. heterozygosity	26.7	25.4	31	57.5	35.6	112.8	38.4	18.9	46.3
*Ra*12									
(n)	36	47	44	68	43	186	47	29	52
246	0.056	0.106	0.057	0.044	0.035	0.048	0.021	0.017	0.087
248	0	0	0	0.007	0	0.003	0	0	0
250	0.153	0.074	0.17	0.169	0.14	0.164	0.17	0.155	0.125
252	0.431	0.287	0.42	0.412	0.407	0.39	0.394	0.379	0.452
254	0.292	0.457	0.295	0.287	0.302	0.304	0.34	0.379	0.288
256	0.069	0.074	0.057	0.081	0.105	0.089	0.074	0.069	0.048
258	0	0	0	0	0.012	0.003	0	0	0
Gene diversity	0.708	0.692	0.709	0.718	0.72	0.725	0.701	0.693	0.693
Allelic richness	4.951	4.963	4.913	5.444	5.314	5.288	4.655	4.682	4.906
F_{IS}	0.02	-0.106	0.103	0.099	-0.002	0.043	-0.031	-0.195	-0.11
Obs. heterozygosity	25	36	28	44	31	129	34	24	40
Exp. heterozygosity	25.5	32.6	31.2	48.8	31	134.8	33	20.2	36.1
*Ra*7									
(n)	33	48	45	67	43	165	48	29	52
172	0	0	0	0	0	0	0	0	0.01

174	0	0.021	0	0	0	0	0	0	0
176	0	0	0	0	0	0.009	0	0	0
178	0	0	0	0	0	0	0.01	0	0
180	0	0	0	0	0.012	0	0	0	0.01
182	0.773	0.75	0.756	0.828	0.802	0.785	0.75	0.879	0.722
184	0	0.01	0.022	0.022	0.012	0.018	0.021	0.017	0.019
186	0.015	0.031	0	0.007	0	0.012	0	0	0.01
188	0.015	0	0	0	0	0	0	0	0
190	0.045	0.042	0.044	0.03	0.012	0.03	0.042	0.017	0
192	0	0.052	0.011	0.022	0.012	0.024	0.042	0	0.029
194	0.106	0.073	0.1	0.06	0.081	0.058	0.042	0.052	0.087
196	0.045	0.021	0.056	0.022	0.058	0.048	0.083	0.017	0.077
198	0	0	0.011	0.007	0.012	0.006	0.01	0.017	0.019
200	0	0	0	0	0	0.009	0	0	0.01
Gene diversity	0.393	0.43	0.418	0.31	0.35	0.377	0.429	0.227	0.469
Allelic richness	5.098	6.355	5.441	5.28	5.276	5.999	6.153	4.732	6.914
F_{IS}	-0.002	0.032	-0.01	-0.011	0.137	0.004	-0.118	-0.065	-0.025
Obs. heterozygosity	13	20	19	21	13	62	23	7	25
Exp. heterozygosity	13	20.7	18.8	20.8	15	62.3	20.6	6.6	24.4
*Prs*240									
(n)	35	51	48	68	42	193	49	31	53
195	0	0	0	0	0.024	0	0	0	0
197	0	0	0	0	0	0	0.02	0	0
199	0.029	0	0	0	0	0	0	0	0
201	0	0	0	0	0	0.003	0.01	0	0
203	0	0	0	0	0	0	0	0	0.009
205	0.014	0.02	0.01	0.022	0	0.013	0.01	0	0.028
207	0.014	0.049	0.021	0.015	0.024	0.01	0.031	0.016	0
209	0	0.02	0.021	0.015	0.012	0.013	0.01	0	0.009
211	0	0.039	0.031	0.022	0.036	0.018	0.041	0.032	0.047
213	0.043	0.039	0.094	0.103	0.024	0.101	0.102	0.097	0.094
215	0.086	0.118	0.115	0.14	0.167	0.106	0.102	0.194	0.142
217	0.1	0.167	0.219	0.213	0.25	0.254	0.224	0.258	0.236
219	0.2	0.118	0.146	0.088	0.119	0.122	0.102	0.113	0.142
221	0.129	0.088	0.125	0.066	0.083	0.104	0.061	0.065	0.066
223	0.214	0.196	0.135	0.14	0.119	0.122	0.194	0.129	0.094
225	0.071	0.01	0.01	0.059	0.012	0.041	0.031	0.048	0.028
227	0.086	0.118	0.042	0.074	0.083	0.06	0.031	0.032	0.075
229	0	0.02	0.031	0.029	0.036	0.021	0.031	0	0.009
231	0.014	0	0	0.015	0.012	0.013	0	0	0.019
237	0	0	0	0	0	0	0	0.016	0
Gene diversity	0.878	0.887	0.88	0.888	0.873	0.869	0.879	0.862	0.88
Allelic richness	10.437	10.937	10.65	11.428	11.318	10.573	11.898	9.991	11.119
F_{IS}	0.089	0.049	0.029	-0.01	0.019	0.028	-0.068	0.027	-0.007
Obs. heterozygosity	28	43	41	61	36	163	46	26	47
Exp. heterozygosity	30.7	45.2	42.2	60.4	36.7	167.7	43.1	26.7	46.7
*Prs*328									
(n)	36	54	48	69	43	193	50	31	53
199	0.014	0	0	0	0	0	0	0	0
203	0.014	0.083	0.01	0.029	0.035	0.049	0.05	0.048	0.066
205	0.556	0.528	0.656	0.594	0.523	0.565	0.67	0.597	0.613
207	0	0	0	0.022	0	0.005	0.03	0	0
209	0.389	0.38	0.323	0.348	0.442	0.378	0.22	0.323	0.311

211	0.014	0.009	0	0	0	0	0	0	0
213	0.014	0	0.01	0	0	0.003	0.03	0.032	0.009
219	0	0	0	0.007	0	0	0	0	0
Gene diversity	0.545	0.575	0.468	0.528	0.535	0.537	0.504	0.544	0.527
Allelic richness	4.222	3.358	2.833	3.685	2.852	3.182	4.504	3.837	3.345
F_{IS}	-0.223	-0.16	-0.335	-0.015	-0.174	-0.139	0.047	-0.187	-0.11
Obs. heterozygosity	24	36	30	37	27	118	24	20	31
Exp. heterozygosity	19.7	31.1	22.5	36.5	23	103.6	25.2	16.9	28

Table 3. Results of exact tests for genotypic (linkage) Disequilibrium over all samples of *Rhomboplites aurorubens*. No test value was significant following sequential Bonferroni correction (α= 0.0009).

Locus pair			$\chi 2$	*df*	*p*-value
Prs260	and	Prs291	24.279	20	0.230
Prs260	and	Prs305	8.592	20	0.987
Prs291	and	Prs305	12.336	20	0.904
Prs260	and	Ra3	8.548	18	0.969
Prs291	and	Ra3	17.481	18	0.490
Prs305	and	Ra3	15.000	18	0.662
Prs260	and	Lca43	15.484	20	0.748
Prs291	and	Lca43	21.024	20	0.396
Prs305	and	Lca43	19.135	20	0.513
Ra3	and	Lca43	18.929	18	0.396
Prs260	and	Lca20	25.088	20	0.198
Prs291	and	Lca20	17.448	20	0.624
Prs305	and	Lca20	11.865	20	0.921
Ra3	and	Lca20	10.768	18	0.904
Lca43	and	Lca20	16.211	20	0.703
Prs260	and	Lca22	15.913	20	0.722
Prs291	and	Lca22	29.389	20	0.080
Prs305	and	Lca22	12.636	20	0.892
Ra3	and	Lca22	10.912	18	0.898
Lca43	and	Lca22	21.141	20	0.389
Lca20	and	Lca22	11.564	20	0.930
Prs260	and	Ra12	15.919	20	0.722
Prs291	and	Ra12	21.853	20	0.349
Prs305	and	Ra12	14.746	18	0.679
Ra3	and	Ra12	10.700	18	0.907
Lca43	and	Ra12	16.362	20	0.694
Lca20	and	Ra12	14.605	20	0.799
Lca22	and	Ra12	18.881	20	0.530
Prs260	and	Ra7	21.569	20	0.364
Prs291	and	Ra7	19.880	20	0.465
Prs305	and	Ra7	15.242	20	0.762
Ra3	and	Ra7	16.845	18	0.534
Lca43	and	Ra7	14.926	20	0.781
Lca20	and	Ra7	28.044	20	0.108
Lc22	and	Ra7	16.404	20	0.691
Ra12	and	Ra7	16.149	20	0.707
Prs260	and	Prs240	16.392	20	0.692
Prs291	and	Prs240	14.317	20	0.814
Prs305	and	Prs240	17.765	20	0.603
Ra3	and	Prs240	8.991	18	0.960
Lca43	and	Prs240	16.545	20	0.682
Lca20	and	Prs240	6.577	20	0.998
Lca22	and	Prs240	16.299	20	0.698
Ra12	and	Prs240	8.312	20	0.990
Ra7	and	Prs240	17.007	20	0.653

Prs260	and	Prs328	14.666	20	0.795
Prs291	and	Prs328	30.257	20	0.066
Prs305	and	Prs328	23.097	20	0.284
Ra3	and	Prs328	12.200	18	0.837
Lca43	and	Prs328	10.397	20	0.960
Lca20	and	Prs328	14.007	20	0.830
Lca22	and	Prs328	16.699	20	0.672
RA12	and	Prs328	35.174	20	0.019
Ra7	and	Prs328	14.626	20	0.797
Prs240	and	Prs328	12.906	20	0.881

Table 4. Genic differentiation over all loci among regional groups of *Rhomboplites aurorubens*. Asterisks denote table-wide significance following sequential Bonferroni adjustment where α = 0.008.

Population pair	$\chi 2$	*df*	Probability
Atlantic and Northern Gulf	49.952	24	0.00144*
Atlantic and Eastern Gulf	44.113	24	0.00740*
Atlantic and Western Gulf	48.833	24	0.00199*
Northern Gulf and Eastern Gulf	32.461	24	0.11597
Northern Gulf and Western Gulf	28.733	24	0.23033
Eastern Gulf and Western Gulf	27.671	24	0.27418

Table 5. Hierarchical Analysis of Molecular Variance (AMOVA) in *Rhomboplites aurorubens*.

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation
Among regional groups	3	2.062	-0.00012 Va	-0.02
Among samples within regional groups	5	2.691	0.00034 Vb	0.07
Within samples	1227	617.714	0.49977 Vc	99.95
Total	1235	622.467	0.50000	
Among Atlantic and Gulf groups	1	0.511	-0.00007 Va	-0.01
Among regional groups within Atlantic and Gulf groups	2	3.742	0.00027 Vb	0.05
Within regional groups	1232	613.214	0.49977 Vc	99.96
Total	1235	617.467	0.50000	

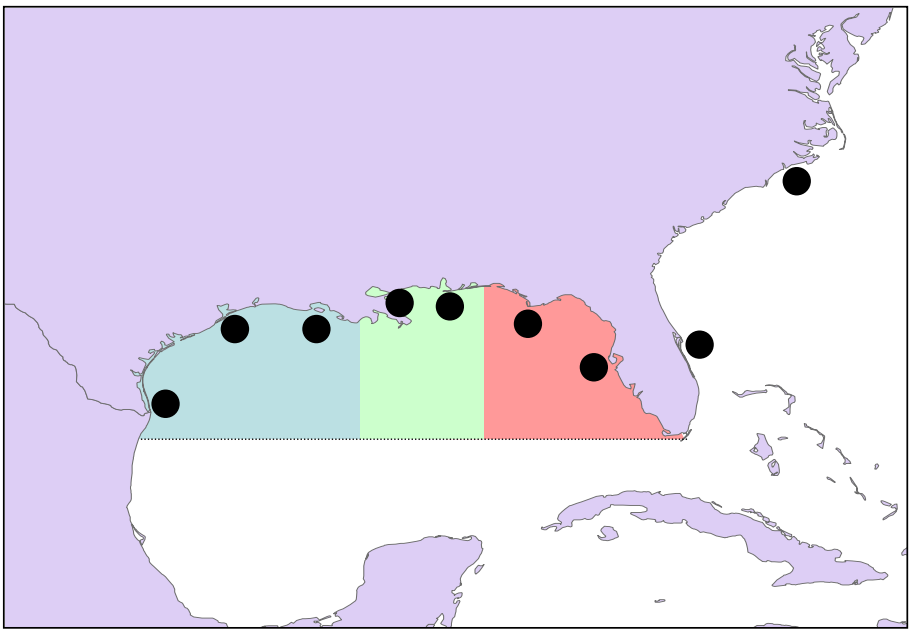

Figure 1. Map of collection locations for *Rhomboplites aurorubens*. For each sampling point identified (A-I), specimens were obtained from proximal fishing grounds. Designations and sample size of each point from west to east are as follows: WG1 (*n*=53), WG2 (*n*=43), WG3 (*n*=52), NG1 (*n*=205), NG2 (*n*=45), EG1 (*n*=70), EG2 (*n*=52), AT1 (*n*=60), and AT2 (*n*=38). *Note*: specimens attributed to sampling point AT1 were collected from various locations along the central-eastern and northeastern waters of the Florida Atlantic. The initial regional grouping for data analysis, as indicated by shading, was: Western Gulf (WG1, WG2, and WG3), Northern Gulf (NG1 and NG2), Eastern Gulf (EG1 and EG2), and Atlantic (AT1 and AT2).

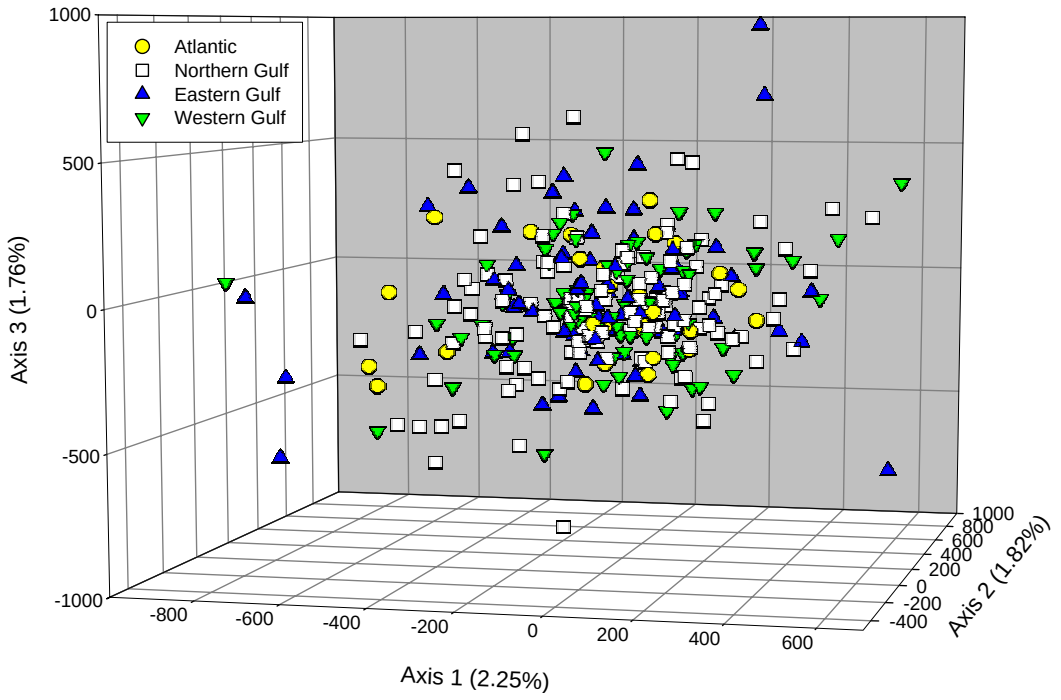

Figure 2. Diagram of the factoral correspondence analysis showing vermilion snapper individuals in multidimensional genotype space. Different symbols correspond to regional group membership as indicated in the legend.

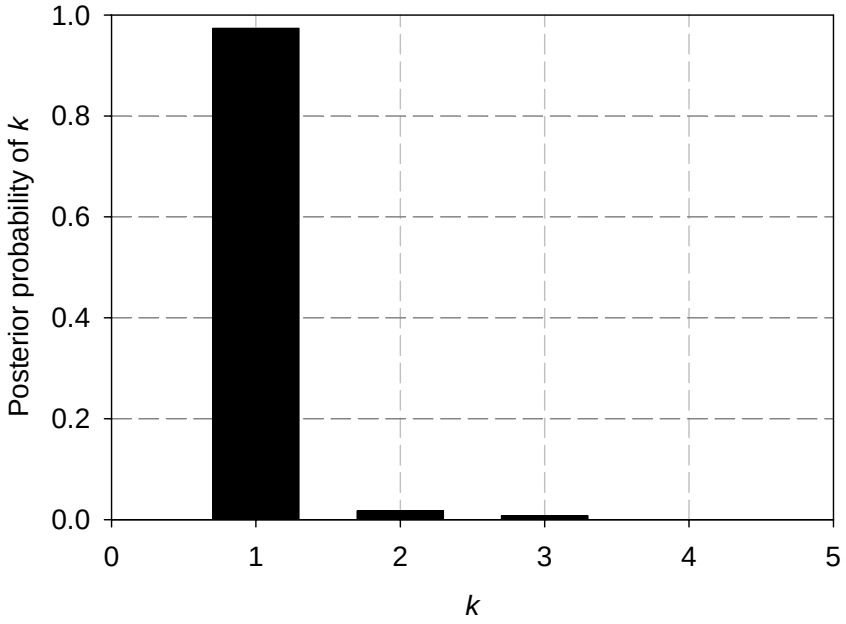

Figure 3. Plot of the posterior distribution of the estimated number of source populations (*k*) for *Rhomboplites aurorubens*. A burn-in value of 1000 was used for estimation of the Bayesian parameters.

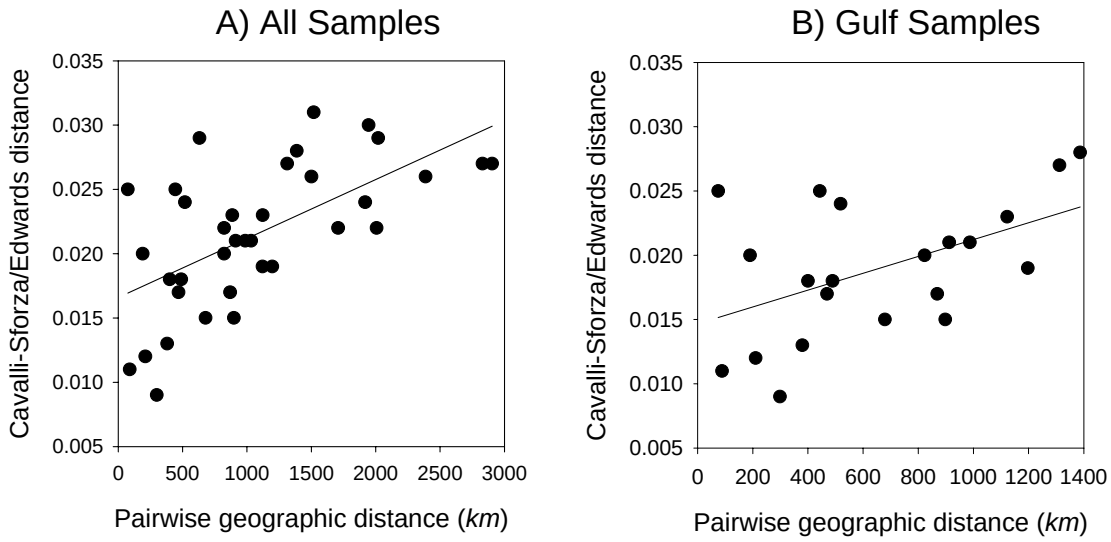

Figure 4. Isolation-by-distance plots for *Rhomboplites aurorubens*. Each point is based on genetic and geographic distance values for sample pairs. The plotted line depicts the regression line for the data. A) All samples, Atlantic and Gulf, included in the plot. B) Only Gulf samples included.