# Genetic Studies of Red Snapper (*Lutjanus campechanus*) in the Northern Gulf of Mexico

ERIC SAILLANT and JOHN R. GOLD
*Center for Biosystematics and Biodiversity*
*Texas A&M University, TAMU-2258*
*College Station, Texas 77843-2258 USA*

ABSTRACT

The Gulf red snapper, *Lutjanus campechanus*, is a highly exploited marine fish in the Gulf of Mexico. Currently, management of Gulf red snapper in U.S. waters is based on a single-stock hypothesis. Herein we report results of a genetic survey of adults (1995 year class) and juveniles (1999 year class) sampled offshore from three geographic localities in the northern Gulf: Port Aransas, Texas; Port Fourchon, Louisiana; and Dauphin Island, Alabama. The study employed 19 microsatellites loci and was designed to test the single-stock hypothesis and estimate effective population size in the Gulf. We also assessed a possible genetic impact of shrimp-trawling on red snapper populations by comparing genetic variability and relatedness between juveniles sampled randomly and juveniles sampled in single tows of a shrimp trawler.

Significant genetic heterogeneity was observed among localities in the 1995 cohort, with the major differences occurring between red snapper sampled offshore of Port Aransas, Texas, and the other two localities. On another hand, no significant genetic heterogeneity was observed among samples from the 1999 cohort. These findings, along with the overall very low $F_{ST}$ value (0.001), suggest considerable genetic mixing within the northern Gulf, consistent with the single-stock hypothesis. A maximum-likelihood estimate of effective population size ($N_e$) of red snapper in the northern Gulf based on the temporal method was 7,075 (95 % CI: 2,933 - >50,000). This estimate of $N_e$ is at least two orders of magnitude lower than current estimates of census size (N).

Levels of genetic variability (gene diversity and allelic richness) between reference (control) and bycatch juveniles were equivalent. Estimates of the variance in genetic relatedness in both reference and bycatch samples were non-significant; however, this variance estimate was positive (0.001) for one of the bycatch samples and close to significance ($P = 0.1$) which might suggest the presence of related individuals in this sample. Further studies employing larger sample size and additional loci are necessary to assess possible genetic impacts of shrimp trawling on red snapper.

KEY WORDS: *Lutjanus campechanus*, genetic stock assessment, shrimp trawl bycatch.

# Estudios Genéticos de la Estructura de la Poblaćion del Pargo Rojo (*Lutjanus campechanus*) en la Zona Norte del Golfo de México

*Lutjanus campechanus* es una especie marina importante para la pesca en el golfo de Mexico. El presente manejo se basa en la hipótesis que hay un solo stock, o poblacion (single-stock). Reportamos aqui resultados de un inventario genético de adultos (cohorte 1995) y juveniles (cohorte 1999) muestreado en tres localidades en la región norte del golfo de Mexico: Port Aransas Texas, Port Fourchon Louisiana, y Duaphin Island, Alabama. Diseñamos el estudio para testear el hipótesis single-stock y estimamos el tamaño de la población a través de análises de 19 loci genéticos de microsatelites-ADN. Adicionalmente evaluamos un posible impacto genético de pesca a la rastra para camarones en la población de *L. campechanus* comparando la variabilidad genética y la relación genética entre juveniles muestreados al azar con variabilidad en juveniles muestreado en un rastreo de un barco camaronero. Observamos heterogeniedad significativa entre localidades para el cohorte 1995, con las diferencias mayores encontrados entre *L. campechanus* muestreados fuera de la costa de Port Aransas, Texas y las otras localidades. En la otra mano, no hubo heterogeniedad significativa entre muestras del cohorte 1999. Estos resultados, tomando en cuenta el FST muy bajo (0.001), indican considerable mezcla genética, consistente con el hipótesis single-stock. Una estimación de tamaño poblacional efectiva (Ne) de la especie en el golfo norte basado en el metodo temporal fue 7,075 (95 % CI: 2,933 - >50,000). Este Ne es por lo menos dos ordenes de magnitud más bajo que estimaciones actuales del tamaño de censo (N). Niveles de variabilidad genética entre juveniles referencia y juveniles capturado durante el rastreo fueron equivalentes. Estimaciones de la varianza en relación genética en juveniles referencia y juveniles capturado durante el rastreo no fueron significativamente diferente. Pero, la estimación fue positiva (0.001) y casi significativa (P = 0.10) para uno de la muestras de rastreo, un resultado que sugiere la presencia de individuos relacionados en este muestreo. Futuros estudios empleando mayores tamaños de muestreo y más loci son necesarios para evaluar los posibles impactos genéticos de pesca a la rastra para camarones en *L. campechanus*.

PALABRAS CLAVES: Pargo rojo, *Lutjanus campechanus*, genéticos

## INTRODUCTION

The Gulf red snapper, *Lutjanus campechanus*, is a highly exploited marine fish distributed along the continental shelf of the Gulf of Mexico (Hoese and Moore 1977). Red snapper abundance in the northern Gulf of Mexico (hereafter, Gulf) has decreased by almost 90 % in the past two decades (Goodyear and Phares 1990) due to overexploitation by commercial and recreational fisheries, high juvenile mortality due to the shrimp trawl fishery, and habitat change (Christman 1997, Gallaway et al.

1999). Important goals for management of red snapper resources include knowledge of stock structure for stock assessment and limitation of impacts of shrimp trawling activities.

Currently, red snapper resources in U.S. waters are managed under a single-stock hypothesis (GMFMC 1989, 1991). This hypothesis is supported by a number of prior genetic studies that employed allozymes (Johnson 1987), mitochondrial (mt)DNA (Gold et al. 1997), and microsatellites (Gold et al. 2001). However, all these studies either involved individuals of mixed cohorts (year classes) or were based on relatively small sample sizes. In this study, we document variation at 19 nuclear-encoded microsatellites within two cohorts sampled at three different geographic areas along the northern Gulf. Both adults (1995 cohort) and juveniles (1999 cohort) were assayed. We also assessed variation in allele frequencies between the two cohorts in order to estimate effective population size or $N_e$ by the temporal method (Waples 1989).

A second issue involving red snapper in the Gulf regards impacts of shrimp trawling on red snapper juveniles. To date, studies on this topic have documented the volume of the bycatch (Gallaway et al. 1998, Gallaway and Cole 1999). In this study, we address the question of whether juvenile red snappers taken as bycatch represent a random sample of the genetic pool of the local population from which they were drawn. Briefly, non-random mortality of genotypes could have the effect of canceling the contribution of the corresponding families, thus reducing the effective number of breeding adults. This in turn could lower the (genetic) effective size of red snapper stock(s). To address this question, we documented variation at 11 nuclear-encoded microsatellites and at mitochondrial (mt)DNA among three samples of red snapper juveniles taken offshore of Galveston, Texas. Two of the samples were obtained from single tows of a shrimp trawler, and one (the reference or 'control' sample) was obtained by sampling randomly in the same geographic area during a groundfish survey.

## MATERIALS AND METHODS

Adult red snapper belonging to the 1995 cohort were sampled in 1999 and 2000 by angling 25-30 miles offshore of Port Aransas (Texas), Port Fourchon (Louisiana), and Dauphin Island (Alabama) (Figure 1). Individual fish were aged by otolith-increment analysis following Wilson and Nieland (2001). Sample sizes for this cohort were 203 (Texas), 286 (Louisiana) and 376 (Alabama). Juveniles (age 0 fish) belonging to the 1999 cohort were sampled by trawling during the summer of 1999 in conjunction with a groundfish survey of the National Marine Fisheries Service (NMFS). Fish were sampled a few at a time (average of 11 fish per trawl) during multiple trawls that differed both spatially and temporally. Sampling localities were offshore of Brownsville, Texas (n = 50), Port Aransas, Texas (n = 47), Galveston, Texas (n = 76), Port Fourchon, Louisiana (n = 77), and Dauphin Island, Alabama (n = 63). Samples from Brownsville and Port Aransas were pooled and are referred hereafter as the Texas sample (n = 97). The sample offshore of Galveston (n = 76)

was used as the '*Reference*' in the analysis of possible genetic impacts of shrimp trawling. For the latter, samples of juvenile red snapper were obtained from two separate shrimp-trawl tows offshore of Galveston, Texas: one (*Bycatch A*) contained 123 juveniles, while the other (*Bycatch B*) contained 40 juveniles.

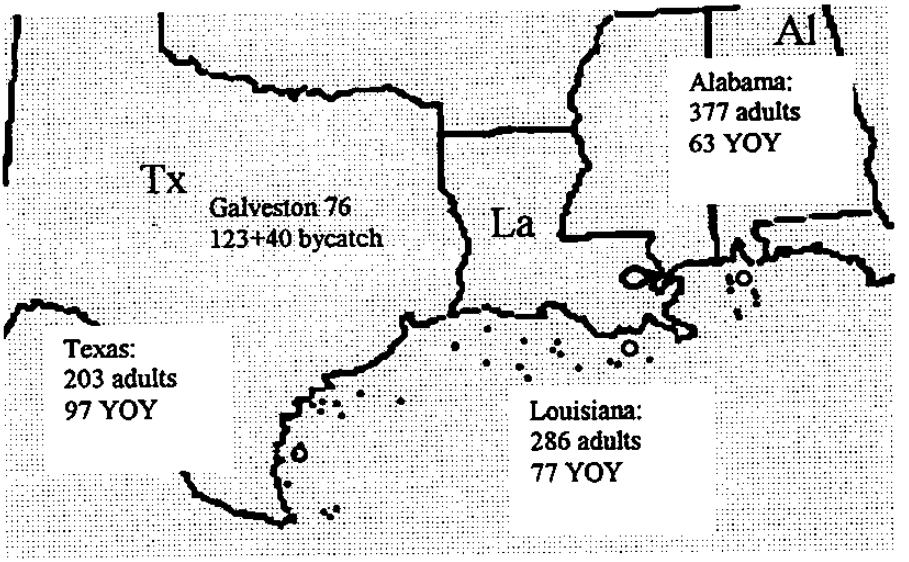


**Figure 1.** Sampling localities for the analysis of stock structure of red snapper and the effect of bycatch on juveniles. ○Adult sampling point, ● juvenile sampling point.

Tissue samples (heart and muscle) were removed from individual fish and stored as described in Gold et al. (2001). For stock structure analysis and estimates of effective population size, adults and juveniles of the 1995 and 1999 cohorts, respectively, were assayed for genotypes at 19 microsatellites, using PCR primers and methods described in Gold et al. (2001). For analysis of possible genetic impacts of shrimp trawling, samples obtained from shrimp trawls and the samples of juveniles obtained offshore of Galveston were assayed for genotypes at 11 microsatellites and for sequence variation in two fragments of mitochondrial (mt)DNA. The mtDNA assay employed single strand conformational polymorphism (SSCP) analysis of a 163 base pair (bp) fragment of the ND-4 gene and a 122 bp fragment of the ND-6 gene. Amplification, electrophoresis, and scoring of SSCP phenotypes followed methods developed in our laboratory (Burridge and Gold unpublished).

Summary statistics, including number of alleles, allelic richness (a measure of number of alleles independent of sample size), and unbiased gene (microsatellites) and nucleon (mtDNA) diversity were computed for each locus in each sample, using F-STAT, version 2.9.3 (Goudet 1995). Homogeneity of allelic richness and gene

diversity between pairs of samples was tested via Wilcoxon signed-rank tests. Departure of genotypic proportions from Hardy-Weinberg equilibrium expectations was measured within samples as Weir and Cockerham's (1984) small *f*; probability of significance ($P_{HW}$) was assessed using a Markov-chain method (Guo and Thompson 1992), as implemented in GENEPOP (Raymond and Rousset 1995) and using 5,000 dememorizations, 500 batches, and 5,000 iterations per batch. Genotypic disequilibrium between pairs of microsatellites within samples was tested by exact tests, as implemented in GENEPOP and employing the same Markov chain parameters. Homogeneity of allele and genotype distributions both among regions and between cohorts within regions (stock structure analysis), and among bycatch and reference samples (bycatch impact analysis), was examined via exact tests; significance of probability values was assessed by a Markov-chain method, as implemented in GENEPOP (Raymond and Rousset 1995) and using the same Markov-chain parameters as above. Homogeneity of mtDNA haplotype frequencies among the bycatch and reference samples was tested using the Monte Carlo simulation approach of Roff and Bentzen (1989), as implemented in REAP (Mc Elroy et al. 1992); significance of probability values was assessed through 1000 bootstrap replicates.

Pseudo-maximum-likelihood estimates of effective population size ($N_e$), based on the temporal method (Waples 1989), were generated following Wang (2001); 95 % confidence intervals were obtained as the range of support associated with a drop of two logarithm units of the likelihood function as inferred from the likelihood distribution (Wang 2001). Estimates of $N_e$ were generated for individual geographic samples and for the northern Gulf; the latter was generated by pooling data by year class (cohort) across sample localities. Life-history data (age structure and weights of males and females belonging to the individual age class) were provided by D. Nieland and C. Wilson of Louisiana State University and used to compute correction factors for temporal-method estimates of $N_e$ (following Jorde and Ryman 1995; 1996) in order to account for effects of overlapping generations. The correction coefficients C and G were estimated as described in Turner et al. (2002) except for the following modifications: Survivorship (S) was estimated to be 0.5 based on the age-structure data examined. The relative birth rate of each age class was estimated by the mean weights of males and females of the corresponding class. Values of C and G obtained were subsequently used to correct $N_e$ by:

$$N_{ec} = N_e \times [C/G]$$

where $N_e$ is the pseudo-maximum-likelihood estimate of variance effective size obtained following Wang (2001).

Microsatellite genotypes were used to estimate relatedness (genetic relationship) between pairs of individuals within bycatch and reference samples. Relatedness (pairwise relationship coefficients) was computed by using the moments estimator of Ritland (1996) and the regression estimator of Lynch and Ritland (1999). A bootstrap distribution (1,000 resamplings, where comparisons between individuals

with identical genotypes were excluded) of estimates of the variance of pair-wise relatedness in each sample was used to test whether the observed variance differed significantly from zero.

## RESULTS

### Population Structure and Variance Effective Population Size

Summary statistics (number of alleles, allelic richness, gene diversity; and results of tests of HW equilibrium) for each samples are given in Appendix 1. Number of alleles among samples ranged from 4-7 at *Prs*260 to 18-23 at *Prs*240, the corresponding Allelic richness range being 2.9-5.3 and 14.3-20.4. Significant departures from HW expectations, following Bonferroni corrections (Rice 1989), were found in three of 114 tests: *Prs*275 in Texas 1995, *Prs*137 in Alabama 1995, and *Prs*248 in Louisiana 1999. Significant, non-random association of genotypes was found in two of 1,026 pairwise comparisons following Bonferroni correction: *Lca*64 and *Prs*248 ($p < 0.001$) and *Prs*328 and *Lca*22 ($p < 0.001$) in the sample form Texas (1995).

Significant differences ($p < 0.001$) in allele and genotype distributions were found among localities in the 1995 year-class. The corresponding $F_{ST}$ value was 0.001. Pairwise comparisons indicated that the sample from Texas differed significantly ($p < 0.002$) from the samples from Louisiana and Alabama. No significant differences ($p > 0.05$) in allele or genotype distributions were found among samples from the 1999 year-class. Both allele ($p = 0.011$) and genotype ($p = 0.017$) distributions differed between the two year classes sampled offshore from Texas; no differences ($p > 0.05$) between year classes at the other two localities were found.

The pseudo-maximum-likelihood estimate (and 95 % confidence intervals) of variance effective population size ($N_e$) for the three localities were 2,658 (1,035 - >50,000), 8,286 (1,418 - >50,000), and 3,210 (1,038 - >50,000) for Texas Louisiana, and Alabama, respectively. The (overall) estimate for the northern Gulf was 7,075 (2,933 - >50,000).

### Genetic Variation and Relatedness in Bycatch Samples

Summary statistics for microsatellites (number of alleles, allelic richness, gene diversity; and results of tests of HW equilibrium) for the *Reference* and two bycatch samples (*Bycatch* A and *Bycatch* B) and analogous data for mtDNA are available from the authors upon request. Number of microsatellite alleles among samples ranged from 4 - 7 at *Prs*260 to 18 - 23 at *Prs*240; allelic richness ranged from 2.87 - 2.94 (*Prs*260) to 12.0 - 13.7 (*Prs*240). For mtDNA, number of haplotypes ranged from 9 – 20, and haplotypic richness ranged from 9.0 - 14.26. Gene (microsatellite) diversities ranged from 0.179 (*Lca*20 in *Bycatch* A) to 0.907 (*Prs* 240 in *Bycatch* B); nucleon diversity (mtDNA) ranged from 0.766 - 0.767. No significant differences in allele/haplotype richness ($0.32 < p < 0.92$) or gene/nucleon diversity

($0.18 < p < 0.93$) were found in pair-wise comparisons of samples. Tests of conformance to HW equilibrium and of genotypic disequilibrium (microsatellites) were non-significant following Bonferroni correction. Tests of homogeneity of allele and genotype distributions (microsatellites) and haplotype distributions (mtDNA) also were non-significant following Bonferroni corrections: ($0.07 < p < 0.63$ for microsatellites; $p = 0.18$ for mtDNA).

Estimates of small $f$ ($F_{IS}$) over all microsatellites were 0.021, 0.022, and 0.063 for *Reference*, *Bycatch* A, and *Bycatch* B, respectively. None of these estimates differed significantly from zero following Bonferroni correction. Estimates of the variance (*Var R*) in both relatedness coefficients was 0 for all three samples except for the regression-based coefficient (Lynch and Ritland 1999) in *Bycatch B*. The variance in this sample was positive (*Var R* = 0.001) and its probability of differing significantly from zero (1,000 bootstrap resamplings) was 0.10.

## DISCUSSION

### Population Structure and Variance Effective Population Size

Analysis of genetic structure based on 19 independent microsatellite loci revealed a weak ($F_{ST} = 0.001$) but significant difference among localities in the 1995 year-class. However, no genetic difference among localities was found in the 1999 year class. Pair-wise homogeneity tests within the 1995 cohort indicated that the sample from Texas differed from the samples from Louisiana and Alabama. The analysis of temporal homogeneity revealed that this sample also differed from the sample obtained in Texas in 1999. Thus the significant heterogeneity in allele and genotype distributions observed here mostly concern one sample (Texas, 1995 year-class) that differs from the other samples and warrants further investigation. We are currently analyzing additional year classes at all three localities. Overall, these results are in accordance with most prior genetic studies of red snapper (Gold et al., 1997, 2001) and suggest that considerable gene flow occurs among red snapper in the northern Gulf. Such a pattern would be consistent with the single-stock model for red snapper in the northern Gulf.

The overall estimate of variance effective population size ($N_e$) for the northern Gulf was 7,075. This estimate is at least three orders of magnitudes less than current estimates of adult census size ($N$) (7 - 20 million, J. Cowan, Louisiana State University, personal communication.). Such a low ratio of effective size to census size is thought to be uncommon, as this ratio is expected to be in the range 0.25 - 0.75 in general (Nunney 1996). However, $N_e/N$ ratios on the order of those observed here have been reported for other marine fishes (Turner et al. 2002, Hauser et al. 2002). Potential factors that may lead to very low $N_e/N$ ratios are fluctuation in population size ($N$), variance in individual reproductive success (within and/or between sexes), and/or variation in productivity of nursery areas (Turner et al. 2002). The estimates of $N_e$ also differed slightly among the sampled localities, although 95 % confidence intervals of estimates at each locality overlapped the

estimates at the other localities. The analysis of additional cohorts will allow to increase accuracy of $N_e$ estimates. Differences in $N_e$ among localities, if confirmed, might suggest the existence of different demographic units (stocks) that would require separate management even if genetic structure is very weak or non-significant. Regardless, our finding of such a low $N_e/N$ ratio for red snapper suggests that red snapper genetic resources are much more limited than what one would expect based on estimates of census size.

**Genetic Variation and Relatedness in Bycatch Samples**

No significant differences in allele diversity (allelic/haplotypic richness, in this case), gene/nucleon diversity, and allele/haplotype and genotype distributions were found between the *Reference* samples and either of the two samples taken from single tows of a shrimp trawler. These results indicate that red snapper taken in the bycatch do not have reduced genetic variation relative to the local population (represented by *Reference* sample), nor do they appear to represent a non-random sample from the local population in terms of allele and genotype frequencies.

We also asked whether red snapper taken as bycatch were more closely related genetically to one another than were individuals drawn at random from the local population. Estimates of the variance (*Var R*) of two different relatedness coefficients did not differ significantly from zero for one of the bycatch samples and for the *Reference* sample. However, the variance of the regression-based coefficient of Lynch and Ritland (1999) was positive and close to significance ($p = 0.010$) for the *Bycatch* B sample. A significantly positive variance would indicate that the sample included individuals that were closely related genetically.

The interpretation of these results is limited by our sample size significant bias when estimating genetic relatedness may be introduced from errors in gene frequency estimation when samples sizes are less than 100 (Lynch and Ritland 1999). Other parameters such as number of loci and pattern(s) of allele distributions at each locus also impact the regression-based coefficient of Lynch and Ritland (1999). Further studies examining larger samples of red snapper from shrimp trawling and examining a larger number of loci are warranted.

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