Stock Structure of Red Snapper in the Northern Gulf of Mexico: Is Their Management as a Single Stock Justified Based on Spatial and Temporal Patterns of Genetic Variation, Otolith Microchemistry, and Growth Rates?

Genetics Subproject

John R. Gold

Center for Biosystematics and Biodiversity
Department of Wildlife and Fisheries Sciences
Texas A&M University
College Station, Texas 77843-2258

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II. ABSTRACT

Allelic variation at 19 nuclear-encoded microsatellite markers was assayed among 2,001 Gulf red snapper (*Lutjanus campechanus*) sampled from four discrete cohorts (year classes) at three offshore localities in the northern Gulf of Mexico (Gulf). Analyses of the genetic data revealed that genetic differences within localities were greater than genetic differences among localities, indicating (spatial) homogeneity across the northern Gulf in these selectively neutral genetic markers. Red snapper sampled offshore of Louisiana had higher (contemporaneous) genetic effective population size than red snapper sampled offshore of Texas and Alabama, potentially reflecting differences among the localities in the number of successfully breeding adults and hence recruitment. The occurrence of differences in contemporaneous effective population size, along with observed differences in age at maturity and growth rates (found in another part of this project) indicate demonstrable demographic differences among these localities and support delineation of red snapper into more than a single management unit in the northern Gulf. Allelic variation at 11 of the microsatellites and at mitochondrial (mt)DNA was examined among two samples of age zero red snapper taken as bycatch during shrimp trawling and compared to reference samples of the same age taken in the same region. No significant differences in allelic richness, gene diversity, or allele frequency were found among the bycatch and reference samples, and red snapper taken as bycatch were no more closely related to one another than were red snapper in the reference sample. Genetic data thus far indicate the absence of a genetic impact of shrimp trawling on red snapper in the northern Gulf.

II. EXECUTIVE SUMMARY

Study Objectives:

1. Sample Gulf red snapper (*Lutjanus campechanus*) from known cohorts (year classes) at three localities in the northern Gulf of Mexico.
    a) Assay allelic variation at a suite of nuclear-encoded microsatellites and test for both temporal (among cohorts within localities) and spatial (among localities) genetic homogeneity.
    b) Estimate contemporaneous (variance) and historical (inbreeding) effective population size(s) of red snapper at each locality.
2. Sample age zero red snapper from individual shrimp trawls and randomly from the same locality.
    a) Assess allelic variation at nuclear-encoded microsatellites and mitochondrial (mt)DNA as a means to detect possible genetic impact(s) of shrimp trawling on red snapper.

Materials and Methods:

For stock-structure analysis, a total of 2,001 red snapper were sampled opportunistically from charterboat/headboat catches at Port Aransas (Texas), Port Fourchon (Louisiana), and Dauphin Island (Alabama) during summers of 1999-2001. All fish were aged by otolith increment analysis and tissues for DNA analyses were saved from individuals of the 1995 and 1997 year classes. Tissues from age zero fish of the 1999 and 2000 year classes were sampled in the fall of 1999 and 2000 during demersal trawl surveys of the northern Gulf carried out by the National Marine Fisheries Service (NMFS). For assessment of genetic impacts of shrimp trawling on red snapper, tissue samples were obtained from 76 of the fish taken during the 1999 demersal survey (above) and from age zero fish obtained as bycatch in two separate tows (one with n = 43 and one with n = 123) of a shrimp trawler.

Allelic variation at 19 nuclear-encoded microsatellites was assayed for stock-structure analysis, while allelic variation at 11 of the microsatellites and in mitochondrial (mt)DNA was assayed to assess genetic impact(s) of shrimp trawling. Methods commonly employed in the Principal Investigator's laboratory were used. Statistical analyses for stock structure included tests of homogeneity in allelic and genotypic distributions both among year classes within

localities (temporal) and among localities (spatial). Estimates of both contemporaneous (variance) and historical (inbreeding) effective size were generated, and a test to detect recent reductions in effective size was carried out. Statistical analyses to detect genetic impacts of shrimp trawling included tests of homogeneity in genetic diversity and allele distributions and estimates of genetic relatedness among individuals.

Conclusions and Recommendations:

Red snapper stock structure: Homogeneity tests revealed significant allele-frequency differences among cohorts at two localities (offshore of Port Aransas, Texas) and offshore of Dauphin Island, Alabama), but not the third (offshore of Port Fourchon, Louisiana). Analysis of molecular variance revealed that genetic differences within localities were greater than genetic differences among localities, indicating (spatial) homogeneity across the northern Gulf in these selectively neutral genetic markers. Estimates of variance (contemporaneous) effective population size (N_{eV}) differed among localities: N_{eV} for the sample from Louisiana was ~ 19,000 versus N_{eV} for the samples from Texas (~ 1,750) and Alabama (~ 2,500). The larger variance effective size of red snapper in Louisiana waters potentially reflects a greater number of successfully breeding adults and hence recruitment. Estimates of inbreeding (historical) effective size (N_{eI}) at all three localities were similar (range of ~1,700 – ~3,000). Given the declines in red snapper abundance since the 1970s, the geographic differences in estimates of N_{eV} and N_{eI} suggest that red snapper offshore of Louisiana have increased in abundance to a greater extent than have red snapper offshore of Texas and Alabama, were less impacted by the reported declines, and/or had larger population sizes just prior to the declines than red snapper at the other two localities. The occurrence of differences in contemporaneous effective population size, along with observed differences in age at maturity and growth rates (found in another part of this overall project) indicate demonstrable demographic differences among these localities and support delineation of red snapper into more than a single management unit in the northern Gulf.

Genetic impact(s) of shrimp trawling: No significant differences in allelic richness, gene diversity, or allele distribution (microsatellites and mtDNA) were found among the bycatch samples and the reference (control) sample. These results indicate that red snapper taken as bycatch do not appear to have reduced genetic variation relative to the local population, nor do they appear to represent a non-random sample from the local population in terms of allele

frequencies. Estimates of the variance of two different relatedness estimators did not differ significantly from zero for the bycatch and reference samples. These results indicate that red snapper in these two samples are not more closely related than would be expected when sampling individuals at random from the local population. However, the variance of the relatedness estimate based on a regression approach was positive for one of the bycatch samples and may indicate that the sample contained some related individuals. In addition, the relatively small sample size and the (comparatively lower) number of genetic markers employed limit inferences about the presence or absence of closely related individuals in the bycatch samples. Continued study of red snapper taken as bycatch and employing larger sample sizes and additional microsatellites is warranted.

II. PURPOSE

The research described below is one part (a subproject) of a large, multidisciplinary and multi-institutional project to assess the population structure of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. This subproject utilized genetic tools and had two research objectives: the first was to examine population structure of red snapper in the northern Gulf via genetic means; the second was to examine whether shrimp trawling and associated bycatch of red snapper impacted genetically red snapper population(s). In terms of time and effort, the first objective (red snapper population structure) comprised about 85% of the total cost and effort. For ease of reading, each section of this report is divided into two subsections, one regarding the work on red snapper population structure, and one regarding work on red snapper taken as bycatch during shrimp trawling.

RED SNAPPER POPULATION STRUCTURE:

Red snapper (*Lutjanus campechanus*) is an important, highly exploited marine fish distributed primarily along the continental shelf in the Gulf of Mexico (hereafter, Gulf) from the Yucatan Peninsula (Mexico) in the southern Gulf to the northeastern Florida coast in U.S. waters (Hoese and Moore 1977). Although the species has comprised an important fishery in U.S. waters since the early 1900s, red snapper resources in U.S. waters have declined by an estimated 90% since the 1970s (Goodyear and Phares 1990). Factors presumably impacting red snapper abundance include overexploitation by directed commercial and recreational fisheries, juvenile mortality associated with bycatch in the shrimp fishery, and habitat change (Christman 1997, Gallaway et al. 1999, Ortiz et al. 2000).

Intensive management of red snapper resources in U.S. waters (the Gulf of Mexico Exclusive Economic Zone or EEZ and adjoining Territorial Sea) has been ongoing since 1984 when the Gulf of Mexico Fishery Management Council (GMFMC) Reef Fish Fishery Management Plan became operative. Currently, assessment and management of red snapper in U.S. follow a unit (single) stock hypothesis (GMFMC 1989, 1991). The Reef fish Stock Assessment Panel was charged by the GMFMC to review the status of red snapper in the northern Gulf and to recommend measures that would enhance efforts to attain 20 percent SPR. Management tools utilized to achieve a spawning potential ratio (SPR) of 20 percent include harvest quotas, minimum size limits, trip quotas for commercial fishers, creel limits for

recreational fishers, and moratoria on issuing commercial reef fish permits. However, the fishery remains overfished (Goodyear 1995; Schirripa and Legault 1997). In addition, because of critical questions regarding red snapper stock assessments, associated scientific data used in assessments, and the potential impact associated with shrimp trawl bycatch, a number of technical reviews (e.g., MRAG, Americas Inc. 1997) of red snapper stock assessment methods were conducted in the mid- to late 1990s. Review panels were not charged specifically to address the unit stock hypothesis, consequently all reviews were based on the assumption that red snapper constitute a single unit stock in the northern Gulf.

An underlying, critical assumption to any fisheries management plan is that the fish being managed belong to a single unit stock (Ricker 1975; Gulland 1965). This assumption is essential to management decisions because measures of growth, natural mortality, reproductive potential, and recruitment can differ significantly for both mixing and non-mixing populations of a single species. Identification of biologically meaningful management units (stocks) and their boundaries within a fishery is thus of profound importance to both assessment and allocation (Hilborn 1985; Sinclair et al. 1985). Accuracy and predictability of stock distribution(s) allow adjustment of fishing quotas for maximum available harvest of surpluses with lowest risk of overharvest, resource injury, or financial loss to the fishing industry. Should separate stocks exist, fishery units could be assessed and managed on a subregional basis, providing the opportunity to adjust regulations to the unique needs of subregional populations and resource users. Alternatively, should only a large, single stock exist, management would need to be based on the premise that policies in one subregion could significantly impact the resource elsewhere. Assessment and allocation are especially critical when such decisions involve politically charged species such as red snapper.

A second reason why knowledge of stock structure is critical to management of a fishery is that stocks within the fishery 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). These differences in theory contribute at the metapopulation or species level to long-term adaptability, survival, and resistance to human-induced or other environmental perturbations. Stocks in different regions often may be independent demographically, even in species (e.g., red snapper) that appear to be distributed more or less continuously. Separate management of regional units is thus desirable to avoid

regional over-exploitation and to maintain potentially adaptive genetic variation (Carvalho and Hauser 1995, Hauser and Ward 1998). Conservation of these genetic resources is especially critical in the context of species or populations under intensive exploitation, as erosion of genetic resources via depletion of (unrecognized) constituent spawning components can directly impact immediate and long-term recruitment potential. In addition, theoretical work on population viability (Lande 1994; Lynch et al. 1995) has shown that subdivided populations subjected to environmental or other stress appear highly prone to a process called "mutational meltdown" where mildly deleterious recessive mutations accumulate, erode fitness, and eventually cause population collapse. Empirical data on this process are not extensive, but the theoretical framework demonstrates that subpopulation extinction can occur extremely rapidly (Bürger and Lynch 1995), particularly where overexploitation or other circumstances (e.g., bycatch loss in the case of red snapper in the northern Gulf) could significantly reduce (genetic) effective population size.

Few data addressing the issue of red snapper stock-structure in the northern Gulf were available when the original management plan was drafted, but subsequent, genetics-based studies generally have been consistent with the existence of a single stock. Johnson (1987) surveyed 67 allozyme loci among 243 red snapper from two localities in the northern Gulf and two localities along the Atlantic coast and found no significant differences among samples in allele frequencies at five marginally polymorphic loci. Camper et al. (1993) and Gold et al. (1997) surveyed variation in mitochondrial (mt)DNA haplotypes among samples of red snapper obtained between 1990 and 1992 from six localities in the northern Gulf and one locality in Mexico. They found no evidence of genetic differences either between or among samples taken in different years at the same locality or among samples taken at different localities within the same years. Similar results were obtained by Heist and Gold (2000) and Gold et al. (2001) in their studies of microsatellite variation. The hypothesis of a unit stock of red snapper in the northern Gulf was *not* supported in a mtDNA study carried out by Bortone and Chapman (1995), and was compatible with mark-recapture and sonic tracking experiments that suggest red snapper juveniles and adults are sedentary and non-migratory (Bradley and Bryan 1975; Beaumariage and Bullock 1976; Fable 1980; Szedlmayer and Shipp 1994; Szedlmayer 1997). The results of Bortone and Chapman (1995), however, may have been compromised because (i) sample sizes were variable, with six or fewer individuals examined at three localities, and (ii) almost no

variation was found in other typically more variable mtDNA sequences (i.e., parts of the cytochrome *b* gene and control region). Bortone and Chapman (1995) suggested that the observed genetic heterogeneity might stem from non-random sampling where individuals related by descent, i.e., from the same spawning aggregation, had remained in close spatial proximity to one another. The non-random sampling suggested by Bortone and Chapman (1995) is similar to what has been termed the Allendorf-Phelps effect (Waples 1998), where different samples represent progeny from limited (and different) spawning events.

The genetic homogeneity observed in most prior genetic studies is consistent with the hypothesis that significant gene flow at one or more life-history stages occurs among red snapper in the northern Gulf (Gold & Richardson 1998a). Goodyear (1995) suggested that extensive mixing of red snapper across the northern Gulf could result from hydrodynamic transport of pelagic eggs and larvae. Red snapper spawn primary during the summer months, with planktonic eggs and larvae averaging around 26-30 days in the water column (Leis 1987, Szedlmayer and Conti 1999). Metamorphosis into benthic juveniles follows, with recruitment to high vertical relief substrates such as reefs and oil platforms occurring the following year (Szedlmayer and Shipp 1994). The notion that gene flow in red snapper may occur primarily during the egg and larval pelagic phase is corroborated by the majority of tagging studies that generally have shown adult red snapper to be sedentary and to exhibit high site fidelity (Beaumariage and Bullock 1976, Fable 1980, Szedlmayer and Shipp 1994, Szedlmayer 1997). However, Patterson et al. (2001) recently documented extensive movement of adult red snapper in the northeastern Gulf; over a mean time at liberty of 404 days, the average distance moved was 29.6 km, with farthest distance moved being 352 km. Patterson et al. (2001) noted that their findings indicated that movement of adults might be sufficient to facilitate mixing of red snapper across the northern Gulf.

Although the genetic homogeneity observed among red snapper in the northern Gulf generally is consistent with the unit stock hypothesis, there are a number of caveats to this interpretation (Gold and Richardson 1998). One is that the genetic homogeneity may reflect historic rather than contemporary gene flow (Camper et al. 1993, Gold et al. 1997, Heist and Gold 2000). As discussed by Gold and Richardson (1998a), present-day red snapper could be subdivided or isolated demographically yet be similar genetically because of substantial gene flow in the recent past. One possibility might be that red snapper colonized the continental shelf

in the northern Gulf following the last glacial retreat, and that there has been insufficient time for genetic differences to accumulate among localities that are presently isolated. Finally, the prior genetic studies of red snapper employed adult fish which, in most cases, were of different ages and perhaps with different migration histories. Mixing of potentially distinct assemblages, whether spatially or temporally discrete, could generate similarity in allele frequencies and preclude detection of existing genetic differences. Analysis of individual cohorts (year classes) and of young-of-the-year fish is expected to provide a more sensitive assessment of stock structure, as confounding influences such as mixing of age and/or genetically distinct assemblages should be minimized (Graves et al. 1992, Jones and Quattro 1999, Buonaccorsi et al. 2001).

This component of the subproject had two primary research goals. The first was to determine whether independent, genetic subpopulations (stocks) of red snapper exist in the northern Gulf. We documented variation in 19 nuclear-encoded microsatellites within four separate cohorts (year classes) across localities and tested for genetic homogeneity both within (temporal) and among (spatial) localities. The second goal was to determine the (genetic) effective population size (N_e) at different localities across the region. Using the same microsatellite loci, we documented genetic variation among cohorts within localities and generated estimates of contemporaneous or variance N_{eV}. The estimates of N_{eV} provided a novel approach to the issue of whether different demographic stocks of red snapper occurred in the northern Gulf, and in addition, provided an innovative, fishery-independent, genetics based method of assessing stock status, condition, and abundance.

GENETIC IMPACTS OF SHRIMP TRAWLING ON RED SNAPPER:

Factors presumably impacting red snapper abundance in the northern Gulf include overexploitation by directed fisheries and mortality of juveniles accidentally caught as bycatch during shrimp trawling operations (Christman 1997; Ortiz et al. 2000). The latter has been addressed by quantitative evaluation of the volume of red snapper bycatch and its composition in terms of age classes (Gallaway et al. 1998; Gallaway and Cole 1999). Estimates of the number of juveniles red snapper taken as bycatch in the shrimp fishery range, for the period 1992-1996, between 26-32 million individuals per year; the majority (65%) of red snapper in the bycatch are young-of-the-year (age 0) fish (Gallaway et al., 1998). Bycatch-induced mortality of red snapper

juveniles may represent an important reduction in the red snapper population given that the estimated number of adults in the northern Gulf is between 7-20 million individuals (J. Cowan, personal communication).

An important question is whether red snapper taken as bycatch represent a random sample of alleles and genotypes from the population from which they were drawn. The question is of importance as non-random mortality when individuals in the bycatch are closely related (e.g., full or half sibs) could reduce the genetic effective size (N_e) of the population by reducing or canceling the contribution of the corresponding families to recruitment. Reductions in N_e may alter long-term sustainability and capacity to respond to changing environments (Crow and Kimura 1970; Allendorf and Waples 1996) because of inbreeding depression and/or accumulation or fixation of deleterious alleles (Frankham 1995; Higgins and Lynch 2001).

Non-random sampling (mortality) of related red snapper in shrimp trawls could arise from behavioral patterns where individuals representing a subset of multiple spawning events tend to remain spatially proximal during part of the early life history. This type of pattern has been hypothesized for herring (Lambert, 1984) on the basis of length-frequency histograms, and for juvenile samples of Atlantic cod on the basis of genetic data (Ruzzante et al. 1996). Similar length-frequency histograms have been reported for red snapper in the northern Gulf (Szedlmayer and Conti 1999). As red snapper in the northern Gulf generally spawn over a period of three to four months (Szedlmayer and Conti 1999; J. Cowan, personal communication), individuals from discrete spawning aggregations involving only a few breeders might remain in spatial association through larval and early juvenile stages.

The goal of this component of the subproject was to determine whether red snapper juveniles taken in shrimp trawls as bycatch represented a random (genetic) sample of the local subpopulation or stock to which they belong. Juvenile red snapper were sampled from two separate shrimp trawls offshore of Galveston, Texas, and assayed for variation at eleven nuclear-encoded microsatellites and mitochondrial (mt)DNA. A reference group for the same geographic area composed of multiple samples that differed both temporally and spatially was assayed for the same genetic markers. Homogeneity in allele and genotype diversity and in allele (microsatellites) and haplotype (mtDNA) distributions among samples was assessed. A 'method-of-moments' estimator (Ritland 1996) and a recently developed 'regression' estimator

(Lynch and Ritland 1999) were used to assess pairwise relatedness within each sample in order to determine whether juveniles sampled during shrimp trawling were more closely related than would be expected if sampling was at random.

III. APPROACH

RED SNAPPER POPULATION STRUCTURE:

Sampling

Adult red snapper were sampled from offshore recreational harvests between 1999 and 2001 from three localities in the northern Gulf (Figure 1, Table 1). Dockside sampling was carried out at Dauphin Island, Alabama (eastern Gulf), Port Fourchon, Louisiana (central Gulf), and Port Aransas, Texas (western Gulf). Sampling was carried out by personnel from the University of South Alabama, Louisiana State University; and Texas A&M University. For the genetics subproject, tissues (heart, spleen, and white muscle) were removed from each specimen, placed into labeled cryopreservation tubes, frozen in liquid nitrogen, and returned to College Station, Texas where they were stored at -80^{0}C. Fork length in mm, total weight in kg, eviscerated body weight in kg, and sex were recorded for all specimens for use in the subprojects at the University of South Alabama and Louisiana State University. Both sagittal otoliths were removed from all specimens and the age of each specimen was estimated from opaque increment count and adjusted for edge condition when necessary. Aging was carried out at Louisiana State University following methods in Wilson and Nieland (2001).

Young of the year (age 0) red snapper were procured in the fall of 1999 and 2000 during demersal trawl surveys of the northern Gulf carried out by the National Marine Fisheries Service (NMFS). Individual fish were sampled using a 12 m shrimp-trawl net, frozen onboard, and returned to College Station where tissues (heart, spleen, and white muscle) were removed from each specimen, placed into labeled cryopreservation tubes, and frozen and stored as described for adult samples. Samples were obtained from offshore localities corresponding to the three geographic regions (Figure 1, Table 1) from which adult samples were obtained, i.e., the eastern Gulf (offshore of Dauphin Island, Alabama), central Gulf (offshore of Port Fourchon, Louisiana), and western Gulf (offshore of Port Aransas, Texas).

Genetic assays

Genomic DNA was isolated from frozen tissues as described in Gold & Richardson (1991). Methods, including PCR primer sequences, for assay of phenotypes (genotypes) at 19 microsatellites followed procedures outlined in Gold et al. (2001).

Data analysis

Genetic variability was measured as number of alleles, allelic richness (a measure of allele diversity independent of sample size), and Hardy-Weinberg expected heterozygosity (gene diversity) at each microsatellite within each regional sample. Genotype proportions at each microsatellite were tested for conformance to Hardy-Weinberg (HW) equilibrium expectations by using either Fisher's exact test (less than five alleles per locus) or an unbiased estimate of the exact-test statistic calculated with a Markov-chain procedure (5,000 dememorisations, 500 batches and 5,000 iterations per batch). Heterozygote deficiency/excess was assessed via F_{IS} (after Weir & Cockerham 1984), as implemented in FSTAT 2.9.3 (Goudet 1995). Genotypic disequilibrium between pairs of microsatellites was tested by an exact test of independence. Tests of HW equilibrium and genotypic disequilibrium were carried out using GENEPOP 3.3 (Raymond and Rousset 1995); significance levels for simultaneous tests were adjusted by following the sequential Bonferroni approach (Rice 1989).

Homogeneity of allele and genotype distributions among cohorts within localities, among all temporal-spatial samples (12 total), and between pairs of samples (66 tests total) was tested via exact tests as implemented in GENEPOP 3.3. Significance of probability from exact tests was assessed using a Markov chain procedure (500 dememorizations, 500 batches, 5,000 iterations per batch). Exact tests of allele distributions (permutations computed on alleles) assumes random mating, whereas tests of genotype distributions (permutations computed on genotypes) does not. Analysis of molecular variance or AMOVA (Excoffier et al. 1992), as implemented in ARLEQUIN 2.000 (Schneider et al. 2000), also was employed to test temporal (among cohorts within localities) and spatial (among localities) genetic homogeneity; both factors (year class and locality) were incorporated in the same hierarchical model where distances between haplotypes were calculated as number of different alleles (infinite allele model or IAM) or sum-of-squared size difference (stepwise mutation or model SMM). Significance of Φ values obtained from AMOVA was estimated from 10,100 (infinite allele model) or 20,022 (stepwise model)

permutations of genotypes. Multilocus estimates of F_{ST} (the θ statistic of Weir and Cockerham 1984) between pairs of samples were obtained using FSTAT.

Short-term or variance effective population size (N_{eV}) was estimated via temporal changes in allele frequencies between/among cohorts (Waples 1989). This 'temporal method' provides an estimate of variance effective size over the time interval between sampling periods, thus providing an estimate of contemporaneous effective size for the population under study. The pseudo-maximum-likelihood method described in Wang (2001) was used to obtain estimates and 95% confidence intervals of N_{eV}. This method performs nearly as well as 'full' likelihood methods when estimating N_{eV} from triallelic loci and provides a more accurate and precise estimate of N_e, relative to estimating N_e from F-statistics, when there are several low-frequency or rare alleles (Wang 2001). Estimates of N_{eV} were generated for individual sample localities (Texas, Louisiana, Alabama) and for the northern Gulf; the latter estimate was obtained by pooling data by year class (cohort) across sample localities. 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).

The analytical method developed by Jorde and Ryman (1995, 1996) was employed to account for effects of overlapping generations on temporal-method estimates of N_e. In a population with overlapping generations, the amount of temporal allele-frequency change is dependent on age-specific survivorship (l_i) and birth rate (b_i). Survivorship was calculated by assuming an equal probability (S) of surviving from one year class to the next and equal probability of survival of males and females. The value of S (0.5) was estimated by examining age-structure data of red snapper caught by commercial and recreational fisheries and used to calculate age-specific survivorship ($l_i = S^{i-1}$) for each age class i. Birth rate was estimated by calculating mean individual (wet) weight, as an indicator of relative gamete contribution, at each age class. Individual weights were averaged across males and females within each age class and this value was multiplied by l_i to obtain the proportional contribution of each age class to offspring (p_i); p_i values were then summed over k age classes. Mean individual weights at each age class were divided by $\sum_{i=1}^{k} p_i$ to produce a standardized birth rate (b_i), corrected to reflect a non-growing population with stable age structure, i.e., $\sum_{i=1}^{k} l_i b_i = 1 = R_0$. [Note: Age-structure and weight data from the commercial and recreational catch are unpublished and were provided by D. Nieland of

Louisiana State University.] Resulting life-history tables were used to calculate a correction factor (C) for overlapping generations by using 100 iterations of Equation 5 in Jorde and Ryman (1996). The value C can be defined as a correction term that is determined by the particular values of l_i and b_i of the population under study. G, the mean generation length in years (5.7), was calculated using Equation 10 in Jorde and Ryman (1996). Values of C and G obtained were subsequently used to correct N_e by

$$N_{ec} = N_e \text{ x } [C/G] \quad (1)$$

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

Long-term or inbreeding effective population size (N_{el}) was estimated by simulations based on the coalescent theory (Kingman 1982). This method uses gene-tree branch lengths as descriptors of $\Theta = 4N_{el}\mu$, where μ is the rate of substitution (mutation) per generation (Hudson 1991); estimates of N_{el} are integrated over the time to common ancestry of all alleles in a population (Avise 2000). The program MIGRATE (Beerli and Felsenstein 2001; Beerli 2002), where Markov Chain-Monte Carlo (MCMC) sampling of gene trees (and accounting for migration) is used to estimate Θ for each sampled population, was employed in the analysis. N_{el} estimates were generated using microsatellite data from the 1995 and 1997 cohorts at each locality. In order to reduce computation time, 30 individuals were selected at random from each sample (six samples total). Computations were performed on a SGI origin 3800 computer (Silicon Graphics Inc.) by using a parallel-processing version of MIGRATE that was run simultaneously on four processors. Each microsatellite was analyzed separately using a Brownian approximation of the stepwise mutation model. Following parallel analysis of each microsatellite, likelihood surfaces were integrated using MIGRATE to generate a likelihood profile of □ over all microsatellites. The MCMC search employed 10 short chains (10,000 genealogies sampled) and 2 long chains (100,000 genealogies sampled); the first 10^4 steps were ignored in each chain in order to avoid bias-towards starting values. Resulting integrated estimates of Θ were used to initialize a more thorough analysis (three replicates runs of each locus, using three long chains with 200,000 genealogies sampled). The assumption of a stepwise mutation model could not be made for the three microsatellites (*Lca* 22, *Prs* 240, and *Prs* 275) that showed occurrence of one base-pair step mutations; these three loci were therefore omitted from the analysis. Estimates of N_{el} and their 95% confidence intervals (CI) were derived from Θ

values and their 95% likelihood-profile CI (Beerli 2002), respectively, by assuming an average mutation rate of 10^{-4} (Jarne and Lagoda, 1996; Garcia de Leon et al., 1998).

The '*M*' test (Garza and Williamson 2001) was used to assess whether recent reductions in effective size (a 'bottleneck') had occurred at each locality. Tests were implemented using microsatellite data (separately) from both the 1995 and 1997 cohorts. The parameter *M* is the mean ratio of the number of alleles to the range in allele size. Under a stepwise model of mutation, *M* is expected to decrease when effective size of a population is reduced; the magnitude of the decrease is positively correlated with the severity and duration of the effective size reduction (Garza and Williamson 2001). The software package {*M_P_Val.exe* and *Critical_M.exe*}, available at http://www.pfeg.noaa.gov/tib/staff/carlos_garza/carlos software.html, was used to estimate and test significance of M; the generalized stepwise mutation model was parameterized as recommended in Garza and Williamson (2001), i.e., p_s (proportion of one-step mutations) = 90% and $\triangle_g$ (average size of non one-step mutations) = 3.5. Values of Θ ($4N_e\mu$, where N_e = inbreeding effective population size and μ = mutation rate) were obtained empirically during estimation of inbreeding effective size. Values of Θ employed were 0.684 and 0.785 (TX '95 and '97), 0.880 and 1.220 (LA '95 and '97), and 0.683 and 0.803 (AL '95 and '97).

GENETIC IMPACTS OF SHRIMP TRAWLING ON RED SNAPPER:

Sampling

Tissue samples (muscle and internal organs) were obtained from juvenile young of the year (age 0) red snapper collected offshore of Galveston, Texas. A total of 76 individuals were sampled in conjunction with a groundfish survey of the National Marine Fisheries Service (NMFS) during the fall of 1999. Juveniles were sampled a few at a time during multiple trawls that differed both spatially and temporally. This sample is referred to as the '*Reference*'. Two additional samples from the same age 0 group were obtained as bycatch in two separate tows of a shrimp trawler within the same area and during the same period: one (*Bycatch A*) contained 123 juveniles, while the other (*Bycatch B*) contained 40 juveniles. Tissue sampling and storage and preparation of genomic DNA followed procedures described in Gold and Richardson (1991).

Genetic assays

All fish were assayed for allelic variation (genotypes) at eleven of the microsatellites described by Gold et al. (2001). Details of PCR amplification, electrophoresis, and scoring followed protocols described in Gold et al. (2001). DNA sequence variation in two fragments of mitochondrial (mt) DNA was assayed by using single strand conformational polymorphism (SSCP), a method that permits detection of differences in nucleotide sequence in polymerase-chain-reaction (PCR) amplifications of DNA (Orita et al.1989). The mtDNA fragments assayed were a 163 base pair (bp) fragment of the ND-4 gene and a 122 bp fragment of the ND-6 gene. These genes encode subunits of the enzyme NADH dehydrogenase. A manuscript detailing methods used in the SSCP assays is in preparation; a synopsis, including PCR primers, is available upon request from the principal investigator. For data analysis, the sequences obtained from both mtDNA genes were combined into a single haplotype for each individual.

Data analysis

Summary statistics for each microsatellite and for mtDNA within each sample were obtained using F-STAT (Goudet 1995). Statistics included number of alleles (microsatellites) and haplotypes (mtDNA), allele and haplotype frequencies, allele and haplotype richness, and unbiased gene (microsatellites) and nucleon (mtDNA) diversity. Allele/haplotype richness represents a measure of the number of alleles/haplotypes independent of sample size. Gene diversity is the average proportion of heterozygotes per (microsatellite) locus in a randomly mating population; nucleon diversity is the haploid equivalent of gene diversity (Nei 1987). Homogeneity of allele richness and of gene diversity between pairs of samples was tested using Wilcoxon signed-rank tests.

Departure of genotype proportions from Hardy-Weinberg equilibrium expectations for each microsatellite within samples were measured as Weir and Cockerham's (1984) f, using F-STAT. Estimates for individual microsatellites were combined to compute a weighted estimate of f over all microsatellites, following recommendations in Weir and Cockerham (1984). Probability of significance was assessed by a Markov-chain method (Guo and Thompson 1992) as implemented in GENEPOP v. 1.2 and using 1000 dememorizations, 100 batches with 1000 iterations per batch (Raymond and Rousset 1995). Genotypic disequilibrium between pairs of microsatellites was assessed using an exact test; significance of probability values was assessed

via 3300 randomizations of genotypes, as implemented in F-STAT. Sequential Bonferroni correction (Rice 1989) was applied for all multiple tests performed simultaneously.

Homogeneity among samples in allele and genotype distributions at the eleven microsatellites was assessed via exact tests, as implemented in F-STAT; significance of probability values was assessed via 5000 randomizations. Homogeneity of mtDNA haplotype frequencies among 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. Sequential Bonferroni correction (Rice 1989) was applied for all multiple tests performed simultaneously.

Microsatellite genotypes were used to estimate relatedness (genetic relationship) between pairs of individuals within samples. Relatedness (pairwise relationship coefficients) was computed using the 'moments' estimator of Ritland (1996) and the 'regression' estimator of Lynch and Ritland (1999). A bootstrap distribution (1000 resamplings, where comparisons between individuals with identical genotypes were excluded) of estimates of the variance of pairwise relatedness in each sample was used to test whether the observed variance differed significantly from zero.

IV. FINDINGS

RED SNAPPER POPULATION STRUCTURE:

Summary statistics for the 19 microsatellites for all 12 temporal-spatial samples (four cohorts x three localities) of red snapper are given in Tables 2a-2d. Statistics included are (i) number of alleles detected, (ii) allelic richness (a measure of allele diversity independent of sample size), (iii) gene diversity (expected heterozygosity), (iv) results of tests of conformity to expected genotype proportions at Hardy-Weinberg (HW) equilibrium, and (v) estimates of F_{IS} (an inbreeding coefficient). Frequencies of individual alleles at each microsatellite within each of the 12 samples are given in Appendix Table A. The number of alleles among samples ranged from a low of 3-6 (*Lca* 20) and 4-7 (*Prs* 260) to 15-23 (*Prs* 240) and 12-25 (*Prs* 248). By cohort (year class), the average number of alleles per microsatellite per sample was 11.74 (1995), 11.23 (1997), 9.26 (1999), and 8.07 (2000). The seemingly fewer number of alleles in the 1999 and 2000 cohorts is a function of the reduced sample sizes (Table 1), as average allelic richness was

nearly the same in all four cohorts, i.e., 7.20 (1995), 7.17 (1997), 7.14 (1999), and 7.10 (2000). Gene diversity essentially paralleled number of alleles: the lowest gene diversities occurred at *Lca* 20 (0.090 – 0.238) and *Prs* 260 (0.279 – 0.462), while the highest gene diversity occurred at *Prs* 240 (0.880 – 0.921) and *Prs* 248 (0.833 – 0.902). These gene diversity values are typical of those reported for microsatellites in other vertebrates, including fish (Turner et al. 1998; DeWoody and Avise 2000).

Following Bonferroni correction (Rice 1989), genotype proportions at 14 of the microsatellites in all 12 samples did not differ significantly from expectations of HW equilibrium (Tables 2a-d). Significant deviations from HW equilibrium following correction were found in seven (of 228 total) tests: two involved *Prs* 137 (the 1995 cohort from Alabama and the 1997 cohort from Louisiana) and two involved *Prs* 229 (the 1997 cohort from both Louisiana and Texas); the remainder involved *Lca* 20 (the 1997 cohort from Texas), *Prs* 248 (the 1999 cohort from Louisiana), and *Prs* 275 (the 1995 cohort from Texas). The absence of a consistent pattern where genotypes at the same microsatellite were out of HW equilibrium in multiple samples indicates the absence of null alleles and that the observed disequilibrium likely stemmed from occurrence of homozygotes for rare alleles pr perhaps from 'dropout' or weak amplification of an allele in a heterozygote. Tests (171 total) of genotypic equilibrium between pairs of loci when all samples were pooled revealed significant genotypic disequilibrium (following Bonferroni correction) four pairwise comparisons: *Lca* 22 vs. *Prs* 328, *Lca* 64 vs. *Prs* 428, *Lca* 107 vs. *Prs* 257, and *Prs* 137 vs. *Prs* 282. However, tests of genotypic equilibrium within each of the 12 samples for these four pairwise comparisons revealed in each case that only one of twelve comparisons was significant following Bonferroni correction. These results indicate that genotypes at these four pairs of microsatellites are randomly associated and that all 19 microsatellites are inherited independently.

Results of exact tests of (temporal) homogeneity of allele and genotype distributions among cohorts (year classes) within each locality are given in Tables 3a (alleles) and 3b (genotypes). Significant heterogeneity over all microsatellites for both allele and genotype distributions was found among cohorts sampled offshore of Texas and Alabama, but not Louisiana. The allele and genotypic heterogeneity among cohorts from Texas was due primarily to microsatellites *Lca* 22 and *Prs* 303; the allele and genotypic heterogeneity among cohorts from Alabama was due primarily to microsatellite *Prs* 240 (Tables 3a & 3b). Because of apparent temporal

heterogeneity (i.e., significant differences among cohorts within localities), exact tests were then carried out among all 12 temporal-spatial samples. Significant heterogeneity among all 12 samples was found at each of four microsatellites (*Lca* 22, *Lca* 91, *Prs* 240, and *Prs* 303) and over all microsatellites (Table 4). Pairwise exact tests between samples over all microsatellites (Table 5) revealed that almost all of the significant differences involved either the 1995 cohort sampled offshore of Texas or the 1997 cohort sampled offshore of Alabama. Collectively, results of the exact tests indicate that the majority of microsatellite differentiation among red snappers in the northern Gulf stem primarily from allele and genotypic differences between or among cohorts and not among localities. This was corroborated by results of AMOVA (Table 6), where the proportion of the molecular variance due to among-cohorts-within-localities was considerably greater than the variance due to among-localities. Neither variance component differed significantly from zero, although the estimated probability that the Φ_{SC} statistic (representing the among-cohorts-within localities component) differed from zero was 0.076. F_{ST} values based on all 19 microsatellites and derived from pairwise comparison of samples are given in Table 7 and demonstrate that the degree of genetic difference among samples is small. As with the pairwise exact tests, almost all of the significant F_{ST} values involved either the 1995 cohort sampled offshore of Texas or the 1997 cohort sampled offshore of Alabama, with estimated (significant) F_{ST} values ranging from 0.0008 to 0.0020 (Table 7).

Estimates of the variance effective size (N_{eV}) and 95% lower and upper confidence intervals for each of the three localities and over all localities (i.e., the northern Gulf), as generated via the pseudo-maximum-likelihood approach (Wang 2001) and corrected for overlapping generations, are shown in Table 8. Estimates of N_{eV} for red snapper from Texas and Alabama were 2,446 and 1,757, respectively, and fell within the 95% confidence levels of one another. The estimated N_{eV} for red snapper from Louisiana was 18,971, with 95% confidence intervals of 3,003 and $> 5 \times 10^4$. The estimated N_{eV} for the sample from Louisiana fell outside of upper-bound confidence intervals for the samples from Texas and Louisiana, while the N_{eV} estimates for the samples from Texas and Alabama fell below the lower-bound confidence interval for the sample from Louisiana. The N_{eV} estimate over all localities, i.e., when samples were pooled by cohorts, was 6,117 with 95% confidence intervals of 3,691 and 14, 235. Recent estimates of the census size (N) of red snapper in the northern Gulf range from 7 to 20 million individuals (J. Cowan, Louisiana State University, personal communication). Assuming these estimates of N represent

lower and upper bounds, the range of values for the ratio of N_e to N (the so-called N_e/N ratio) would be approximately 3×10^{-4} to 9×10^{-4}. Estimates of the inbreeding effective size (N_{eI}) and 95% lower and upper confidence intervals for the 1995 and 1997 cohorts at each of the three localities are shown in Table 9. Estimates N_{eI} for all six samples ranged from ~1,700 – 3,000; estimates based on the 1997 cohort at all three localities were uniformly higher that estimates based on the 1995 cohort, and estimates for the cohorts sampled offshore of Louisiana were higher than estimates for cohorts sampled offshore of Texas and Alabama.

Results of 'M' tests for the 995 and 1997 cohorts at each of the three localities are shown in Table 10. M is the mean ratio of the number of alleles to the range in allele size, M_c is the critical value of M under a specified mutation model (i.e., the 'cutoff' above which 95% of simulated values of M occur under a hypothesis of mutation-drift equilibrium), and P represents the probability of obtaining the observed value of M in an equilibrium population. M values for both cohorts at all three localities are not significantly less than estimated values of M_c, indicating that they either have not experienced a recent reduction in effective size or have recovered sufficiently from a prior reduction such that significant 'gaps' in the microsatellite allele-size distribution no longer exist. Of interest is the observation that probability values for the samples from Texas and Alabama are considerably lower than probability values for the sample from Louisiana.

GENETIC IMPACTS OF SHRIMP TRAWLING ON RED SNAPPER:

Summary statistics, including number of alleles (microsatellites) and haplotypes (mtDNA), and results of tests of HW equilibrium (microsatellites), for each of the three samples are given in Table 11. The distribution of alleles at each microsatellite and of mtDNA haplotypes by sample may be obtained from the Principal Investigator. The number of microsatellite alleles among samples ranged from 3-4 (*Prs* 260) to 12-18 (*Prs* 240); the number of mtDNA haplotypes ranged from 9 to 14. Estimates of *f* for the 11 microsatellites ranged from -0.088 at *Prs* 55 in *Bycatch B* to 0.342 at *Lca* 20 in *Bycatch B* (Table 11); estimates over all microsatellites were 0.021, 0.022, and 0.063 for *Reference*, *Bycatch A*, and *Bycatch B*, respectively. No significant departure from genotype proportions to proportions expected under HW equilibrium was observed following Bonferroni correction. Tests of genotypic disequilibrium at pairs of loci within samples also were non-significant following Bonferroni correction.

Estimates of allele/haplotype richness and of gene/nucleon diversity for each sample also are given in Table 11. Estimates of allele richness (microsatellite) ranged from 2.87 - 2.94 (*Prs* 260) to 12.0 - 13.70 (*Prs* 240); haplotype richness (mtDNA) ranged from 9.00 to 14.26. Estimates of gene diversity (microsatellites) ranged from 0.179 at *Lca* 20 in *Bycatch A* to 0.907 at *Prs* 240 in *Bycatch B*; estimates of nucleon diversity (mtDNA) ranged from 0.766 - 0.767. No significant differences in allele richness ($0.32 < P < 0.92$) or gene diversity ($0.18 < P < 0.93$) were found in pairwise comparisons of samples. Tests of homogeneity of microsatellite allele and mt DNA haplotype distributions among samples also were non-significant ($0.12 < P < 0.97$ for the 11 microsatellites; $P = 0.18$ for mtDNA haplotypes).

The distributions of the two pairwise relatedness coefficients appeared nearly identical in all three samples (Figure 2). Estimates of the variance (*Var R*) in both relatedness coefficients did not differ significantly from zero at a threshold probability level of 0.05 in any of the three samples. However, the estimate for *Bycatch B*, based on Lynch and Ritland's (1999) regression approach, was positive (*Var R* = 0.001); its probability of differing significantly from zero (1000 bootstrap resamplings) was 0.10.

V. EVALUATION

RED SNAPPER POPULATION STRUCTURE:

Allelic richness and gene diversity among the samples of red snapper were roughly average in comparison to those found in other marine and anadromous fishes (DeWoody and Avise 2000). This suggests that overall genetic diversity among red snapper in the northern Gulf has not been impacted significantly by the observed declines in abundance. Significant deviations from HW and genotypic equilibrium were found in seven of 228 tests and four of 171 tests, respectively. However, none of the deviations from either equilibrium displayed a consistent pattern involving either the same sample (cohort) or the same microsatellite. Consequently, all twelve samples were assumed to be in both Hardy-Weinberg and genotypic equilibrium at all 19 microsatellites.

Initial homogeneity testing revealed significant differences in both allele and genotype distributions among year classes or cohorts at the offshore localities from both Texas and Alabama. While important because genetic differences among cohorts within localities is

indicative of reduced effective population size (see below), the finding of heterogeneity among cohorts precluded the 'standard' approach of pooling year classes or temporal samples within localities to test whether (spatial) genetic differences occurred among localities. Homogeneity testing between pairs of samples revealed that most of the genetic differences were due to the samples from the 1995 cohort sampled offshore of Port Aransas (Texas) and the 1997 cohort sampled offshore of Dauphin Island (Alabama). Results of the pairwise homogeneity tests thus indicated that the majority of allelic and genotypic differences among red snapper in the northern Gulf stemmed from differences between or among cohorts within localities rather that among localities. Results from AMOVA confirmed that the among-cohorts-within-localities genetic variance was considerably greater than the among-localities genetic variance.

Results of homogeneity testing in this study are consistent with the majority of prior genetic studies (Camper et al. 1993; Heist and Gold 2000; Gold et al. 1997, 2001) in that no consistent, detectable genetic differences appear to exist found among geographic samples of red snapper in the northern Gulf. The exception is Bortone and Chapman's (1995) study where small-scale geographic differences and restriction sites in a ribosomal RNA sequence were reported. Given that virtually all other genetic studies of red snapper have failed to identify consistent spatial differences, Bortone and Chapman's (1995) hypothesis that their sampling may have been non-random, leading to what's now termed an Allendorf-Phelps effect (Waples 1998), may have been correct. Of importance to note is that almost all of the prior genetic studies have involved genetics markers that for one reason or another are assumed to be selectively neutral. That is, different alleles at most of these markers are assumed either to be functionally equivalent (e.g., samesense substitutions in third codon positions in protein-coding mitochondrial DNA genes) or non-coding and non-functional in terms of cellular/organismal phenotypes (e.g., microsatellites). Selectively neutral genetic markers are generally employed in studies of stock structure because significant differences in allele frequencies can be attributed to reduced or non-existent gene flow, leaving little doubt as to whether the different 'stocks' belong to geographically isolated units.

Estimates of contemporaneous or variance effective size (N_{eV}), corrected for overlapping generations, differed among the three sample localities. The N_{eV} estimate (~19,000) generated from the cohorts sampled offshore of Port Fourchon, Louisiana, was ~7-10 times greater than the estimates for the cohorts sampled offshore of Dauphin Island, Alabama (~2,500), and Port

Aransas, Texas (~1,750), and fell outside the 95% confidence intervals estimated for both of the latter two localities (and vice-versa). These results suggest that current effective population size of red snapper offshore of Port Fourchon is larger than the current effective size at the other two localities. An additional point to note is that even though the N_{eV} estimates for the red snapper sampled offshore of Texas and Alabama fell within the 95% confidence intervals of each other, their geographic locations (i.e., on either side of the sampling locality in offshore Louisiana waters) does not necessarily mean that both are affected by similar factors lowering effective size relative to the samples from offshore of Louisiana (see below). Estimates of long-term or inbreeding effective size (N_{eI}) were essentially the same for the samples from Texas and Alabama (N_{eI} estimates ranging from ~1,700 – 2,000) and generally paralleled the estimates of variance effective size (N_{eV}) for these two localities. Estimates of N_{eI} for the cohorts sampled offshore of Louisiana were 6-8 times lower than the estimate of N_{eV} (N_{eI} ~ 2,800 versus N_{eV} ~ 19,000).

The two estimates of N_e (N_{eV} and N_{eI}) differ in important ways. N_{eV} provides an estimate of variance effective size based on changes in allele frequencies over the time interval between sampling periods (Waples 1989), thus providing an estimate of effective size for a contemporaneous population. N_{eI}, alternatively, provides a long(er)-term estimate of effective size integrated over the time to common ancestry of all alleles in the population (Avise 2000). N_{eV} and N_{eI} are expected to differ under conditions such as fluctuating effective population size (Crow and Denniston 1988). Thus, if a population suffers a bottleneck but recovers with a burst of exponential population growth, the temporal-method estimate (i.e., N_{eV}) is expected to reflect the current adult census size, whereas the coalescent-method estimate (N_{eI}) will tend to reflect the adult census size from the date of the bottleneck. On the surface, and given the reported declines since the 1970s in red snapper abundance in the northern Gulf (Goodyear and Phares 1990), the geographic differences in the estimates of N_{eV} and N_{eI} would seem to suggest that red snapper offshore of Louisiana have increased in abundance to a greater extent than have red snapper offshore of Texas and Alabama and/or were less impacted by the reported declines. It also is possible that the size of the red snapper population in waters offshore of Louisiana historically has been larger than those in the other two localities. However, these inferences should be considered more in the way of hypotheses, in large part because one of the central assumptions in using the program MIGRATE (i.e., constant population size over time) is very

likely unrealistic. In addition, the coalescent approach used to generate estimates of N_{el} reflects the time scale necessary to reestablish equilibrium between genetic drift and mutation (Avise 2000), an equilibrium unlikely to have been reached in the short generational time span between the 1970s and the present day. Results of the 'M' test of allele distributions indicated that red snapper at the localities sampled either had not experienced a recent (detectable) reduction in effective size or had recovered sufficiently such that the 'signature' of a bottleneck was no longer evident. This may suggest that red snapper at all three localities are recovering from the reported declines. However, sensitivity of the 'M' test decreases with theta (Θ) values less than one (Garza and Williamson 2001) and observed theta values for the 1995 and 1997 year classes (cohorts) sampled from Texas and Alabama ranged from 0.683 to 0.803. Alternatively, probability values that observed values of M were less than the estimated values of M_c for the samples from Texas and Alabama were lower than that for the sample from Louisiana, consistent with the estimates of lower N_{ev}.

While identifying which of the factors leading to reduced N_e among the three localities would be of interest, more important from the perspective of stock structure of red snapper in the northern Gulf is that such differences may exist and potentially identify different *demographic* assemblages. Independent evidence that discrete demographic assemblages of red snapper may exist at the three localities was provided by the other subprojects in this Marfin-funded project (Wilson 2001), where it was found that red snapper sampled offshore of Texas were significantly smaller at age than snapper sampled in waters offshore of Louisiana and Alabama, and that female red snapper sampled off Alabama differed significantly from those sampled off Louisiana relative to age at maturity (Alabama females matured earlier). These latter findings, together with known ecological differences that distinguish the western Gulf from areas to the east (Gallaway et al. 1998), are consistent with the hypothesis that red snapper from the three localities represent demographically different units, with the highest levels of successful recruitment and productivity occurring in waters offshore of Louisiana. To our knowledge, this is the first instance where genetic data has been used to suggest the existence of discrete (demographic) stocks in the absence of significant allele-frequency differences among geographic localities. A final point raised by the life-history differences reported in Wilson (2001) is that there may also be genetic differences among red snapper at the three localities that could not be assessed with the genetic markers used here. As noted earlier in this report, the

genetic markers (microsatellites) used in this study are presumed to be selectively neutral and to reflect the presence or absence of gene flow among geographic localities. Homogeneity of selectively neutral markers does not in itself preclude differences in genes reflecting life-history traits that might be under the influence of natural selection. Thus, red snapper demographic units could differ in genes affecting critical life-history traits (e.g., age at maturity, size at age) yet have sufficient gene flow among them to homogenize frequencies of selectively neutral alleles. In theory, differences in genes (frequencies of alleles) at individual life-history traits would be detectable only if the genes themselves could be assayed or if an allele at selectively neutral marker (e.g., a microsatellite) was very closely linked on a chromosome to an allele that significantly affected variation at a the life-history trait (so-called genetic hitch-hiking).

Current estimates of adult red snapper abundance in the northern Gulf range from 7-20 million (J. Cowan, Louisiana State University, personal communication). The estimates of N_e generated in this study, regardless of the analytical approach, indicate that the effective size of red snapper in the northern Gulf is roughly four orders of magnitude less than current estimates of adult census size (i., e., the ratio N_e/N is $\sim 10^{-4}$). This result was somewhat surprising, as red snapper have a long reproductive life span and overlapping generations, a type of life-history that is expected to increase the ratio of N_e (effective size) to N (census size) by limiting variance among individuals in lifetime reproductive success (Hill 1979; Jorde and Ryman 1995; Waite and Parker 1996). The ratio of N_e to N has been the source of considerable discussion in the literature (Nunney and Elam 1994; Frankham 1995; Nunney 1996). Theoretical expectations (Nunney and Elam 1994; Nunney 1996) are that N_e/N ratios should rarely be less than 0.25; empirically derived N_e/N values, however, are generally lower, averaging around 0.1 (Frankham 1995). However, very low N_e/N ratios have now been reported for several marine fish species, including red drum (Turner et al. 2003), a snapper species in waters off of New Zealand (Hauser et al. 2003), and Pacific northwest rockfish (Gomez-Uchida and Banks 2003). Expectations for N_e/N ratios are based in large part on Wright's (1931) model of effective population size, where N_e is the size of the *ideal* population that will result in the same amount of genetic drift as the actual population being considered. Wright (1931) envisioned the *ideal* population to be one where (i) mating is panmictic (random), (ii) there is a 1:1 sex ratio, (iii) generations are non-overlapping, (iv) there is a Poisson variance in reproductive success, and (v) the population

number (census size) is temporally stable. A detailed ecological model where these factors were considered may be found in Nunney (1999).

The observed differences in N_{eV} among the geographic samples of red snapper may reflect differences in N_e/N ratios among the geographic localities. If so, this would suggest the existence of differences among the localities in one or more of the conditions that are hypothesized to affect N_e/N ratios. Given that (i) microsatellite genotypes at all three sample localities appear to be in Hardy-Weinberg equilibrium (this study), (ii) sex ratios observed in commercial and recreational catches are approximately 1:1 (D. Nieland, Louisiana State University, personal communication), and corrections for overlapping generations were included in the estimates of N_{eV}, putative differences in N_e/N ratios among the three localities could be hypothesized to stem from differences in individual reproductive success, fluctuating population sizes, and/or differences in habitat productivity. In red drum, the occurrence of detectable heterogeneity in selectively neutral genetic markers (microsatellites) and (very approximates) estimates that adult census size of red drum in the northern Gulf has not changed appreciably over the last decade led Turner et al. (2003) to hypothesize that the effects on N_e/N of fluctuations in population size were small in relation to variation in reproductive success and/or habitat productivity.

Identifying the factors that might be involved in generating low N_e/N ratios in red snapper in the northern Gulf clearly requires further research. Preliminary data in the PI's laboratory, based on nested-clade analysis of mitochondrial DNA sequence variation, is consistent with the notion that red snapper in the northern Gulf may be distributed in a series of semi-isolated units, separated in part by geographic distance, that expand and contract over time. This could indicate that temporal fluctuations in population size may play a significant role in generating the low N_e/N ratios. Habitat productivity also may play a role, given the ecological differences that distinguish the western Gulf from areas to the east (Gallaway et al. 1998).

A final point to note is that the N_{eV} estimator of Wang (2001) used in this study does not permit separation of the parameter m (rate of *genetic* migration) from the parameter Ne_V (variance effective population size). Consequently, the observed differences in Ne_V among red snapper from the three localities in the northern Gulf could reflect differences in variance effective size (essentially the number of successfully reproducing adults), differences in rates and dynamics of migration, or both. Significant migration, particularly if it is asymmetric, can bias

estimates of Ne_V either upward or downward, depending on whether the migration occurs in the short or long term (Wang and Whitlock 2003). Thus, while the genetic data obtained in this study are consistent with the hypothesis (model) that red snapper in the northern Gulf comprise a 'metapopulation' with different demographic units or stocks, significant questions that would impact management planning remain. Primary among these are (i) the extent to which asymmetric migration of red snapper occurs across the northern Gulf, and (ii) the effect (magnitude, direction, and dynamics) such migration might have on variance effective population size. Persistent, long-term migration, for example, could result in overestimates of Ne_V and consequently an underestimate of the potential for a demographic unit to experience a severe decline due to genetic factors. Alternatively, the observed differences in Ne_V among localities could simply reflect fragmentation and reduced gene flow where differences in Ne_V primarily arise from different degrees (magnitude) of genetic drift. A recent proposal to the MARFIN Program from the PI's laboratory was designed to answer these questions.

GENETIC IMPACTS OF SHRIMP TRAWLING ON RED SNAPPER:

No significant differences in allelic richness or gene diversity were found between the bycatch samples or between the bycatch samples and the reference or 'control' sample, nor was there any indication of allele frequency differences among the three samples. These results indicate that red snapper in the bycatch samples do not appear to have reduced genetic variation relative to the local population, nor do they appear to represent a non-random sample from the larger, local population in terms of allele frequencies. We also assessed whether red snapper taken as bycatch were more closely related to one another than are individuals drawn from multiple samples within the local population. The occurrence of closely related (full or half sibs) individuals within a trawl sample might suggest that bycatch mortality affects families non-randomly, thereby reducing the number of families contributing to recruitment and ultimately the effective size (N_e) of the population. Estimates of the variance of two different relatedness estimators were zero for one of the bycatch samples and for the 'control' sample. These results indicate that red snapper in these two samples are not more closely related than would be expected when sampling individuals at random from the local population. However, the variance of the relatedness estimate based on the regression approach of Lynch and Ritland (1999) was positive for the second bycatch samples (*Bycatch B*), and the probability that this

variance differed significantly from zero (1000 bootstrap resamplings) was 0.10. A positive variance may indicate that the sample contained some related individuals. This result, although not significant at the threshold level of 0.05, might be noteworthy given the size (n = 40) of the *Bycatch B* sample, given that significant bias when estimating genetic relatedness may be introduced from errors in gene frequency estimation when samples sizes are less than 100 or so (Lynch and Ritland 1999). Additional parameters that impact the regression estimator of Lynch and Ritland (1999) are number of loci and 'evenness' of allele distributions at each locus. The expected single-locus sampling variance declines with increasing number of unlinked loci, and an even allele-frequency distribution provides the greatest power of inference (Lynch and Ritland 1999). We employed only 11 microsatellites, and the distributions of alleles at five of the microsatellites (*viz.*, *Lca* 22, *Lca* 107, *Prs* 240, *Prs* 303, and *Prs* 333) were not especially even. These caveats limit inferences about the presence or absence of closely related individuals in the bycatch samples examined here, and indicate that continued study employing larger sample sizes and additional loci is warranted.

Publications (to date)

Saillant, E., T.A. Mousseau, and J.R. Gold. 2003. Genetic variation and relatedness of juvenile red snapper (*Lutjanus campechanus*) sampled from shrimp trawls in the northern Gulf of Mexico. Transactions of the American Fisheries Society 132: 1229-1235.

Saillant, E. and J.R. Gold. 2003. Genetic studies of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. Proceedings of the Gulf and Caribbean Research Institute (In Press).

Presentations

Saillant, E. and Gold J.R. 2002. Population Structure of Red Snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. Annual Meeting of the American Society of Ichthyologists and Herpetologists, Kansas City, MO.

Saillant, E., and J.R. Gold. 2003. Population structure and effective size of red snapper (Lutjanus campechanus) in the northern Gulf of Mexico. Symposium "Use of Genetic Markers for Management and Conservation". Annual Meetings of the American Fisheries Society, Quebec City, Quebec.

Saillant, E. C.L. Pruett, and J.R. Gold. 2003. Effective population size of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. Annual Meeting of the Society for the Study of Evolution, Davis, CA.

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Table 3b. Probability values of (temporal) 'genotypic' homogeneity at each of 19 microsatellites among four cohorts (1995, 1997, 1999, and 2000 year classes) of red snapper (*Lutjanus campechanus*) at three geographic localities in the northern Gulf of Mexico. Probability values are based on exact tests, with 1000 permutations. Bold indicates $P < 0.05$ following (sequential) Bonferroni correction.

Table 4. Probability values of genic and genotypic homogeneity at 19 microsatellites among spatial and temporal samples (12 total) of red snapper (*Lutjanus campechanus*) sampled from the northern Gulf of Mexico. Tests of genic homogeneity assume random mating within samples, whereas tests of genotypic homogeneity do not. Probability values are based on exact tests; significance was assessed via a Markov chain method, using 500 demomorizations, 500 batches, 5000 iterations per batch. Bold indicates $P < 0.05$ following (sequential) Bonferroni correction.

Table 5. Probability values of genic (upper diagonal) and genotypic (lower diagonal) homogeneity at 19 microsatellites among all (12 total) temporal and spatial samples of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico. Tests of genic homogeneity assume random mating within samples, whereas tests of genotypic homogeneity do not. Probability values are based on exact tests; significance was assessed via a Markov chain method, using 500 demomorizations, 500 batches, 5000 iterations per batch. Bold indicates $P < 0.05$ following (sequential) Bonferroni correction.

Table 6. Results of hierarchical analysis of molecular variance (AMOVA) among sampling localities (spatial) and among cohorts (year classes) within localities (temporal). Distance(s) between/among alleles is based on the sum of square size differences assuming a stepwise mutation model. Degrees of freedom are in parentheses.

Table 7. Estimates of F_{ST} from pairwise comparisons for allele distributions at 19 microsatellites among all (12 total) temporal and spatial samples of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico. Boldface indicates F_{ST} values that differ significantly from zero. Zero values are given for those F_{ST} estimates that were less than zero (negative).

Table 8. Pseudo-maximum-likelihood (after Wang 2001) estimates of contemporaneous (variance) effective population size (N_{eV}). Estimates of N_eV were corrected for overlapping generations (after Jorde and Ryman 1995, 1996).

Table 9. Estimates of theta long-term (inbreeding) effective population size (N_{el}) of the 1995 and 1997 cohorts (year classes) of red snapper (*Lutjanus campechanus*) at three localities in the northern Gulf of Mexico. A mutation rate (μ) of 10^{-4} was employed, using the equation $N_{el} = \Theta/4\mu$. Θ values were derived from MIGRATE (Beerli and Felsenstein 2001).

Table 10. Results of *M** tests to detect recent reductions in (contemporaneous) effective population size among red snapper (*Lutjanus campechanus*) sampled from the 1995 and 1997 cohorts (year classes) at three localities in the northern Gulf of Mexico.

Table 11. Summary statistics for each of eleven microsatellites and mitochondrial (mt)DNA for three samples of red snapper (*Lutjanus campechanus*) obtained offshore of Galveston, Texas.

Appendix Table A. Allele frequencies at 19 microsatellites in red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico.

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REFERENCES

Allendorf, F.W. and R.S. Waples. 1996. Conservation and genetics of salmonid fishes, p. 238-280. *In:* Conservation genetics: case histories from nature, J.C. Avise and J.L. Hamrick (eds). Chapman and Hall, New York, NY.

Avise, J.C. 2000. Phylogeography: the history and formation of species. Harvard Univ. Press, Cambridge, MA.

Beaumariage, D.S. and L.H. Bullock. 1976. Biological research on snappers and groupers as related to fishery management requirements, p. 86-94. *In*: Proceedings: colloquium on snapper-grouper resources of the western central Atlantic Ocean, H.R. Bullis and A.C. Jones (eds). Rep. No. 17, Florida Sea Grant College Program, Gainesville, FL.

Beerli, P. 2002. MIGRATE: documentation and program, part of LAMARC. Version 1.5, available at http://evolution.genetics.washington.edu/lamarc.html.

Beerli, P. and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. U.S.A. 98: 4563-4568.

Bortone, S.A. and R.W. Chapman. 1995. Identification of stock structure and recruitment patterns for the red snapper, *Lutjanus campechanus*, in the Gulf of Mexico. Final report for Marfin Program Grant Number NA17FF0379-03. SE Regional Office, National Marine Fisheries Service, Petersburg, FL.

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 Carib. Fish. Inst. 27:77-106.

Buonaccorsi V.P., E. Starkey and J.E. Graves. 2001. Mitochondrial and nuclear DNA analysis of population subdivision among young-of-the-year Spanish mackerel (*Scomberomorous maculatus*) from the western Atlantic and Gulf of Mexico. Mar. Biol. 138:37-45.

Bürger, R. and M. Lynch. 1995. Evolution and extinction in a changing environment: a quantitative-genetic analysis. Evolution 49:151-163.

Camper, J.D., R.C. Barber, L.R. Richardson, and J.R. Gold. 1993. Mitochondrial DNA variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Mol. Mar. Biol. Biotech. 2:154-161.

Carvalho, G.R. and L. Hauser. 1995. Molecular genetics and the stock concept in fisheries, p. 55-80. *In*: Molecular genetics in fisheries, G.R. Carvalho and T.J. Pitcher (eds.). Chapman and Hall, London.

Christman, M.C. 1997. Peer review of red snapper (*Lutjanus campechanus*) research and management in the Gulf of Mexico: statistics review. Office of Science and Technology, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Silver Spring, MD.

Crow, J.F. and C. Denniston, 1988 Inbreeding and variance effective population numbers. Evolution 42: 482-495

Crow, J.F. and M. Kimura. 1970. An introduction to population genetics theory. Burgess, Minneapolis. MN.

DeWoody J. and J.C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes as compared with other animals. J. Fish Biol. 56: 461-473.

Excoffier, L., P. Smouse, and J. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial 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.

Frankham, R. 1995. Effective population-size adult-population size ratios in wildlife: a review. Genet. Res. 66:95-107.

Gallaway, B.J., J.C. Cole, R. Meyer and P. Roscigno. 1999. Delineation of essential habitat for juvenile red snapper in the northwestern Gulf of Mexico. Trans. Am. Fish. Soc. 128, 713-726.

Gallaway B.J., M. Longnecker, J.G. Cole and R.M. Meyer. 1998. Estimates of shrimp trawl bycatch of red snapper, *Lutjanus campechanus*, in the Gulf of Mexico, p. 817-839. *In*: Fishery stock assessment models, F. Funk, T.J. Quinn II, J. Heifetz, J.N. Ianelli, J.E. Powers, J.F. Schweigert, P.J. Sullivan and C.-I. Zhang (eds). Alaska Sea Grant College Program Report, No. AK-SG-98-01. Univ. of Alaska, Fairbanks, AK.

Garza, J.C. and E.G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Mol. Ecol. 10: 305-318.

GMFMC {Gulf of Mexico Fishery Management Council}. 1989. Amendment number 1 to the reef fish fishery management plan. Gulf of Mexico Fishery Management Council, Tampa, FL.

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. Gulf of Mexico Fishery Management Council, Tampa, FL.

Gold, J.R. and L.R. Richardson. 1991. Genetic studies in 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. Genetic homogeneity among geographic samples of snappers and groupers: Evidence of continuous gene flow? Proc. Gulf Carib. Fish. Res. Inst. 50: 709-726.

Gold, J.R., E. Pak and L.R. Richardson. 2001. Microsatellite variation among red snapper (*Lutjanus* campechanus) from the Gulf of Mexico. Mar. Biotechnol. 3, 293-304.

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

Gomez-Uchida, D. and M.A. Banks. 2003. Fishing effects upon molecular variation in overfished rockfish species. Annual Meeting of the American Fisheries Society, Quebec City, Quebec, Canada.

Goodyear, C.P. 1995. Red snapper in U.S. waters of the Gulf of Mexico. Contr. #MIA-95/96-05. Southeast Fisheries Center, Miami Laboratory, Miami, FL.

Goodyear, C.P. and P. Phares 1990. Status of red snapper stocks of the Gulf of Mexico: report for 1990. National Marine Fisheries Service, Southeast Fisheries Centre, Miami Laboratory, CRD 89/90-05, Miami, FL.

Goudet, J. 1995. F-STAT (1.2), a program for IBM compatible PCs to calculate Weir and Cockerham's (1984) estimators of *F*-statistics. J. Hered. 139:463-471.

Graves, J.E., J.R. McDowell, A.M. Beardsely and D.R. Scoles. 1992. Stock structure of the bluefish *Pomatomus saltatrix* along the mid-Atlantic coast. Fish. Bull. US 90:703-710.

Gulland, J.A. 1965. Fish stock assessment: a manual of basic methods. John Wiley & Sons, New York, NY.

Guo S. and E. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion from multiple alleles. Biometrics 48:361-372.

Hauser, L. and R.D. Ward. 1998. Population identification in pelagic fish: the limits of molecular markers, 191-224. *In*: Carvalho, G.R. (Ed.) Advances in molecular ecology, G.R. Carvalho (ed.). IOS Press, Amsterdam.

Hauser, L., G.J. Adcock, P.J. Smith, J.H.B. Ramírez, and G.R. Carvalho. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proc. Nat. Acad. Sci. 99: 11742-11747.

Heist, E.J. and J.R. Gold. 2000. DNA microsatellites and genetic structure of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico. Trans. Am. Fish. Soc. 129:469-479.

Higgins, K. and M. Lynch. 2001. Metapopulation extinction caused by mutation accumulation. Proc. Natl. Acad. Sci. USA 98:2928-2933.

Hilborn, R. 1985. Apparent stock-recruitment relationships in mixed stock fisheries. Can. J. Fish. Aquat. Sci. 42: 718-723.

Hill, W.G. 1979. Note on effective population-size with overlapping generations. Genetics 92: 317-322.

Hoese, H.D. and R.H. Moore. 1977. Fishes of the Gulf of Mexico. Texas A&M University Press, College Station, TX.

Jarne, P. and P.J.L. Lagoda. 1996. Microsatellites, from molecules to populations and back. Trends Ecol. Evol. 11: 424-429.

Johnson, A.G. 1987. An investigation of the biochemical and morphometric characteristics of red snapper (*Lutjanus campechanus*) from the southern United States. Unpubl. MS, National Marine Fisheries Service, Panama City Laboratory, Panama City, FL.

Jones, W.J. and J.M. Quattro. 1999. Genetic structure of summer flounder (*Paralichthys dentatus*) populations north and south of Cape Hatteras. Mar. Biol. 133:129-135.

Jorde, P.E. and N. Ryman. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. Genetics 139: 1077-1090.

Jorde, P. E. and N. Ryman. 1996. Demographic genetics of brown trout (*Salmo trutta*) and estimates of effective population size from temporal change of allele frequencies. Genetics 143: 1369-1381.

Kingman, J.F.C. 1982. The coalescent. Stoch. Proc. App. 13: 235-248.

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

Lambert, T.C. 1984. Larval cohort succession in herring (*Clupea harengus*) and capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 41:1552-1654.

Lande, R. 1994. Risk of population extinction from fixation of new deleterious mutations. Evolution 48: 1460-1469.

Lynch, M., and K. Ritland. 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152:1753-1766.

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

McElroy, D., E. Bermingham, and I. Kornfield. 1992. REAP-the restriction enzyme analysis package. J. Hered. 83:157-158.

MRAG Americas Inc. 1997. Consolidated report on the peer review of red snapper (*Lutjanus campechanus*) research and management in the Gulf of Mexico. Report to the Office of Science and Technology, National Marine Fisheries Service, Silver Spring, MD.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York, NY.

Nunney, L. 1996. Influence of variation in female fecundity on effective population size. Biol. J. Linn. Soc. 59: 411-425.

Nunney, L. 1999. The effective size of a hierarchically structured population. Evolution 53: 1-10.

Nunney, L. and D.R. Elam. 1994. Estimating the effective size of conserved populations. Conserv. Biol. 8: 175-184.

Orita, M., Suzuki, Y., Sekiya, T., Hayashi, K., 1990. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5, 874-879.

Ortiz, M., C.M. Legault and N.M. Ehrhardt. 2000. An alternative method for estimating bycatch from the US shrimp trawl fishery in the Gulf of Mexico, 1972-1995. Fish. Bull. US 98, 583-599.

Patterson, W.F., J.C. Watterson, R.L. Shipp and J.H. Cowan. 2001. Movement of tagged red snapper in the northern Gulf of Mexico. Trans. Am. Fish. Soc. 130, 533-545.

Raymond, M. and F. Rousset. 1995. GENEPOP (Version 1.2): population genetics software for exact test ecumenism. J. Hered. 86:248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Bd. Canada 191: 1-382.

Ritland, K. 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. Genet. Res. 67:175-185.

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

Ruzzante, D.E., C.T. Taggart and D. Cook. 1996. Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability. Can. J. Fish. Aquat. Sci. 53:2695-2705.

Schirripa, M.J. and C.M. Legault. 1997. Status of the red snapper in the U.S. waters of the Gulf of Mexico: Updated through 1996. National Marine Fisheries Service, Southeast Fisheries Science Center. Miami Laboratory, Cont. MIA-97/98-05:1-40, Miami, FL.

Schneider, S., D. Roessli and L. Excoffier. 2000. ARLEQUIN ver. 2000: a software for population genetic analysis. Genetics & Biometry Lab., Univ. Geneva, Switzerland.

Sinclair, M., V.C. Anthony, T.D. Iles and R.N. O'Boyle. 1985. Stock assessment problems in Atlantic herring *(Clupea harengus)* in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 42:888-898.

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

Szedlmayer, S.T. and J Conti. 1999. Nursery habitats, growth rates, and seasonality of age-0 red snapper, *Lutjanus campechanus*, in the northeast Gulf of Mexico. Fish. Bull. US 97:626-635.

Szedlmayer, S.T. and R.L. Shipp. 1994. Movement and growth of red snapper, *Lutjanus campechanus*, from an artificial reef area in the northeastern Gulf of Mexico. Bull. Mar. Sci. 55:887-896.

Turner, T.F., L.R. Richardson and J.R. Gold. 1998. Polymorphic microsatellite DNA markers in red drum (*Sciaenops ocellatus*). Mol. Ecol. 7: 1771-1772.

Turner, T.F., J.P. Wares and J.R. Gold. 2003. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162: 1329-1339.

Waite, T.A. and P. G. Parker. 1996. Dimensionless life histories and effective population size. Conserv. Biol. 10: 1456-1462.

Wang, J.L. 2001. A pseudo-likelihood method for estimating effective population size from temporally spaced samples. Genet. Res. 78: 243-257.

Wang, J. and M.C. Whitlock (2003) Estimating effective population size and migration rates from genetic samples over space and time. Genetics 163: 429-446

Waples, R.S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379-391.

Waples, R.S. 1998. Separating the wheat from the chaff - patterns of genetic differentiation in high gene flow species. J. Hered. 89:438-450.

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

Wilson, C.A. 2001. Growth Rates Portion: Stock Structure of Red Snapper in the Northern Gulf of Mexico: Is Their Management as a Single Stock Justified Based on Spatial and Temporal Patterns of Genetic Variation, Otolith Microchemistry, and Growth Rates? Final report to Marfin Project No. NA87FF0424, National Marine Fisheries Service Regional Office, St. Petersburg, FL.

Wilson, C.A. and D.L. Nieland. 2001. Age and Growth of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana. Fish. Bull. US 99:653-664.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16: 114-138.

Table 1. Sample localities, cohorts (year classes), and number of individuals assayed for allelic variation at nineteen microsatellites among red snapper (*Lutjanus campechanus*) sampled from the northern Gulf of Mexico. State abbreviations are as in legend to Figure 1.

	Number of individuals				
Cohort Locality	1995	1997	1999	2000	Total
Port Aransas, TX	203	211	97	65	567
Port Fourchon, LA	286	272	77	32	667
Dauphin Island, MS	377	274	63	44	758
Total	866	757	237	242	2,001

Table 2a. Summary statistics for 19 microsatellites from the 1995 year class (cohort) of red snapper (*Lutjanus campechanus*) sampled from three regions in the northern Gulf of Mexico. A_R is allelic richness; H_E is expected heterozygosity (gene diversity); P_{HW} is probability of conforming to expected Hardy-Weinberg genotypic proportions; and F_{IS} is an inbreeding coefficient. Bold indicates significant departures from HW equilibrium following (sequential) Bonferroni correction.

Micro-satellite	Texas	Louisiana	Mississippi-Alabama	Micro-satellite	Texas	Louisiana	Mississippi-Alabama
Lca 20				*Prs* 240			
#alleles	5	6	5	# alleles	20	23	21
A_R	3.39	3.37	3.00	A_R	15.84	14.94	14.96
H_E	0.170	0.215	0.172	H_E	0.917	0.901	0.885
P_{HW}	0.088	0.820	0.006	P_{HW}	0.010	0.124	0.093
F_{IS}	0.053	-0.009	0.097	F_{IS}	0.065	0.079	-0.012
Lca 22				*Prs* 248			
# alleles	14	14	17	# alleles	21	23	21
A_R	8.45	9.62	8.92	A_R	13.40	12.61	12.88
H_E	0.686	0.741	0.712	H_E	0.889	0.851	0.874
P_{HW}	0.166	0.332	0.016	P_{HW}	0.464	0.398	0.304
F_{IS}	0.013	0.002	0.055	F_{IS}	-0.010	0.006	0.047
Lca 43				*Prs* 257			
# alleles	10	9	11	# alleles	16	16	16
A_R	6.41	5.98	6.19	A_R	12.95	12.57	12.50
H_E	0.535	0.553	0.530	H_E	0.903	0.909	0.904
P_{HW}	0.608	0.767	0.674	P_{HW}	0.343	0.140	0.390
F_{IS}	-0.028	-0.006	0.017	F_{IS}	0.021	0.013	0.005
Lca 64				*Prs* 260			
# alleles	12	13	14	# alleles	4	7	5
A_R	7.19	7.54	6.97	A_R	3.45	3.39	3.50
H_E	0.777	0.778	0.764	H_E	0.361	0.390	0.339
P_{HW}	0.251	0.704	0.029	P_{HW}	0.509	0.704	0.188
F_{IS}	0.027	-0.025	0.014	F_{IS}	-0.011	-0.005	-0.045
Lca 91				*Prs* 275			
# alleles	6	7	7	# alleles	9	9	11
A_R	4.49	4.16	4.43	A_R	5.46	4.91	5.25
H_E	0.608	0.559	0.580	H_E	0.635	0.595	0.590
P_{HW}	0.002	0.956	0.160	P_{HW}	**0.000**	0.618	0.186
F_{IS}	0.002	-0.066	-0.021	F_{IS}	**0.105**	-0.014	0.011

Lca 107

# alleles	11	11	12
A_R	8.67	8.59	7.90
H_E	0.809	0.806	0.796
P_{HW}	0.870	0.447	0.768
F_{IS}	0.013	0.006	-0.031

Prs 55

# alleles	8	9	7
A_R	3.30	3.82	3.60
H_E	0.158	0.228	0.209
P_{HW}	0.368	0.320	0.106
F_{IS}	-0.030	0.001	0.073

Prs 137

# alleles	13	17	13
A_R	7.83	7.92	8.33
H_E	0.706	0.700	0.711
P_{HW}	0.126	0.333	**0.000**
F_{IS}	0.049	0.071	**0.125**

Prs 221

# alleles	16	19	20
A_R	9.78	10.26	9.73
H_E	0.791	0.802	0.792
P_{HW}	0.037	0.048	0.099
F_{IS}	0.018	0.050	0.053

Prs 229

# alleles	8	8	7
A_R	5.96	5.44	5.39
H_E	0.470	0.508	0.486
P_{HW}	0.691	0.402	0.778
F_{IS}	0.038	0.088	0.005

Prs 282

# alleles	14	14	13
A_R	8.57	8.62	8.06
H_E	0.664	0.669	0.623
P_{HW}	0.255	0.046	0.079
F_{IS}	-0.006	0.072	-0.035

Prs 303

# alleles	7	11	13
A_R	5.02	5.77	5.29
H_E	0.365	0.416	0.375
P_{HW}	0.744	0.558	0.006
F_{IS}	-0.029	-0.012	-0.026

Prs 328

# alleles	6	6	8
A_R	3.70	4.06	3.53
H_E	0.555	0.557	0.557
P_{HW}	0.008	0.003	0.036
F_{IS}	0.072	-0.086	0.020

Prs 333

# alleles	8	8	6
A_R	4.22	3.98	4.70
H_E	0.288	0.294	0.371
P_{HW}	0.011	0.639	0.002
F_{IS}	0.055	0.013	0.128

Table 2b. Summary statistics for 19 microsatellites from the 1997 year class (cohort) of red snapper (*Lutjanus campechanus*) sampled from three regions in the northern Gulf of Mexico. A_R is allelic richness; H_E is expected heterozygosity (gene diversity); P_{HW} is probability of conforming to expected Hardy-Weinberg genotypic proportions; and F_{IS} is an inbreeding coefficient. Bold indicates significant departures from HW equilibrium following (sequential) Bonferroni correction.

Micro-satellite	Texas	Louisiana	Mississippi-Alabama	Micro-satellite	Texas	Louisiana	Mississippi-Alabama
Lca 20				*Prs* 240			
#alleles	6	6	6	# alleles	20	22	18
A_R	3.72	3.35	3.78	A_R	14.22	15.37	14.34
H_E	0.238	0.184	0.206	H_E	0.897	0.898	0.885
P_{HW}	0.120	0.563	0.006	P_{HW}	0.003	0.008	0.329
F_{IS}	0.042	0.043	0.082	F_{IS}	0.092	0.043	-0.021
Lca 22				*Prs* 248			
# alleles	16	15	15	# alleles	21	21	22
A_R	9.88	9.36	9.45	A_R	12.58	12.91	12.67
H_E	0.769	0.757	0.771	H_E	0.872	0.867	0.882
P_{HW}	**0.000**	0.891	0.084	P_{HW}	0.159	0.613	0.333
F_{IS}	**0.106**	0.004	-0.009	F_{IS}	0.001	0.030	0.017
Lca 43				*Prs* 257			
# alleles	9	11	12	# alleles	17	18	17
A_R	6.22	6.48	6.19	A_R	13.24	12.86	13.51
H_E	0.587	0.536	0.528	H_E	0.908	0.898	0.915
P_{HW}	0.325	0.665	0.988	P_{HW}	0.299	0.109	0.456
F_{IS}	0.010	-0.049	-0.003	F_{IS}	0.011	0.008	0.005
Lca 64				*Prs* 260			
# alleles	11	11	13	# alleles	5	6	7
A_R	7.33	6.93	6.90	A_R	3.70	3.41	3.88
H_E	0.784	0.765	0.769	H_E	0.367	0.344	0.429
P_{HW}	0.084	0.732	0.496	P_{HW}	0.274	0.781	0.323
F_{IS}	0.027	0.020	0.012	F_{IS}	-0.019	0.026	-0.002
Lca 91				*Prs* 275			
# alleles	6	7	7	# alleles	7	8	9
A_R	4.22	4.38	4.43	A_R	5.00	5.07	4.61
H_E	0.560	0.575	0.570	H_E	0.608	0.612	0.579
P_{HW}	0.893	0.931	0.005	P_{HW}	0.709	0.346	0.441
F_{IS}	-0.070	0.039	0.030	F_{IS}	0.034	0.015	0.031

Lca 107

# alleles	10	11	11
A_R	7.90	8.07	8.15
H_E	0.799	0.798	0.775
P_{HW}	0.259	0.675	0.350
F_{IS}	-0.104	-0.015	-0.045

Prs 55

# alleles	6	6	6
A_R	4.34	3.62	3.52
H_E	0.266	0.210	0.221
P_{HW}	0.102	0.523	0.199
F_{IS}	-0.017	-0.052	0.051

Prs 137

# alleles	13	12	13
A_R	7.88	7.43	8.00
H_E	0.721	0.694	0.715
P_{HW}	0.141	**0.000**	0.046
F_{IS}	0.008	**0.105**	0.019

Prs 221

# alleles	19	17	18
A_R	9.78	10.26	9.73
H_E	0.800	0.792	0.802
P_{HW}	0.110	0.007	0.801
F_{IS}	0.016	0.100	-0.025

Prs 229

# alleles	7	9	9
A_R	5.05	5.08	5.51
H_E	0.495	0.464	0.527
P_{HW}	**0.000**	**0.000**	0.018
F_{IS}	**0.081**	**0.181**	0.118

Prs 282

# alleles	13	12	12
A_R	8.46	8.40	7.89
H_E	0.636	0.639	0.614
P_{HW}	0.882	0.554	0.143
F_{IS}	-0.051	0.028	0.022

Prs 303

# alleles	10	12	9
A_R	5.33	5.17	6.05
H_E	0.375	0.400	0.400
P_{HW}	0.536	0.350	0.763
F_{IS}	-0.010	-0.011	-0.055

Prs 328

# alleles	6	5	6
A_R	3.54	3.45	3.71
H_E	0.542	0.545	0.568
P_{HW}	0.321	0.112	0.182
F_{IS}	0.090	-0.018	0.007

Prs 333

# alleles	6	6	7
A_R	3.84	4.52	4.20
H_E	0.342	0.320	0.323
P_{HW}	0.579	0.811	0.409
F_{IS}	0.029	-0.022	0.032

Table 2c. Summary statistics for 19 microsatellites from the 1999 year class (cohort) of red snapper (*Lutjanus campechanus*) sampled from three regions in the northern Gulf of Mexico. A_R is allelic richness; H_E is expected heterozygosity (gene diversity); P_{HW} is probability of conforming to expected Hardy-Weinberg genotypic proportions; and F_{IS} is an inbreeding coefficient. Bold indicates significant departures from HW equilibrium following (sequential) Bonferroni correction.

Micro-satellite	Texas	Louisiana	Mississippi-Alabama
Lca 20			
#alleles	3	5	4
A_R	2.59	3.17	3.76
H_E	0.120	0.203	0.179
P_{HW}	1.000	0.181	1.000
F_{IS}	-0.049	0.170	-0.064
Lca 22			
# alleles	11	10	10
A_R	8.50	8.39	8.43
H_E	0.722	0.741	0.729
P_{HW}	0.072	0.806	0.964
F_{IS}	0.115	-0.121	-0.067
Lca 43			
# alleles	9	8	6
A_R	6.51	5.15	6.48
H_E	0.593	0.532	0.635
P_{HW}	0.388	0.205	0.012
F_{IS}	0.070	0.086	0.175
Lca 64			
# alleles	10	9	11
A_R	6.84	7.64	7.40
H_E	0.775	0.772	0.777
P_{HW}	0.572	0.671	0.920
F_{IS}	-0.009	-0.010	0.019
Lca 91			
# alleles	5	7	5
A_R	4.29	4.15	5.47
H_E	0.588	0.586	0.599
P_{HW}	0.911	0.605	0.043
F_{IS}	-0.056	0.003	0.031
Prs 240			
# alleles	18	18	18
A_R	14.27	14.55	16.22
H_E	0.904	0.891	0.915
P_{HW}	0.747	0.299	0.134
F_{IS}	-0.025	0.024	0.032
Prs 248			
# alleles	20	15	20
A_R	14.03	14.34	11.97
H_E	0.878	0.896	0.860
P_{HW}	0.054	**0.000**	0.629
F_{IS}	-0.043	**0.087**	0.003
Prs 257			
# alleles	14	13	14
A_R	12.54	13.25	12.18
H_E	0.889	0.917	0.904
P_{HW}	0.002	0.680	0.027
F_{IS}	0.021	-0.033	0.139
Prs 260			
# alleles	5	5	4
A_R	3.54	2.93	4.07
H_E	0.399	0.279	0.392
P_{HW}	0.274	1.000	0.946
F_{IS}	0.050	-0.069	-0.093
Prs 275			
# alleles	6	7	6
A_R	4.42	4.79	5.88
H_E	0.593	0.564	0.611
P_{HW}	0.302	0.190	0.224
F_{IS}	0.026	-0.059	0.117

Lca 107

# alleles	11	10	11
A_R	9.05	8.57	9.05
H_E	0.823	0.796	0.818
P_{HW}	0.198	0.662	0.040
F_{IS}	0.043	0.086	-0.002

Prs 55

# alleles	7	3	7
A_R	3.93	4.09	2.43
H_E	0.248	0.138	0.175
P_{HW}	0.691	0.202	1.000
F_{IS}	0.058	0.151	-0.089

Prs 137

# alleles	10	11	11
A_R	7.70	7.88	9.08
H_E	0.688	0.721	0.732
P_{HW}	0.029	0.002	0.301
F_{IS}	-0.015	0.154	0.111

Prs 221

# alleles	13	12	13
A_R	9.72	8.91	8.57
H_E	0.805	0.788	0.748
P_{HW}	0.605	0.450	0.579
F_{IS}	0.043	-0.088	0.003

Prs 229

# alleles	6	6	7
A_R	5.16	5.88	5.73
H_E	0.537	0.589	0.556
P_{HW}	0.521	0.643	0.502
F_{IS}	0.119	-0.014	0.002

Prs 282

# alleles	13	12	12
A_R	7.65	8.92	7.39
H_E	0.622	0.661	0.623
P_{HW}	0.883	0.313	0.497
F_{IS}	0.029	0.136	0.109

Prs 303

# alleles	9	6	8
A_R	5.31	5.38	4.28
H_E	0.421	0.393	0.427
P_{HW}	0.255	0.809	0.242
F_{IS}	0.100	-0.038	0.108

Prs 328

# alleles	7	4	5
A_R	4.02	3.53	3.42
H_E	0.556	0.540	0.568
P_{HW}	0.563	0.234	0.245
F_{IS}	0.054	0.158	0.022

Prs 333

# alleles	6	6	6
A_R	4.27	4.48	4.66
H_E	0.268	0.280	0.339
P_{HW}	0.907	0.878	0.311
F_{IS}	-0.113	-0.022	-0.064

Table 2d. Summary statistics for 19 microsatellites from the 2000 year class (cohort) of red snapper (*Lutjanus campechanus*) sampled from three regions in the northern Gulf of Mexico. A_R is allelic richness; H_E is expected heterozygosity (gene diversity); P_{HW} is probability of conforming to expected Hardy-Weinberg genotypic proportions; and F_{IS} is an inbreeding coefficient.

Micro-satellite	Texas	Louisiana	Mississippi-Alabama
Lca 20			
#alleles	3	4	3
A_R	2.61	2.95	3.59
H_E	0.090	0.121	0.230
P_{HW}	1.000	1.000	1.000
F_{IS}	-0.028	-0.037	-0.087
Lca 22			
# alleles	11	9	8
A_R	9.44	7.61	7.73
H_E	0.733	0.672	0.707
P_{HW}	0.030	0.508	0.937
F_{IS}	-0.079	-0.201	-0.118
Lca 43			
# alleles	8	7	6
A_R	6.77	5.95	6.44
H_E	0.571	0.547	0.594
P_{HW}	0.847	0.670	0.310
F_{IS}	0.003	-0.028	-0.072
Lca 64			
# alleles	11	7	7
A_R	8.21	6.82	6.23
H_E	0.786	0.801	0.784
P_{HW}	0.928	0.173	0.717
F_{IS}	0.022	0.064	-0.014
Lca 91			
# alleles	7	5	6
A_R	4.62	5.70	4.91
H_E	0.574	0.565	0.620
P_{HW}	0.086	0.097	0.142
F_{IS}	-0.072	0.115	0.230
Prs 240			
# alleles	17	16	15
A_R	14.40	15.00	14.45
H_E	0.880	0.921	0.881
P_{HW}	0.795	0.010	0.200
F_{IS}	-0.082	-0.005	0.098
Prs 248			
# alleles	18	12	12
A_R	14.36	11.35	10.74
H_E	0.902	0.861	0.833
P_{HW}	0.628	0.274	0.811
F_{IS}	-0.006	-0.016	-0.119
Prs 257			
# alleles	16	14	13
A_R	13.30	12.85	13.18
H_E	0.907	0.898	0.910
P_{HW}	0.211	0.522	0.608
F_{IS}	-0.007	-0.041	0.039
Prs 260			
# alleles	5	4	4
A_R	3.72	3.84	3.85
H_E	0.338	0.398	0.462
P_{HW}	0.703	0.010	0.814
F_{IS}	0.044	0.371	0.016
Prs 275			
# alleles	6	5	5
A_R	5.38	4.69	4.80
H_E	0.649	0.586	0.608
P_{HW}	0.226	0.312	0.075
F_{IS}	0.028	-0.094	0.215

Lca 107

# alleles	10	9	7
A_R	8.93	6.87	8.39
H_E	0.827	0.795	0.794
P_{HW}	0.771	0.213	0.543
F_{IS}	0.015	-0.136	0.027

Prs 55

# alleles	6	4	2
A_R	3.87	2.00	3.26
H_E	0.149	0.119	0.213
P_{HW}	1.000	1.000	0.015
F_{IS}	-0.047	-0.051	0.126

Prs 137

# alleles	9	11	11
A_R	7.52	9.33	9.67
H_E	0.685	0.759	0.693
P_{HW}	0.407	0.249	0.813
F_{IS}	0.057	0.135	0.016

Prs 221

# alleles	14	13	8
A_R	10.43	7.77	10.43
H_E	0.790	0.753	0.800
P_{HW}	0.638	0.282	0.265
F_{IS}	-0.012	0.129	0.034

Prs 229

# alleles	5	6	5
A_R	4.35	4.69	5.57
H_E	0.530	0.436	0.550
P_{HW}	0.271	1.000	0.082
F_{IS}	0.101	-0.002	0.215

Prs 282

# alleles	11	10	10
A_R	8.48	9.33	9.15
H_E	0.670	0.675	0.702
P_{HW}	0.887	0.476	0.364
F_{IS}	0.059	0.167	0.062

Prs 303

# alleles	8	4	6
A_R	6.19	5.53	3.94
H_E	0.466	0.478	0.387
P_{HW}	0.494	0.375	0.853
F_{IS}	0.043	0.150	-0.057

Prs 328

# alleles	5	5	4
A_R	3.63	3.69	4.22
H_E	0.541	0.531	0.588
P_{HW}	0.945	1.000	0.875
F_{IS}	-0.024	-0.001	-0.004

Prs 333

# alleles	4	4	5
A_R	3.46	4.82	3.47
H_E	0.237	0.356	0.280
P_{HW}	1.000	1.000	0.197
F_{IS}	-0.105	-0.142	-0.057

Table 3a. Probability values of (temporal) 'genic' homogeneity at each of 19 microsatellites among four cohorts (1995, 1997, 1999, and 2000 year classes) of red snapper (*Lutjanus campechanus*) at three geographic localities in the northern Gulf of Mexico. Probability values are based on exact tests, with 1000 permutations. Bold indicates $P < 0.05$ following (sequential) Bonferroni correction.

Micro-satellite	Texas	Louisiana	Mississippi-Alabama	Micro-satellite	Texas	Louisiana	Mississippi-Alabama
Lca 20	0.119	0.788	0.062	*Prs* 240	0.026.	0.519	**0.000**
Lca 22	**0.000**	0.368	0.049	*Prs* 248	0.788	0.304	0.765
Lca 43	0.420	0.144	0.025	*Prs* 257	0.341	0.156	0.076
Lca 64	0.685	0.411	0.163	*Prs* 260	0.321	0.277	0.051
Lca 91	0.016	0.059	0.126	*Prs* 275	0.673	0.665	0.847
Lca 107	0.466	0.635	0.404	*Prs* 282	0.363	0.722	0.161
Prs 55	0.029	0.287	0.906	*Prs* 303	**0.004**	0.075	0.119
Prs 137	0.540	0.459	0.067	*Prs* 328	0.577	0.485	0.825
Prs 221	0.102	0.966	0.593	*Prs* 333	0.180	0.757	0.897
Prs 229	0.213	0.060	0.247	Overall	**0.000**	0.264	**0.000**

Table 3b. Probability values of (temporal) 'genotypic' homogeneity at each of 19 microsatellites among four cohorts (1995, 1997, 1999, and 2000 year classes) of red snapper (*Lutjanus campechanus*) at three geographic localities in the northern Gulf of Mexico. Probability values are based on exact tests, with 1000 permutations. Bold indicates $P < 0.05$ following (sequential) Bonferroni correction.

Micro-satellite	Texas	Louisiana	Mississippi-Alabama	Micro-satellite	Texas	Louisiana	Mississippi-Alabama
Lca 20	0.038	0.687	0.109	*Prs* 240	0.057	0605.	**0.000**
Lca 22	**0.000**	0.158	0.025	*Prs* 248	0.871	0.421	0.599
Lca 43	0.450	0.130	0.043	*Prs* 257	0.393	0.211	0.085
Lca 64	0.718	0.470	0.167	*Prs* 260	0.329	0.398	0.075
Lca 91	0.027	0.100	0.313	*Prs* 275	0.703	0.564	0.932
Lca 107	0.523	0.513	0.602	*Prs* 282	0.377	0.740	0.304
Prs 55	0.057	0.189	0.699	*Prs* 303	**0.002**	0.088	0.039
Prs 137	0.642	0.652	0.187	*Prs* 328	0.776	0.388	0.913
Prs 221	0.185	0.893	0.570	*Prs* 333	0.145	0.845	0.747
Prs 229	0.122	0.072	0.411	Overall	**0.000**	0.296	**0.000**

Table 4. Probability values of genic and genotypic homogeneity at 19 microsatellites among spatial and temporal samples (12 total) of red snapper (*Lutjanus campechanus*) sampled from the northern Gulf of Mexico. Tests of genic homogeneity assume random mating within samples, whereas tests of genotypic homogeneity do not. Probability values are based on exact tests; significance was assessed via a Markov chain method, using 500 demomorizations, 500 batches, 5000 iterations per batch. Bold indicates $P < 0.05$ following (sequential) Bonferroni correction.

Micro-Satellite	Genic homogeneity	Genotypic homogeneity
Lca 20	0.193	0.123
Lca 22	**0.001**	**0.000**
Lca 43	0.033	0.067
Lca 64	0.093	0.109
Lca 91	**0.000**	**0.001**
Lca 107	0.435	0.460
Prs 55	0.134	0.107
Prs 137	0.194	0.437
Prs 221	0.761	0.747
Prs 229	0.038	0.042
Prs 240	**0.000**	**0.000**
Prs 248	0.461	0.564
Prs 257	0.056	0.084
Prs 260	0.102	0.173
Prs 275	0.817	0.887
Prs 282	0.104	0.219
Prs 303	**0.001**	**0.000**
Prs 328	0.417	0.550
Prs 333	0.720	0.619
Overall	**0.000**	**0.000**

Table 5. Probability values of genic (upper diagonal) and genotypic (lower diagonal) homogeneity at 19 microsatellites among all (12 total) temporal and spatial samples of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico. Tests of genic homogeneity assume random mating within samples, whereas tests of genotypic homogeneity do not. Probability values are based on exact tests; significance was assessed via a Markov chain method, using 500 demomorizations, 500 batches, 5000 iterations per batch. Bold indicates $P < 0.05$ following (sequential) Bonferroni correction.

	TX '95	*LA '95*	*AL '95*	*TX '97*	*LA '97*	*AL '97*	*TX '99*	*LA '99*	*AL '99*	*TX '00*	*LA '00*	*AL '00*
TX '95	---	**0.000**	**0.000**	**0.000**	0.019	**0.000**	0.010	0.142	0.190	0.064	0.109	0.024
LA '95	0.002	---	0.009	0.004	0.660	**0.000**	0.395	0.417	0.431	0.270	0.048	0.151
AL '95	0.001	0.030	---	**0.000**	0.735	**0.000**	0.120	0.199	0.118	0.459	0.111	0.054
TX '97	**0.000**	0.012	**0.000**	---	0.073	0.035	0.017	0.076	0.007	0.387	0.271	0.065
LA '97	0.039	0.743	0.739	0.078	---	0.025	0.196	0.494	0.170	0.418	0.029	0.045
AL '97	**0.000**	**0.000**	**0.000**	0.056	0.068	---	0.005	0.012	0.115	0.147	0.035	0.104
TX '99	0.016	0.409	0.164	0.026	0.242	0.002	---	0.326	0.502	0.819	0.202	0.639
LA '99	0.234	0.479	0.296	0.089	0.631	0.014	0.594	---	0.306	0.611	0.089	0.063
AL '99	0.201	0.681	0.294	0.011	0.307	0.196	0.569	0.326	---	0.520	0.300	0.745
TX '00	0.074	0.406	0.781	0.551	0.642	0.337	0.926	0.638	0.552	---	0.585	0.473
LA '00	0.042	0.019	0.072	0.087	0.007	0.006	0.085	0.056	0.130	0.457	---	0.079
AL '00	0.018	0.178	0.134	0.075	0.082	0.073	0.755	0.152	0.850	0.502	0.041	---

Table 6. Results of hierarchical analysis of molecular variance (AMOVA) among sampling localities (spatial) and among cohorts (year classes) within localities (temporal). Distance(s) between/among alleles is based on the sum of square size differences assuming a stepwise mutation model. Degrees of freedom are in parentheses.

Variance component	Observed partition			
	Variance	% total	Φ values	P*
Among localities (2)	-0.0368	-0.07	-0.0007	0.690
Among cohorts/within localities (9)	0.0523	0.09	0.009	0.076
Within samples (3990)	55.6494	99.97	---	

* Probability based on 20,022 permutations.

Table 7. Estimates of F_{ST} from pairwise comparisons for allele distributions at 19 microsatellites among all (12 total) temporal and spatial samples of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico. Boldface indicates F_{ST} values that differ significantly from zero. Zero values are given for those F_{ST} estimates that were less than zero (negative).

Sample	*TX '95*	*LA '95*	*AL '95*	*TX '97*	*LA '97*	*AL '97*	*TX '99*	*LA '99*	*AL '99*	*TX '00*	*LA '00*	*AL '00*
TX '95	---	**0.0012**	**0.0010**	**0.0013**	0.0010	**0.0020**	0.0010	0.0003	0.0003	0.0000	0.0000	0.0011
LA '95		---	0.0006	0.0007	0.0000	**0.0009**	0.0004	0.0000	0.0000	0.0002	0.0004	0.0000
AL '95			---	**0.0008**	0.0000	**0.0015**	0.0008	0.0000	0.0002	0.0005	0.0000	0.0002
TX '97				---	0.0005	0.0002	0.0011	0.0000	0.0005	0.0009	0.0000	0.0008
LA '97					---	0.0006	0.0004	0.0000	0.0000	0.0000	0.0005	0.0002
AL '97						---	0.0015	0.0002	0.0013	0.0014	0.0015	0.0006
TX '99							---	0.0000	0.0000	0.0000	0.0000	0.0000
LA '99								---	0.0000	0.0000	0.0000	0.0000
AL '99									---	0.0000	0.0000	0.0000
TX '00										---	0.0000	0.0000
LA '00											---	0.0002
AL '00												---

Table 8. Pseudo-maximum-likelihood (after Wang 2001) estimates of contemporaneous (variance) effective population size (N_{eV}). Estimates of N_eV were corrected for overlapping generations (after Jorde and Ryman 1995, 1996).

Locality	N_{eV}	95% lower bound	95% upper bound
Texas	2,446	1,363	7,922
Louisiana	18,971	3,003	$>5x10^4$
Alabama	1,757	1,114	3,592
Overall	6,117	3,691	14,235

Table 9. Estimates of theta long-term (inbreeding) effective population size (N_{el}) of the 1995 and 1997 cohorts (year classes) of red snapper (*Lutjanus campechanus*) at three localities in the northern Gulf of Mexico. A mutation rate (μ) of 10^{-4} was employed, using the equation $N_{el} = \Theta/4\mu$. Θ values were derived from MIGRATE (Beerli and Felsenstein 2001).

Sample	N_{el}	95% lower bound	95% upper bound
Texas – 1995	1,710	1,334	1,857
Texas – 1997	1,955	1,837	2,097
Louisiana – 1995	2,200	2,052	2,367
Louisiana – 1997	3,047	2,870	3,242
Alabama – 1995	1,707	1,585	1,845
Alabama – 1997	2,007	1,872	2,147

Table 10. Results of M^* tests to detect recent reductions in (contemporaneous) effective population size among red snapper (*Lutjanus campechanus*) sampled from the 1995 and 1997 cohorts (year classes) at three localities in the northern Gulf of Mexico.

Sample	Avg. M	Avg. M_c	Probability
Texas '95	0.838	0.821	0.090
Texas '97	0.835	0.817	0.101
Louisiana '95	0.899	0.810	0.549
Louisiana '97	0.883	0.798	0.543
Alabama '95	0.852	0.820	0.152
Alabama '97	0.883	0.816	0.393

* Based on Θvalues of 0.684 and 0.785 (TX '95 and '97), 0.880 and 1.220 (LA '95 and '97), and 0.683 and 0.803 (AL '95 and '97) derived from MIGRATE (Beerli and Felsenstein 2001).

Table 11. Summary statistics for each of eleven microsatellites and mitochondrial (mt)DNA for three samples of red snapper (*Lutjanus campechanus*) obtained offshore of Galveston, Texas.

Genetic marker	*Lca*20	*Lca*22	*Lca*43	*Lca*91	*Lca*107	*Prs*55	*Prs*229	*Prs*240	*Prs*260	*Prs*303	*Prs*333	mtDNA[a]
Reference												
n	76	76	76	76	75	76	76	75	74	76	76	75
# alleles	5	11	6	6	12	5	6	17	3	6	4	14
P_{HW}	0.024	0.524	0.261	0.667	0.701	0.166	0.685	0.775	0.637	0.150	1.000	--
Allelic richness	3.75	8.02	5.19	4.38	8.81	3.52	5.07	13.70	2.94	3.63	2.85	12.04
Gene diversity[a]	0.250	0.676	0.529	0.611	0.801	0.217	0.504	0.878	0.370	0.268	0.225	0.766
f	0.211	0.027	0.046	0.095	0.049	0.093	0.035	0.002	0.081	0.079	0.004	--
Bycatch A												
n	120	118	120	116	122	123	123	117	121	115	123	122
# alleles	5	12	8	6	9	6	8	18	4	10	7	20
P_{HW}	0.276	0.093	0.278	0.099	0.437	0.281	0.314	0.393	0.862	0.842	0.198	--
Allelic richness	3.20	8.00	5.82	4.18	7.95	3.64	5.59	13.37	3.31	5.55	4.25	14.26
Gene diversity[a]	0.179	0.735	0.481	0.562	0.810	0.197	0.511	0.880	0.348	0.390	0.284	0.789
f	0.115	0.002	0.030	0.111	0.002	0.092	0.015	0.000	0.021	0.041	0.032	--
Bycatch B												
N	40	39	40	40	39	40	40	24	38	40	40	40
# alleles	4	10	6	5	8	4	5	12	3	7	6	9
P_{HW}	0.099	0.096	0.322	0.330	0.428	1.000	0.617	0.413	1.000	0.536	0.075	--

Allelic richness	3.20	9.15	5.44	4.14	7.54	3.20	4.84	12.00	2.87	5.72	5.14	9.00
Gene diversity[a]	0.227	0.732	0.600	0.562	0.751	0.207	0.583	0.907	0.295	0.407	0.389	0.767
f	0.342[b]	0.054	0.084	0.201	0.024	0.088	0.014	0.083	0.019	0.046	0.102	--

n -- sample size; P_{HW} - probability of conforming to expected Hardy-Weinberg proportions; *f* - inbreeding coefficient

[a] -- value for mtDNA is nucleon diversity (after Nei, 1987); [b] -- Significant probability ($P < 0.05$) that $f > 0$ before but not after sequential Bonferroni correction

Appendix Table A. Allele frequencies at 19 microsatellites in red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico.

Microsatellite	1995 cohort			1997 cohort			1999 cohort			2000 cohort		
(allele*)	AL 95	LA 95	TX 95	AL 97	LA 97	TX 97	AL 99	LA 99	TX 99	AL 00	LA 00	TX 00
Lca 20 (N)	199	373	286	211	269	272	95	63	77	65	44	32
203	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
205	0.002	0.000	0.000	0.007	0.004	0.007	0.000	0.016	0.000	0.000	0.011	0.000
209	0.009	0.003	0.005	0.004	0.019	0.007	0.006	0.008	0.000	0.000	0.000	0.000
211	0.000	0.000	0.010	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
213	0.066	0.063	0.053	0.063	0.059	0.066	0.078	0.056	0.047	0.031	0.068	0.023
215	0.883	0.908	0.910	0.901	0.888	0.870	0.890	0.905	0.937	0.938	0.875	0.954
217	0.040	0.024	0.023	0.024	0.026	0.047	0.026	0.016	0.016	0.031	0.045	0.023
219	0.000	0.001	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
Lca 22 (N)	198	376	281	208	266	244	94	63	77	62	43	31
215	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
227	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
229	0.002	0.000	0.003	0.000	0.002	0.012	0.000	0.000	0.005	0.000	0.000	0.000
231	0.002	0.003	0.008	0.010	0.004	0.010	0.013	0.000	0.000	0.016	0.000	0.000
232	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
233	0.014	0.008	0.003	0.006	0.011	0.007	0.000	0.008	0.011	0.016	0.000	0.008
235	0.448	0.476	0.503	0.420	0.389	0.399	0.435	0.452	0.468	0.532	0.465	0.452
236	0.025	0.025	0.003	0.037	0.032	0.022	0.013	0.008	0.053	0.000	0.000	0.056
237	0.021	0.027	0.030	0.035	0.039	0.053	0.013	0.024	0.005	0.000	0.000	0.024
239	0.196	0.214	0.225	0.209	0.227	0.188	0.221	0.230	0.213	0.177	0.256	0.234
240	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
241	0.103	0.100	0.073	0.119	0.130	0.178	0.123	0.095	0.096	0.113	0.081	0.065
243	0.071	0.056	0.068	0.068	0.070	0.041	0.058	0.032	0.048	0.000	0.047	0.048
245	0.041	0.029	0.025	0.039	0.030	0.034	0.058	0.071	0.037	0.081	0.093	0.048
247	0.039	0.028	0.028	0.031	0.036	0.024	0.019	0.048	0.048	0.048	0.012	0.032
249	0.014	0.021	0.013	0.014	0.015	0.019	0.045	0.000	0.016	0.016	0.012	0.016
251	0.012	0.009	0.018	0.008	0.011	0.007	0.000	0.032	0.000	0.000	0.023	0.016
253	0.005	0.001	0.003	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000

255	0.002	0.003	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
257	0.000	0.000	0.000	0.004	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
Lca 43 (N)	202	340	275	210	272	272	96	63	76	65	44	32
162	0.113	0.074	0.097	0.088	0.096	0.114	0.059	0.119	0.135	0.094	0.125	0.062
164	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000
172	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
176	0.033	0.041	0.027	0.033	0.026	0.045	0.013	0.024	0.021	0.031	0.023	0.054
178	0.000	0.000	0.000	0.002	0.002	0.000	0.000	0.000	0.005	0.000	0.000	0.000
180	0.002	0.001	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
182	0.002	0.000	0.002	0.002	0.000	0.002	0.000	0.008	0.000	0.000	0.000	0.000
184	0.087	0.084	0.109	0.119	0.101	0.114	0.158	0.183	0.115	0.125	0.045	0.108
186	0.645	0.669	0.663	0.662	0.669	0.617	0.658	0.563	0.609	0.656	0.614	0.638
188	0.098	0.090	0.064	0.051	0.070	0.074	0.099	0.071	0.078	0.063	0.114	0.069
190	0.007	0.004	0.005	0.004	0.006	0.010	0.000	0.008	0.010	0.000	0.000	0.008
192	0.009	0.034	0.020	0.029	0.026	0.024	0.013	0.024	0.021	0.031	0.068	0.054
194	0.002	0.000	0.010	0.004	0.002	0.000	0.000	0.000	0.005	0.000	0.000	0.000
196	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
198	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0.000	0.000	0.000	0.002	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.008
202	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lca 64 (N)	197	377	286	211	271	271	96	63	77	65	44	32
139	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000
151	0.000	0.003	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
153	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
155	0.002	0.004	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008
157	0.021	0.003	0.005	0.002	0.006	0.007	0.006	0.000	0.005	0.016	0.000	0.008
159	0.016	0.020	0.013	0.011	0.009	0.014	0.006	0.032	0.000	0.031	0.000	0.015
161	0.002	0.007	0.005	0.004	0.007	0.000	0.000	0.008	0.000	0.000	0.000	0.000
163	0.281	0.284	0.307	0.284	0.279	0.265	0.292	0.341	0.229	0.219	0.250	0.277
165	0.283	0.276	0.264	0.277	0.292	0.284	0.266	0.214	0.292	0.313	0.193	0.300
167	0.231	0.268	0.218	0.262	0.240	0.230	0.260	0.230	0.281	0.203	0.318	0.200
169	0.079	0.070	0.086	0.094	0.092	0.083	0.084	0.103	0.083	0.109	0.102	0.062
171	0.063	0.054	0.074	0.042	0.055	0.071	0.039	0.048	0.073	0.109	0.114	0.092
173	0.009	0.008	0.018	0.007	0.009	0.033	0.013	0.008	0.021	0.000	0.011	0.015

175	0.007	0.003	0.005	0.007	0.009	0.007	0.019	0.000	0.000	0.000	0.000	0.015
177	0.002	0.000	0.000	0.000	0.002	0.002	0.000	0.000	0.005	0.000	0.000	0.008
179	0.002	0.000	0.003	0.004	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
181	0.003	0.001	0.000	0.000	0.000	0.002	0.006	0.016	0.005	0.000	0.011	0.000
Lca 91 (N)	201	375	285	202	262	268	95	62	77	65	44	30
132	0.009	0.012	0.010	0.009	0.011	0.007	0.000	0.008	0.000	0.033	0.000	0.008
134	0.018	0.013	0.065	0.026	0.010	0.015	0.013	0.016	0.026	0.017	0.045	0.008
136	0.430	0.476	0.480	0.438	0.447	0.502	0.448	0.508	0.500	0.567	0.455	0.492
138	0.505	0.437	0.396	0.481	0.479	0.433	0.461	0.379	0.400	0.350	0.420	0.431
140	0.033	0.052	0.047	0.039	0.038	0.032	0.065	0.056	0.063	0.000	0.045	0.046
142	0.004	0.005	0.002	0.002	0.013	0.010	0.013	0.016	0.011	0.017	0.034	0.008
144	0.002	0.004	0.000	0.004	0.002	0.000	0.000	0.016	0.000	0.017	0.000	0.008
Lca 107 (N)	189	375	286	211	269	264	94	61	77	65	44	31
95	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
97	0.003	0.003	0.008	0.000	0.004	0.002	0.006	0.008	0.011	0.000	0.011	0.008
99	0.031	0.023	0.032	0.038	0.026	0.033	0.019	0.049	0.027	0.016	0.023	0.054
101	0.068	0.081	0.079	0.076	0.054	0.076	0.065	0.057	0.043	0.081	0.045	0.069
103	0.255	0.303	0.272	0.294	0.275	0.268	0.260	0.254	0.234	0.210	0.273	0.208
105	0.311	0.280	0.291	0.290	0.351	0.313	0.331	0.295	0.287	0.339	0.330	0.308
107	0.091	0.101	0.095	0.083	0.097	0.095	0.110	0.098	0.133	0.129	0.068	0.100
109	0.100	0.107	0.101	0.114	0.100	0.095	0.078	0.131	0.090	0.117	0.125	0.085
111	0.093	0.073	0.082	0.074	0.058	0.088	0.097	0.057	0.122	0.048	0.091	0.123
113	0.005	0.004	0.019	0.002	0.009	0.000	0.013	0.025	0.016	0.000	0.000	0.015
115	0.031	0.023	0.019	0.023	0.024	0.028	0.006	0.025	0.032	0.000	0.034	0.031
117	0.003	0.003	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
119	0.007	0.000	0.003	0.006	0.002	0.000	0.013	0.000	0.005	0.000	0.000	0.000
Prs 55 (N)	184	377	277	211	272	272	94	63	77	64	43	32
180	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
182	0.000	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
188	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
190	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
192	0.005	0.004	0.003	0.002	0.009	0.005	0.006	0.008	0.005	0.000	0.000	0.016
194	0.002	0.003	0.003	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000

196	0.025	0.012	0.011	0.024	0.017	0.038	0.013	0.000	0.005	0.000	0.000	0.008
198	0.875	0.886	0.916	0.886	0.879	0.853	0.929	0.905	0.862	0.938	0.884	0.922
200	0.074	0.080	0.057	0.070	0.085	0.073	0.019	0.087	0.101	0.063	0.093	0.039
202	0.016	0.007	0.005	0.013	0.009	0.019	0.019	0.000	0.005	0.000	0.012	0.008
204	0.000	0.005	0.003	0.006	0.002	0.009	0.006	0.000	0.011	0.000	0.012	0.008
208	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
Prs 137 (N)	201	376	286	211	271	272	96	63	77	65	44	32
155	0.000	0.003	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
157	0.010	0.008	0.005	0.006	0.020	0.002	0.013	0.048	0.000	0.000	0.023	0.000
159	0.007	0.012	0.002	0.009	0.015	0.019	0.000	0.000	0.016	0.016	0.000	0.015
161	0.175	0.156	0.157	0.169	0.164	0.147	0.162	0.135	0.135	0.188	0.114	0.185
163	0.000	0.001	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000
165	0.023	0.012	0.027	0.017	0.013	0.017	0.045	0.024	0.016	0.016	0.023	0.023
167	0.002	0.005	0.002	0.002	0.000	0.005	0.000	0.000	0.016	0.031	0.011	0.008
169	0.028	0.025	0.037	0.024	0.033	0.040	0.032	0.056	0.026	0.031	0.057	0.015
171	0.472	0.457	0.470	0.471	0.448	0.445	0.435	0.468	0.474	0.406	0.523	0.485
173	0.017	0.017	0.025	0.020	0.022	0.019	0.013	0.048	0.016	0.016	0.034	0.015
175	0.212	0.230	0.214	0.233	0.234	0.232	0.253	0.167	0.266	0.219	0.148	0.223
177	0.024	0.043	0.032	0.031	0.031	0.059	0.026	0.016	0.016	0.063	0.034	0.015
179	0.021	0.020	0.022	0.015	0.013	0.009	0.006	0.024	0.021	0.015	0.023	0.000
181	0.005	0.003	0.002	0.002	0.004	0.002	0.000	0.008	0.000	0.000	0.000	0.000
183	0.003	0.001	0.000	0.002	0.000	0.002	0.000	0.008	0.000	0.000	0.011	0.000
185	0.000	0.005	0.002	0.000	0.000	0.000	0.006	0.000	0.000	0.016	0.000	0.000
189	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Prs 221 (N)	197	376	282	211	270	271	96	63	77	65	44	32
220	0.004	0.001	0.003	0.002	0.000	0.005	0.000	0.000	0.010	0.000	0.000	0.000
222	0.032	0.040	0.048	0.030	0.041	0.036	0.026	0.016	0.026	0.000	0.034	0.038
224	0.069	0.068	0.061	0.081	0.076	0.073	0.084	0.071	0.104	0.047	0.125	0.108
226	0.170	0.180	0.140	0.173	0.146	0.185	0.227	0.175	0.141	0.188	0.125	0.115
228	0.309	0.314	0.320	0.328	0.317	0.310	0.312	0.413	0.255	0.375	0.284	0.377
230	0.021	0.021	0.025	0.020	0.024	0.038	0.013	0.008	0.016	0.031	0.011	0.015
232	0.027	0.027	0.033	0.017	0.028	0.017	0.006	0.024	0.042	0.016	0.000	0.008
234	0.255	0.263	0.282	0.247	0.257	0.249	0.240	0.222	0.313	0.281	0.307	0.208
236	0.046	0.032	0.041	0.050	0.043	0.026	0.039	0.040	0.036	0.031	0.023	0.046

238	0.025	0.015	0.015	0.013	0.015	0.010	0.026	0.008	0.010	0.000	0.011	0.031
240	0.011	0.009	0.003	0.007	0.011	0.009	0.006	0.008	0.036	0.000	0.034	0.015
242	0.002	0.007	0.000	0.004	0.000	0.002	0.006	0.008	0.005	0.000	0.000	0.000
244	0.004	0.001	0.000	0.004	0.011	0.002	0.000	0.008	0.000	0.000	0.011	0.000
246	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
248	0.000	0.001	0.003	0.000	0.004	0.002	0.006	0.000	0.000	0.000	0.000	0.000
250	0.005	0.007	0.010	0.009	0.013	0.014	0.000	0.000	0.000	0.000	0.011	0.008
252	0.005	0.004	0.008	0.007	0.004	0.002	0.006	0.000	0.005	0.000	0.000	0.008
254	0.004	0.001	0.000	0.002	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000
256	0.004	0.003	0.005	0.002	0.007	0.005	0.000	0.000	0.000	0.031	0.011	0.015
258	0.000	0.007	0.005	0.004	0.002	0.002	0.000	0.000	0.000	0.000	0.011	0.008
260	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
266	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Prs 229 (N)	201	376	285	211	269	271	95	63	77	65	44	32
121	0.000	0.000	0.000	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
123	0.028	0.013	0.017	0.011	0.035	0.019	0.013	0.048	0.021	0.000	0.045	0.008
125	0.007	0.003	0.015	0.004	0.002	0.009	0.019	0.000	0.000	0.000	0.000	0.000
127	0.681	0.699	0.714	0.714	0.664	0.687	0.610	0.643	0.653	0.734	0.648	0.654
129	0.121	0.104	0.087	0.133	0.123	0.147	0.130	0.119	0.137	0.078	0.091	0.146
131	0.044	0.059	0.042	0.037	0.035	0.031	0.042	0.040	0.078	0.016	0.045	0.038
133	0.111	0.102	0.104	0.090	0.126	0.104	0.136	0.127	0.137	0.156	0.159	0.154
135	0.009	0.017	0.015	0.007	0.011	0.002	0.000	0.024	0.011	0.016	0.011	0.000
137	0.000	0.003	0.005	0.002	0.002	0.000	0.013	0.000	0.000	0.000	0.000	0.000
Prs 240 (N)	140	372	276	188	240	234	95	61	77	63	39	27
164	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
180	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008
182	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
184	0.002	0.003	0.007	0.006	0.010	0.000	0.000	0.000	0.000	0.037	0.013	0.000
186	0.013	0.019	0.014	0.011	0.019	0.021	0.006	0.016	0.011	0.000	0.000	0.016
188	0.062	0.054	0.032	0.060	0.033	0.066	0.019	0.066	0.053	0.074	0.064	0.079
190	0.049	0.032	0.046	0.032	0.038	0.035	0.019	0.025	0.026	0.019	0.026	0.056
192	0.027	0.024	0.039	0.021	0.010	0.016	0.026	0.041	0.011	0.000	0.051	0.016
194	0.072	0.059	0.057	0.079	0.073	0.059	0.032	0.049	0.105	0.074	0.103	0.048
196	0.031	0.009	0.029	0.028	0.010	0.008	0.019	0.016	0.016	0.019	0.000	0.000

198	0.065	0.074	0.071	0.049	0.069	0.077	0.071	0.074	0.063	0.037	0.038	0.024
200	0.036	0.032	0.064	0.045	0.044	0.051	0.091	0.057	0.047	0.093	0.064	0.040
202	0.226	0.266	0.164	0.233	0.198	0.202	0.247	0.205	0.211	0.185	0.282	0.278
204	0.109	0.114	0.129	0.114	0.131	0.093	0.104	0.123	0.105	0.074	0.103	0.111
205	0.000	0.000	0.000	0.004	0.023	0.003	0.000	0.000	0.000	0.000	0.000	0.000
206	0.103	0.086	0.121	0.111	0.198	0.170	0.123	0.066	0.105	0.093	0.115	0.079
208	0.060	0.070	0.068	0.062	0.060	0.069	0.058	0.090	0.058	0.056	0.051	0.103
210	0.053	0.051	0.054	0.038	0.029	0.048	0.045	0.025	0.063	0.093	0.013	0.032
212	0.036	0.027	0.032	0.036	0.015	0.040	0.058	0.033	0.063	0.111	0.013	0.048
214	0.025	0.036	0.025	0.026	0.021	0.019	0.045	0.041	0.042	0.019	0.026	0.008
216	0.013	0.015	0.014	0.017	0.006	0.011	0.019	0.025	0.011	0.000	0.026	0.040
218	0.005	0.013	0.021	0.009	0.008	0.003	0.006	0.016	0.005	0.000	0.013	0.016
220	0.009	0.007	0.000	0.013	0.004	0.008	0.006	0.033	0.005	0.000	0.000	0.000
222	0.002	0.001	0.004	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000
224	0.002	0.004	0.007	0.004	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000
264	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Prs 248 (N)	195	372	285	211	272	271	96	63	77	65	44	32
202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000
212	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008
216	0.002	0.003	0.010	0.007	0.000	0.007	0.000	0.000	0.010	0.000	0.000	0.000
218	0.004	0.007	0.003	0.004	0.004	0.005	0.000	0.008	0.000	0.000	0.000	0.000
220	0.007	0.007	0.013	0.006	0.006	0.002	0.013	0.000	0.005	0.016	0.011	0.008
222	0.007	0.005	0.000	0.006	0.004	0.000	0.006	0.000	0.005	0.000	0.000	0.008
224	0.053	0.077	0.079	0.076	0.092	0.073	0.058	0.071	0.104	0.109	0.057	0.077
226	0.002	0.011	0.013	0.018	0.009	0.005	0.013	0.000	0.010	0.000	0.000	0.015
228	0.309	0.250	0.218	0.273	0.213	0.256	0.227	0.294	0.266	0.281	0.330	0.208
230	0.121	0.152	0.118	0.135	0.160	0.147	0.097	0.127	0.130	0.141	0.148	0.108
232	0.051	0.089	0.062	0.061	0.055	0.062	0.065	0.056	0.057	0.047	0.045	0.069
234	0.130	0.110	0.133	0.116	0.110	0.118	0.097	0.135	0.094	0.141	0.080	0.100
236	0.098	0.075	0.108	0.101	0.121	0.102	0.123	0.095	0.073	0.094	0.159	0.115
238	0.067	0.079	0.077	0.052	0.057	0.069	0.078	0.063	0.068	0.094	0.068	0.085
240	0.051	0.042	0.069	0.061	0.064	0.055	0.072	0.048	0.047	0.000	0.034	0.077
242	0.028	0.035	0.028	0.031	0.039	0.026	0.058	0.048	0.042	0.016	0.045	0.015
244	0.016	0.011	0.013	0.006	0.006	0.014	0.006	0.008	0.026	0.000	0.000	0.031
246	0.012	0.011	0.008	0.009	0.013	0.026	0.019	0.008	0.010	0.000	0.011	0.015

248	0.012	0.009	0.008	0.011	0.017	0.009	0.026	0.000	0.010	0.016	0.011	0.015
250	0.009	0.007	0.015	0.009	0.007	0.005	0.006	0.008	0.016	0.000	0.000	0.023
252	0.016	0.008	0.008	0.007	0.006	0.002	0.013	0.016	0.005	0.016	0.000	0.000
254	0.004	0.005	0.013	0.004	0.013	0.007	0.006	0.016	0.010	0.000	0.000	0.023
256	0.000	0.001	0.003	0.002	0.004	0.005	0.006	0.000	0.000	0.000	0.000	0.000
258	0.004	0.005	0.003	0.006	0.002	0.000	0.006	0.000	0.010	0.000	0.000	0.000
260	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000

Prs 257 (N)	165	269	273	206	246	266	93	63	77	58	40	31
109	0.004	0.004	0.000	0.002	0.004	0.002	0.000	0.000	0.011	0.000	0.013	0.000
112	0.104	0.080	0.088	0.085	0.045	0.075	0.045	0.079	0.081	0.048	0.088	0.078
115	0.055	0.063	0.042	0.047	0.057	0.061	0.065	0.032	0.048	0.032	0.088	0.052
118	0.145	0.175	0.191	0.211	0.142	0.170	0.156	0.159	0.237	0.226	0.188	0.190
121	0.101	0.067	0.067	0.064	0.073	0.063	0.091	0.103	0.129	0.048	0.125	0.078
124	0.126	0.138	0.112	0.120	0.114	0.138	0.104	0.159	0.065	0.048	0.113	0.129
127	0.055	0.074	0.076	0.060	0.071	0.075	0.052	0.024	0.048	0.065	0.063	0.078
130	0.088	0.087	0.109	0.090	0.110	0.061	0.078	0.087	0.075	0.048	0.063	0.095
133	0.086	0.072	0.088	0.081	0.079	0.078	0.104	0.095	0.108	0.145	0.075	0.060
136	0.068	0.076	0.061	0.070	0.100	0.090	0.071	0.095	0.070	0.048	0.025	0.095
139	0.079	0.076	0.070	0.064	0.059	0.070	0.078	0.087	0.048	0.113	0.038	0.026
142	0.044	0.048	0.039	0.053	0.057	0.053	0.071	0.032	0.048	0.097	0.075	0.060
145	0.016	0.024	0.024	0.019	0.043	0.024	0.045	0.032	0.022	0.065	0.000	0.026
148	0.026	0.007	0.012	0.024	0.030	0.017	0.026	0.016	0.011	0.000	0.038	0.009
151	0.002	0.004	0.015	0.004	0.002	0.017	0.013	0.000	0.000	0.000	0.013	0.009
154	0.002	0.004	0.003	0.006	0.010	0.002	0.000	0.000	0.000	0.016	0.000	0.017
157	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
160	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
163	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
166	0.000	0.000	0.000	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Prs 260 (N)	189	376	283	211	272	272	95	63	77	65	44	32
105	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
109	0.002	0.001	0.000	0.002	0.002	0.005	0.000	0.000	0.000	0.000	0.000	0.000
111	0.760	0.801	0.786	0.796	0.733	0.782	0.838	0.762	0.753	0.766	0.705	0.800
113	0.007	0.004	0.011	0.006	0.013	0.007	0.006	0.016	0.005	0.016	0.023	0.008
115	0.000	0.001	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

117	0.173	0.130	0.173	0.145	0.162	0.126	0.143	0.167	0.184	0.125	0.205	0.154
123	0.058	0.055	0.074	0.050	0.085	0.078	0.013	0.048	0.053	0.094	0.068	0.031
129	0.000	0.000	0.000	0.002	0.006	0.000	0.000	0.008	0.000	0.000	0.000	0.008
Prs 275 (N)	199	374	286	211	273	272	97	63	77	65	44	32
122	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
123	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
132	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
133	0.000	0.005	0.000	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.008
135	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000
136	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
138	0.007	0.009	0.008	0.009	0.005	0.012	0.006	0.024	0.005	0.000	0.000	0.015
140	0.010	0.016	0.020	0.018	0.007	0.014	0.019	0.024	0.005	0.000	0.023	0.000
142	0.098	0.102	0.103	0.110	0.103	0.107	0.058	0.079	0.098	0.078	0.136	0.123
144	0.563	0.563	0.485	0.535	0.577	0.543	0.578	0.548	0.552	0.547	0.568	0.469
146	0.290	0.279	0.347	0.300	0.280	0.294	0.318	0.294	0.309	0.344	0.239	0.346
148	0.024	0.019	0.025	0.022	0.022	0.028	0.019	0.024	0.031	0.000	0.034	0.031
150	0.002	0.005	0.005	0.002	0.004	0.000	0.000	0.008	0.000	0.000	0.000	0.008
152	0.002	0.001	0.003	0.002	0.000	0.002	0.000	0.000	0.000	0.016	0.000	0.000
Prs 282 (N)	202	377	285	211	273	272	96	63	77	65	44	32
113	0.039	0.017	0.030	0.039	0.016	0.012	0.026	0.008	0.010	0.016	0.000	0.000
117	0.005	0.003	0.000	0.000	0.000	0.000	0.000	0.008	0.005	0.000	0.000	0.000
119	0.253	0.256	0.252	0.235	0.223	0.220	0.260	0.254	0.266	0.250	0.205	0.262
121	0.511	0.554	0.515	0.548	0.575	0.557	0.519	0.556	0.552	0.516	0.500	0.508
123	0.014	0.012	0.015	0.020	0.026	0.026	0.019	0.008	0.016	0.000	0.034	0.038
125	0.054	0.048	0.072	0.048	0.049	0.055	0.045	0.048	0.042	0.047	0.080	0.069
127	0.040	0.032	0.035	0.033	0.046	0.040	0.039	0.079	0.057	0.063	0.034	0.038
129	0.009	0.019	0.005	0.013	0.013	0.031	0.032	0.008	0.010	0.031	0.023	0.015
131	0.012	0.008	0.017	0.009	0.009	0.007	0.006	0.008	0.005	0.016	0.000	0.015
133	0.019	0.020	0.017	0.022	0.020	0.017	0.013	0.008	0.005	0.031	0.023	0.023
135	0.002	0.007	0.002	0.007	0.009	0.005	0.000	0.000	0.005	0.000	0.057	0.008
137	0.012	0.015	0.010	0.011	0.005	0.017	0.019	0.008	0.021	0.016	0.034	0.015
139	0.028	0.009	0.017	0.015	0.007	0.009	0.013	0.008	0.005	0.000	0.011	0.008
141	0.000	0.000	0.010	0.000	0.000	0.005	0.006	0.000	0.000	0.000	0.000	0.000
143	0.002	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000

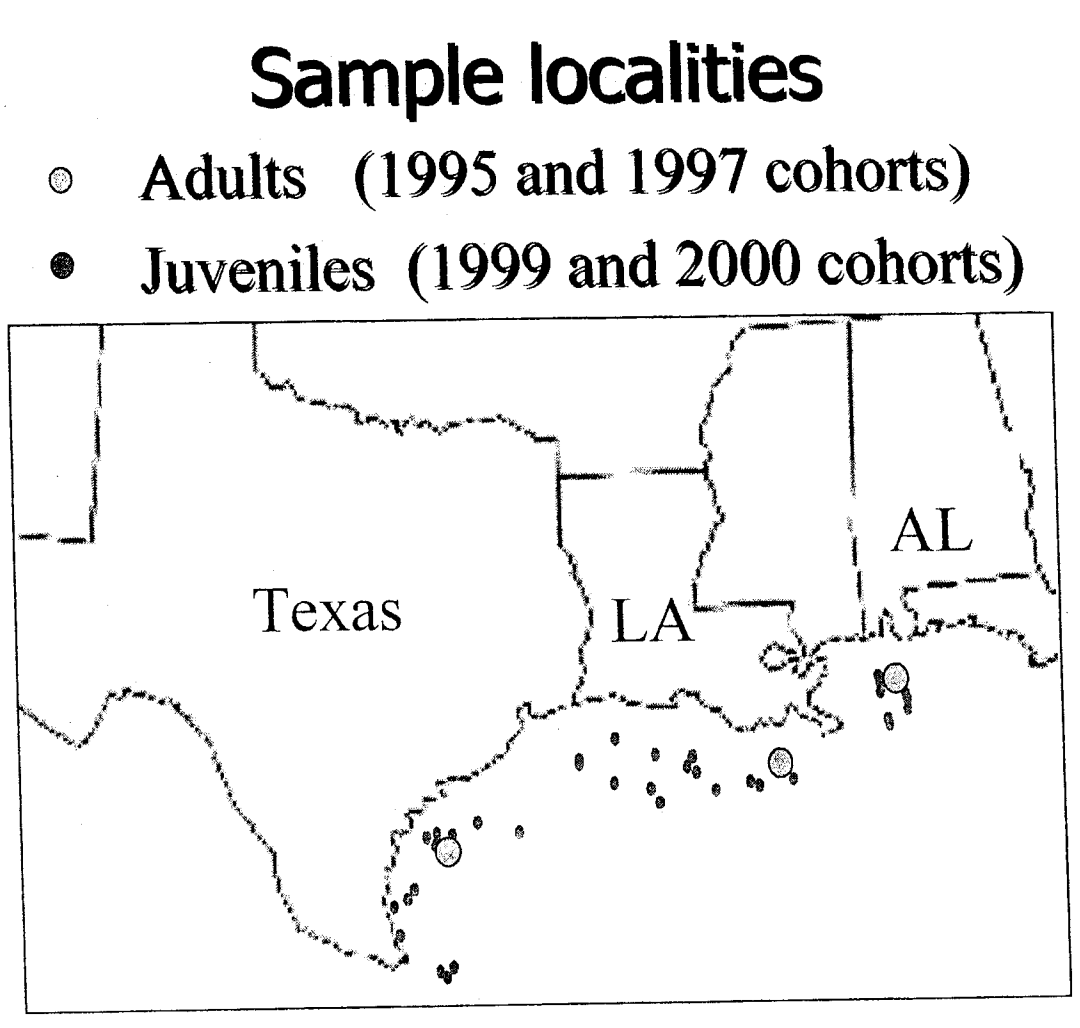
Prs 303 (N)	200	374	285	211	270	272	95	63	76	65	44	32
118	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
122	0.002	0.000	0.000	0.000	0.000	0.002	0.000	0.008	0.011	0.000	0.000	0.000
124	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
126	0.002	0.003	0.003	0.000	0.002	0.000	0.007	0.000	0.005	0.000	0.000	0.000
128	0.035	0.040	0.053	0.057	0.037	0.036	0.072	0.079	0.079	0.000	0.091	0.046
130	0.753	0.781	0.788	0.763	0.765	0.780	0.770	0.738	0.747	0.703	0.773	0.715
132	0.119	0.112	0.118	0.119	0.113	0.126	0.105	0.159	0.126	0.172	0.102	0.146
134	0.023	0.028	0.023	0.031	0.030	0.017	0.020	0.000	0.011	0.000	0.000	0.008
136	0.021	0.021	0.018	0.011	0.019	0.019	0.013	0.008	0.011	0.078	0.000	0.038
138	0.018	0.005	0.000	0.007	0.013	0.007	0.007	0.008	0.005	0.016	0.034	0.023
140	0.004	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
142	0.002	0.003	0.000	0.000	0.006	0.000	0.007	0.000	0.000	0.000	0.000	0.008
144	0.002	0.003	0.000	0.002	0.009	0.005	0.000	0.000	0.000	0.016	0.000	0.015
146	0.002	0.000	0.000	0.000	0.004	0.002	0.000	0.000	0.000	0.016	0.000	0.000
148	0.002	0.000	0.000	0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
150	0.000	0.001	0.000	0.000	0.002	0.007	0.000	0.000	0.000	0.000	0.000	0.000

Prs 328 (N)	200	377	286	211	273	272	97	63	77	65	44	32
196	0.003	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
198	0.002	0.001	0.005	0.000	0.000	0.002	0.000	0.000	0.000	0.016	0.011	0.000
200	0.009	0.005	0.005	0.006	0.009	0.000	0.000	0.008	0.005	0.000	0.011	0.008
202	0.484	0.468	0.518	0.439	0.473	0.526	0.506	0.437	0.454	0.547	0.466	0.485
204	0.456	0.471	0.420	0.511	0.454	0.427	0.455	0.492	0.490	0.422	0.443	0.477
206	0.033	0.049	0.048	0.039	0.057	0.033	0.026	0.063	0.036	0.016	0.068	0.023
208	0.010	0.005	0.005	0.004	0.007	0.009	0.006	0.000	0.005	0.000	0.000	0.008
210	0.000	0.000	0.000	0.002	0.000	0.000	0.006	0.000	0.005	0.000	0.000	0.000
214	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000

Prs 333 (N)	202	371	283	211	272	272	94	61	77	65	44	32
135	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
145	0.009	0.008	0.020	0.013	0.007	0.002	0.013	0.016	0.011	0.016	0.000	0.000
147	0.007	0.008	0.005	0.006	0.004	0.000	0.006	0.000	0.000	0.000	0.000	0.000
149	0.832	0.780	0.837	0.816	0.814	0.799	0.844	0.803	0.851	0.797	0.841	0.869
151	0.117	0.137	0.109	0.114	0.112	0.140	0.091	0.131	0.090	0.094	0.125	0.092

153	0.021	0.028	0.010	0.024	0.033	0.021	0.026	0.025	0.021	0.031	0.011	0.023
155	0.014	0.031	0.015	0.022	0.029	0.036	0.019	0.016	0.021	0.063	0.023	0.015
157	0.000	0.005	0.002	0.006	0.000	0.002	0.000	0.008	0.005	0.000	0.000	0.000
163	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure 1


Sample localities
Adults (1995 and 1997 cohorts)
Juveniles (1999 and 2000 cohorts)
Texas
LA
AL