Population structure and genetic demography of red snapper (*Lutjanus campechanus*) in the U.S. South Atlantic and connectivity with red snapper in the Gulf of Mexico

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**I. Executive Summary**

Genetic population structure of red snapper, *Lutjanus campechanus*, sampled from five localities along the southeastern coast of the United States (Atlantic) and from three localities in the northeastern Gulf of Mexico (eastern Gulf) was assessed using genotypes at16 nuclear-encoded microsatellites and 590 base pairs of the mitochondrialy encoded (mtDNA) ND4 gene. Genotypes at all 16 microsatellites in all localities sampled did not deviate significantly from the expectations of Hardy-Weinberg equilibrium, following Bonferroni correction. MtDNA haplotypes at the eight sample localities consisted primarily of two common haplotypes and numerous rare haplotypes. Conventional approaches (i.e., global exact tests, pairwise tests of *FST* or *ФST* = 0, hierarchical analysis of molecular variance) to test homogeneity of microsatellite alleles and genotypes and mtDNA haplotypes between and among localities and between pooled localities in the Atlantic versus pooled localities in the eastern Gulf, were non-significant. Mantel tests of a correlation between genetic and geographic distance (significant for mtDNA but non-significant for microsatellites) and a nearest-neighbor analysis of mtDNA suggested an isolation-by distance effect, possibly reflecting female philopatry or limited spatial geographic movement of females relative to males. A metric of selective neutrality of mtDNA haplotypes was significant at seven of the localities prior to Bonferroni correction and remained significant following correction at two localities, suggesting possible demographic differences among the localities. A Bayesian approach to estimation of F-statistics produced significant, non-zero estimates of the parameters *θI* and θ*II* (reflecting historical and contemporaneous variance in microsatellite allele frequencies, respectively) among sample localities in the Atlantic, among sample localities in the eastern Gulf, and between sample localities in the Atlantic (pooled) versus sample localities in the eastern Gulf (pooled). Attempts to estimate contemporaneous effective size largely resulted in infinite point estimates or infinitely bounded confidence intervals. Estimates of average, long-term genetic effective size (*NeLT*) among localities ranged from 826 to 2,111 but did not differ significantly from one another; estimates of *NeLT* for localities from the Atlantic (pooled) and localities from the Gulf (pooled) were 3,930 and 4,114, respectively. The estimate of *NeLT* for all eight localities (pooled) was 6,267. The sum of the estimates of *NeLT* for the five localities in the Atlantic was 6,450, considerably larger that the global estimate (3,930) for the region. This is consistent with the ‘propagule pool’ model where migrants come primarily from a nearby subpopulation or stock and where a metapopulation is subdivided into groups with different demographic rates (e.g. average survival and/or reproduction). The sum of the estimates of *NeLT* for the three localities in the eastern Gulf was 4,414, close to the global estimate (4,114) for that region, suggesting few demographic differences among the three eastern-Gulf localities. The sum of the estimates of *NeLT* for the two regions (8,005) is larger than the global estimate for all eight localities (6,267), suggesting that the two regions differ demographically. Estimates of average, long-term migration rates (*m*) between the two regions were 0.0033 (Gulf into the Atlantic) and 0.0021 (Atlantic into the Gulf) and did not differ significantly from one another. Because the estimates of *NeLT* and of *m* for the two regions did not differ, we used an average estimate of *NeLT* (4,022) and of *m* (0.0027) to generate a long-term *mNe* estimate of 10.86, the effective number of migrants moving in either direction from one region to the other. Estimates of *NeLT* represent a weighted harmonic mean of effective size (*Ne*) over a period of 4*N*e generations, with greater weight on more recent generations and on smaller values of *Ne*. The estimate of *m* (0.33%) between the two regions is considerably less than the 10% rate, suggested for contemporaneous migration, beyond which populations are not considered to be demographically independent. Results of the study are consistent with slight genetic and demographic heterogeneity among localities within the two regions, particularly within the Atlantic, and between the two regions. The heterogeneity may reflect the metapopulation structure hypothesized previously for red snapper in the northern Gulf. In summary, there is evidence that genetic and demographic heterogeneity occurs among red snapper across the geographic region surveyed. The signal, however, is weak and precludes definition of geographic boundaries of subpopulations or stocks. More robust genetic approaches (e.g., RADseq) that utilize next-generation sequencing to screen thousands of genetic markers, and have the capability to identify genomic regions under selection are the next logical step in assessing population structure, genetic demography, and connectivity of red snapper across its range in U.S. waters.

**II. Purpose**

Red snapper *Lutjanus campechanus* (Poey 1860) have historically supported important commercial and recreational fisheries along the Atlantic Coast of the southeastern United States (hereafter Atlantic) and the northern (U.S.) coast of the Gulf of Mexico (hereafter Gulf) (Allman and Grimes 2002). Commercial landings of red snapper in the Gulf, for example, averaged 2.6 million pounds between 2007 and 2011, with an average dockside value of $9.6 million; while recreational fishing in the Gulf in 2011 involved > 375,000 target trips and $52.8 million in output impact (GMFMC 2013). Until recently, the major focus in terms of management has been red snapper in the Gulf, where the stock has been considered over-fished and to be undergoing overfishing since at least the late 1980s when the initial rebuilding plan was formulated (Strelcheck and Hood 2007). Although red snapper in the Gulf remain over-fished (NOAA 2012; SEDAR 2013), an assessment of red snapper in the U.S. South Atlantic indicated that red snapper are not only overfished since 1960 but that overfishing is occurring at several times the sustainable level (SEDAR 2008). Factors impacting the decline in red snapper are thought to include high mortality due to directed fisheries, habitat alteration and degradation and mortality of juveniles taken as unintentional harvest (bycatch) in the shrimp fishery, which appears to have been a factor in the Gulf (Schirripa and Legault 1997; Christman 1997; Ortiz et al. 2000).

Red snapper resources in the Atlantic and Gulf are currently managed as separate stocks (Cowan 2011). Genetics studies of adult red snapper, utilizing both nuclear and mitochondrial DNA (mtDNA) markers, have found little evidence of significant population structure across the northern Gulf (Gold et al. 1997: Pruett et al. 2005; Saillant and Gold 2006); Saillant et al. (2010), however, did detect a significant, positive spatial autocorrelation of microsatellite genotypes among age 0 fish sampled within a geographic range of 50–100 km. The lone genetic study of red snapper from both the Atlantic and the Gulf (Garber et al. 2004) involved sequences of the hypervariable, mitochondrial (mtDNA) control region among four localities in the Gulf (140 fish) and one locality in the Atlantic (35 fish). No differences in mtDNA haplotype frequencies were detected, consistent with the null hypothesis that red snapper in the five localities comprised a single, genetic population. MtDNA haplotype diversities (the probability of randomly sampling different haplotypes) in the localities sampled, however, ranged from 0.936 to 1.000 (average across localities of 0.973), indicating that most samples were comprised of unique, singleton mtDNA haplotypes. Effective testing of statistical homogeneity (the null hypothesis) was thus seriously constrained, leaving equivocal the question of whether red snapper from the two regions comprised a unit stock.

Alternatively, life-history data, results of tagging, and/or otolith microchemistry indicate there could be different stocks both within the Gulf and between the Gulf and Atlantic. Significant differences in red snapper reproductive biology (Jackson et al. 2007), growth rate (Fischer et al. 2004), and effective population size (Saillant and Gold 2006) have been found among localities in the Gulf, and Brown-Peterson et al. (2009) found differences in reproductive biology between red snapper sampled along the east coast of Florida and the Dry Tortugas. Studies of red snapper in the Gulf, based on tagging and/or ultrasonic telemetry, have been more equivocal as some (Fable 1980; Szedlmayer 1997; Schroepfer and Szedlmayer 2006; Strelcheck et al. 2007) have shown relatively high site fidelity, while others (Watterson et al. 1998; Patterson et al. 2001; Patterson and Cowan 2003) have reported lower site fidelity. However, there is little to no evidence from tagging studies for movement of red snapper between the Atlantic and the Gulf, and what limited data there are indicate high site fidelity, at least in the Atlantic. Burns et al. (2004) tagged and released roughly 5,000 red snapper in the Atlantic and Gulf (~40% were released in the Atlantic between Cape Canaveral, Florida, to Georgia). Approximately 44% of the more than 400 recaptures were taken within less than 2 km of the tagging site and only 2% or so of the recaptures had moved more than ≥160 km. Two smaller studies carried out in the Atlantic also indicated relatively little movement away from the tagging site (SEDAR 2008).

In this study, we used nuclear-encoded microsatellites and sequences of mtDNA to assess genetic population structure of red snapper sampled in the Atlantic and the eastern Gulf. Characterizing population structure is essential because failure to recognize population structure within an exploited fishery may lead to over-exploitation and depletion of a localized, undetected stock and result in the loss of unique genetic resources inherent in that stock (Carvalho and Hauser 1994; Begg et al. 1999; Hilborn et al. 2003). Loss of genetic resources can compromise long-term sustainability (Hilborn et al*.* 2003), and for fisheries undergoing rebuilding, failure to recognize cryptic stocks can result in failure to anticipate recruitment in those non-identifiewd units (Ruzzante et al*.* 1999). We also attempted to estimate the effective number of breeders (*Nb*) and the average, long-term effective (*NeLT*) size at each sample locality. *Nb* is an estimate of the effective number of breeding individuals in a subpopulation (Waples 1990), while *NeLT* reflects the long-term, relative effects of genetic drift and selection. As long-term sustainability requires maintenance of sufficient genetic resources (Allendorf and Waples 1996), stocks with small *Nb* and/or *Ne* potentially may suffer reduced capacity to respond to changing or novel environmental pressures (Frankham 1995; Higgins and Lynch 2001). Finally, we estimated the average, long-term migration rates between the Gulf and the Atlantic.

**III. Approach**

A total of 669 adult red snapper were sampled dockside between 2008 and 2011 from boats fishing offshore of North Carolina (NC), South Carolina (SC), Georgia (GA), Daytona, Florida (DA), and Melbourne, Florida (ML) in the U.S. South Atlantic (hereafter Atlantic), and offshore of Sarasota, Florida (SA), the Florida Middle Grounds (MG), and Panama City, Florida (PC) in the eastern Gulf of Mexico (hereafter Gulf). Approximate fishing localities are indicated in Figure 1. Tissue samples (fin clips) were obtained by personnel from several state or federal agencies (see Acknowledgements), fixed in 10% DMSO buffer (Seutin *et al*. 1991), and mailed to our laboratory in College Station. DNA was extracted following a modified chelex extraction protocol (Estoup *et al.* 1996); following final centrifugation, 1 µL of the supernatant was used as the template in subsequent polymerase-chain-reaction (PCR) amplification.

All fish were genotyped at 16 nuclear-encoded microsatellites, following multiplex PCR protocols described in Renshaw et al. (2006) and using PCR primers described in Bagley and Geller (1998) and Gold et al. (2001). Amplicons were electrophoresed and visualized on 6% polyacrylamide gels, using an ABI Prism 377 automated sequencer (Applied Biosystems). Allele-calling was conducted manually, using Genescan v.3.1.2 (Applied Biosystems Inc., Warrington, UK) and Genotyper v.2.5 (Perkin Elmer). A fragment of the mitochondrially-encoded NADH dehydrogenase subunit 4 (ND4) gene was amplified for 20 individuals from each locality, using primers ND4LB (Bielawski and Gold 2002) and NAP2 (Arevalo et al. 1994). Thirty microliter PCR reactions consisted of 1x reaction buffer, 1.45 mM MgCl2, 0.25 mM of each dNTP, 30 pmol of each primer, 0.1 U/µL *Taq* polymerase, and 2 µL of DNA template. Reaction conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. Amplified products were purified with ExoSAP-ITTM PCR cleanup kit (GE Healthcare, Piscataway, NJ, USA) and sequenced bi-directionally, using BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems). Five microliter sequencing reactions consisted of 10–50 ng of template, 0.5 μL of BigDye master mix, 0.875 μL of BigDye 5x reaction buffer, and 32 pmol of forward or reverse primer. Sequencing conditions consisted of denaturation at 96°C for 1 min followed by 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Amplifications were electrophoresed on an ABI 3100 Sequencer (Applied Biosystems) through 50 cm capillaries. Sequence chromatograms were aligned and trimmed to a common 590 base pair region, using Sequencher 4.8 (Gene Codes Corporation).

Number of alleles, allelic richness, unbiased gene diversity (expected heterozygosity), and the inbreeding coefficient *FIS* (Weir and Cockerham1984))were calculated for each microsatellite in each locality, using Fstat v.2.9.3.2 (Goudet 2001). Conformance to expectations of Hardy-Weinberg equilibrium (HWE) was tested for each microsatellite in each locality, using exact tests as implemented in Genepop v.4.0.7 (Raymond & Rousset 1995; Rousset 2008). Parameters of the Markov Chain employed in estimation were 10,000 dememorizations, 1,000 batches, and 10,000 iterations per batch. Sequential Bonferroni correction (Rice 1989) was applied for all multiple tests performed simultaneously. Possible occurrence of scoring error due to stuttering, large allele dropout, and/or null alleles was evaluated for each microsatellite in each locality, using Microchecker (van Oosterhout *et al*. 2004). Likelihood-ratio tests of genotypic disequilibrium between pairs of microsatellite within each locality were carried out using Arlequin v.3.5 (Excoffier & Lischer 2010). Homogeneity of allelic richness and unbiased gene diversity among localities was assessed using Friedman rank tests, as implemented in R (R Core Team 2013). For mtDNA sequences, number of haplotypes, haplotype diversity (*h*), and nucleotide diversity (*π*) were obtained for each sample, using Arlequin. Selective neutrality of mtDNA sequences was assessed using Fu and Li’s (1993) *D\** and *F\** statistics and Fu’s (1997) *FS* statistic, as implemented in Dnasp v.5 (Librado & Rozas 2009) and Arlequin, respectively.

Tests of homogeneity of allele and genotype distributions (microsatellites) and haplotype distribution (mtDNA) among localities employed exact tests as implemented in Genepop; exact probabilities were estimated using the same Markov chain approach as above for tests of HWE. The degree of divergence in microsatellites and mtDNA between pairs of localities was estimated as *F*ST and *ФST*, respectively, using Arlequin. For mtDNA, *ФST* values were estimated under a Tamura-Nei substitution model (Tamura and Nei 1993) with a gamma shape parameter of 0.198, as selected by jModelTest v.2.1.1 (Guindon and Gascuel 2003, Darriba et al. 2012). Significance of *FST* and *ФST* values between pairs of localities was assessed by permuting individuals between localities 10,000 times. Correction for multiple testing involved sequential Bonferroni adjustment. Hierarchical analysis of molecular variance (Amova), as implemented in Arlequin, was conducted for both microsatellites and mtDNA by grouping Atlantic localities (NC, SC, GA, DA, and ML) and Gulf localities (SA, MG, and PC) separately; significance of the between-group component of variance was assessed by permuting sample localitiesbetween groups 10,000 times. Mantel tests, implemented in Arlequin, were used to test for correlation between genetic distance and geographic distance for both microsatellites and mtDNA. Distance matrices contained pairwise measures of genetic distance, coded as *FST*/1-*FST* (microsatellites) or *ФST*/1- *ФST* (mtDNA), and linear coastline geographic distance, and were permuted 10,000 times to assess significance. Hudson’s (2000) *Snn* test was applied to the mtDNA data set to determine whether ‘nearest neighbor’ haplotypes (in terms of sequence identity) were sampled within the same locality more often than would be expected in a panmictic population. The test was performed considering each sample locality separately, the Atlantic sample localities pooled, and the Gulf sample localities pooled. To visualize relationships among haplotypes between the Atlantic and Gulf, a minimum-spanning network of mtDNA haplotypes was constructed using Network v.4.6.11 (<http://www.fluxus-engineering.com/>).

Two alternative approaches to testing spatial genetic homogeneity, based on microsatellite data, were used to assess between/among population divergence. The first employed the Bayesian framework in Hickory v 1.1 (Holsinger and Lewis 2002). This approach relaxes the assumption that allele frequencies are uncorrelated among populations, an assumption that does not necessarily hold when a small to moderately large number of populations are sampled (Song et al. 2006). Under this framework, Hickory estimates a number of parameters, including: *θI*, which corresponds to Wright’s (1951) *FST* and reflects variance in allele frequencies across evolutionary time, and *θII*, an analogue to Weir and Cockerham’s (1984) *θ* and which reflects contemporaneous variation between/among populations. Hickory also provides estimates of rho (*ρ*), the among-population correlation of allele frequencies (Holsinger and Lewis 2002). Microsatellite data were separated into three partitions for separate Hickory runs: (i) the five sample localities from the Atlantic; (ii) the three sample localities from the Gulf; and (iii) all sample localities from the Atlantic (pooled) and all sample localities from the Gulf (pooled). Each partition was were run in duplicate under the ‘full’ model in Hickory, with a burn-in period of 500,000 steps, followed by 2 x 108 steps, with samples taken every 100 steps. The R package Boa (Smith 2005) was used to ensure convergence of posterior distributions, combine chains between replicates, and compute 95% HPD estimates for combined chains.

Estimates of the effective number of breeders (*Nb*) were generated for each sample locality, using microsatellite data and the linkage disequilibrium method implemented in LdNe (Waples 2006, Waples & Do 2008). Rare alleles below a frequency of 0.02 were excluded from calculations, following Portnoy *et al*. (2009); confidence intervals were obtained by jackknifing. Estimates of average, long-term effective population size (*NeLT*) for each sample locality and for Atlantic localities (pooled) and Gulf localities (pooled) and estimates of average, long-term migration rate (*m*) between the Atlantic and Gulf were generated using microsatellite data and Migrate-n. A random subsample (n = 50; the smallest individual sample size) was drawn from each locality and replicate runs were combined to generate parameter estimates of theta (*θ*) and *M* (mutation-scaled migration rate), where *θ* = 4*Neμ* (*Ne* is the average, long-term effective population size [*NeLT*] and *μ* is the modal mutation rate across all microsatellites per generation) and *M* = *m/μ*. Estimates of *µ* were obtained using the Bayesian coalescent approach implemented in Msvar v1.3 (Beaumont 1999, Storz and Beaumont 2002). Boa (Smith 2005) was used to estimate the 95% highest posterior density (HPD) interval for the modal value of *µ*. Lower and upper bounds for *NeLT* and *m* were estimated using 95% HPD intervals of *θ* and *M* generated by Migrate-n. Estimates of *m* (migration rate) were generated only for pooled sampled from the Atlantic versus pooled samples from the Gulf.

**IV. Findings**

Summary statistics for microsatellite data are given in Appendix 1. The mean (± SE) number of alleles across microsatellites ranged from 7.44 ± 1.11(PA) to 9.69 ± 1.12 (ML); mean (± SE) allelic richness ranged from 7.40 ± 1.11 (PC) to 8.12 ± 0.97 (ML); and mean (± SE) gene diversity ranged from 0.583± 0.05 (PC) to 0.596 ± 0.05 (ML). Significant deviation from HWE equilibrium prior to Bonferroni correction was found in 13 of 128 tests; no significant deviations were found following correction. Possible null alleles, as inferred by Microchecker, were detected at *Lca*107 (NC), *Ra*6 (SA), *Lca*43 (MG), and *Prs*221 (PC). Following Bonferroni correction, two pairwise tests of genotypic disequilibrium were significant: *Lca*20-*Lca*107 and *Lca*20-*Prs*328, both in SC. Friedman rank tests of homogeneity across sample localities in allelic richness (*AR*) and gene diversity (*HE*) were non-significant (*AR*: *χ*2[7,15] = 4.01, *P* = 0.778; *HE*: *χ*2[7,15] = 3.12, *P* = 0.874). Summary statistics for mtDNA data also are given in Appendix 1. A total of 39 haplotypes were found among the 160 individuals surveyed. Estimates of haplotype (*h*) and nucleotide (*π*) diversity ranged from 0.658 (PC) to 0.905 (ML) and 0.050 (MG) to 0.185 (ML), respectively. Fu & Li’s (1993) *D\** and *F\** statistics were negative in all sample localities and significant prior to but not following Bonferroni correction in SC and MG; all other *D\** and *F\** statistics did not differ significantly from zero. Fu’s (1997) *FS* statistic was negative and significant prior to Bonferroni correction in all sample localities except GA; following correction, *FS* statistics differed significantly from zero only in SC and SA.

Global exact tests of homogeneity in microsatellite allele and genotype distributions and in mtDNA haplotype distribution across all 16 microsatellites were non-significant (allelic: *P* = 0.072; genotypic: *P* = 0.150; haplotypic: *P* = 0.347). Pairwise estimates of *FST* (microsatellites) and *ΦST* (mtDNA) are given in Table 1; none of the pairwise comparisons *FST* or *ΦST* values differed significantly from zero. Hierarchical Amova revealed that the component of molecular variance allocated to between regions (Atlantic versus Gulf) was non-significant for both microsatellites (% variance = 0.02, *P* = 0.319) and mtDNA (% variance = 1.21, *P* = 0.106). Mantel tests of correlation between genetic and geographic distances were non-significant (*P* = 0.161) for microsatellites, but significant (*P* = 0.023) for mtDNA. Hudson’s nearest-neighbor test (mtDNA only) was non-significant (*P* = 0.191) when considering all sample localities separately, but significant (*P* = 0.038) when samples were pooled within Gulf and Atlantic regions. The minimum-spanning network of mtDNA haplotypes by regional locality (Gulf and/or Atlantic) is presented in Figure 2. The distribution of haplotypes in all eight sample localities (Appendix 2) consisted primarily of two common haplotypes (#2 and #4) and numerous rare haplotypes. A total of 21 haplotypes were unique to the Atlantic, while 12 haplotypes were unique to the Gulf. At least four putative clades of two or three haplotypes were found in the Atlantic; none were found in the Gulf.

Bayesian analysis of population structure, using Hickory, produced estimates of *θI* and *θII* (Table 2) that differed significantly from zero among sample localities in the Atlantic, among sample localities in the Gulf, and between sample localities in the Atlantic (pooled) versus sample localities in the Gulf (pooled). Estimate(s) *θI* were an order of magnitude greater than estimates of *θII*. For all three approaches, mean estimates of *ρ* from replicate runs were greater than 0.95 and differed significantly from zero.

Estimates of *Nb* (not shown but available upon request) for seven of the eight sample localities had infinite upper bounds. The lone exception was SC where the upper bound was greater than 10,000. Estimates (and 95% confidence limits) of theta (*θ*) and estimates of average long-term effective size (*NeLT*) for each sample locality, for localities from the Atlantic (pooled) and localities from the Gulf (pooled) and for all eight localities (pooled) are given in Table 3; *NeLT*values were estimated according to *θ* = 4*Neμ* and a modal value of *µ*, obtained from Msvar, of 3.00 x 10-4. Estimates of *NeLT* across sample localities ranged from 826.15 (PC) to 2,111.28 (SA); none of the estimates of *NeLT* differed significantly from one another. Estimates of *NeLT* for Atlantic localities (pooled) and Gulf localities (pooled) were 3,930.50 and 4,114.07, respectively, and did not differ significantly from another. The estimate of *NeLT* for all eight localities pooled was 6,267.08. Finally, the estimate of average, long-term migration rate (*m*) from the Atlantic to the Gulf was 0.21% (95% CI: 0.04% – 0.77%), while the rate from the Gulf to the Atlantic was 0.33% (95% CI: 0.07% – 1.11%). Because the estimates of *NeLT* and of *m* for the two regions did not differ, we used an average estimate of *NeLT* (4,022) and of *m* (0.0027) to generate an average, long-term estimate of 10.86 (*mNe*), the effective number of migrants moving in either direction from one region to the other.

**V. Evaluation**

Conventional approaches (i.e., global exact tests, pairwise tests of *FST* or *ФST*= 0, hierarchical Amova) to test spatial homogeneity of microsatellite alleles and genotypes and mtDNA haplotypes between and among localities and between pooled localities in the Atlantic versus pooled localities in the Gulf, were non-significant. We also found no differences in levels of (microsatellite) variability among localities. These findings are consistent with most prior genetics studies (Gold et al. 1997, Heist and Gold 2000, Gold et al. 2001; Pruett et al. 2005; Saillant and Gold 2006) of adult red snapper in the northern Gulf where significant spatial heterogeneity of alleles, genotypes, or haplotypes was not detected. Spatial genetic differences do exist in the Gulf, however, as Saillant et al. (2010) found significant heterogeneity in microsatellite allele and genotype distributions among age 0 fish sampled at small spatial scales in the western and central Gulf and a significant, positive spatial autocorrelation of microsatellite genotypes within the geographic range 50–100 km. These findings were consistent with a metapopulation model suggested by Pruett et al. (2005) and Saillant et al. (2006). In the latter, significant differences in variance effective size (*NeV*) also were reported across localities and were correlated with findings of differences in growth rates (Fischer et al., 2004) and in size and age of female maturation (Jackson e al. 2007). The lone genetic study of red snapper sampled from both the Atlantic and Gulf (Garber et al. 2004) involved sequences of the hypervariable, mitochondrial (mtDNA) control region sampled from four localities in the Gulf (140 fish) and one locality in the Atlantic (35 fish). No differences in mtDNA haplotype frequencies were detected, consistent with the null hypothesis that red snapper from the five localities comprised a single, genetic population. Most of the mtDNA haplotypes in that study, however, were singleton (unique) mtDNA haplotypes, seriously constraining the power to test the null hypothesis of homogenity.

Other approaches used to test spatial genetic homogeneity within and between the two regions (Atlantic and Gulf) were not consistent with a single, well-mixed genetic unit. Mantel tests revealed a weak but significant correlation between genetic and geographic distance in mtDNA haplotypes, but not microsatellite genotypes, and analysis of mtDNA haplotypes indicated that ‘nearest-neighbor’ haplotypes were sampled within the same geographic locality more often than would be expected in a randomly sampled panmictic population. These results indicate that mtDNA haplotypes are not distributed uniformly across the two regions and that female-mediated gene flow, but not necessarily male-mediated, could be negatively correlated with coastline geographic distance. This pattern of isolation by distance would then suggest either female philopatry or limited spatial geographic movement of females relative to males. Most examples of philopatry, including female philopatry, in non-anadromous, marine fishes not restricted to coral reefs involve sharks (Heuter et al. 2004; Keeney et al 2005) although there is evidence of philopatry in two estuarine-dependent sciaenids (Gold et al. 1999; Thorrold et al. 2001). Pruett et al. (2005) used nested-clade analysis of red snapper mtDNA haplotypes from the Gulf and found historically restricted, female gene flow due to isolation by distance. Alternatively, Gold et al. (1997) did not find evidence of a significant spatial autocorrelation (also indicative of isolation by distance) of mtDNA haplotypes from adult red snapper sampled over three years at nine localities in the Gulf stretching from northern through the Gulf to the Florida panhandle. The isolation-by-distance of mtDNA haplotypes found in this study covered the area from the Florida panhandle to the North Carolina coast, including samples from the west and east coasts of Florida, possibly suggesting reduced (female) gene flow around peninsular Florida. In addition, a network of mtDNA haplotypes revealed at least four clades of two or three haplotypes occurring in the Atlantic but not the Gulf, and Fu’s (1997) *FS* metric was negative and significant before Bonferroni correction at seven of the eight localities sampled and at the samples from South Carolina (Atlantic) and Sarasota (Gulf) following correction. The difference in number of small clades detected may stem in large part from the difference in sample size (477 individuals from the Atlantic vs. 191 individuals from the Gulf). The *FS* approach, however, detects population growth and/or genetic hitch-hiking (Fu 1997), and negative *FS* values (based on mtDNA data) indicate either a ‘selective sweep’ or a recent reduction in female effective size , both of which can result in an excess of rare mtDNA variants over those expected under a neutral model (Kaplan et al. 1989). Together, these observations suggest geographic limits to red snapper female gene flow and possible demographic differences affecting female effective size.

Bayesian estimates of the population parameters *θI* (long-term heterogeneity) and *θII* (contemporaneous heterogeneity) were significant and non-zero among localities in the Atlantic, among localities in the Gulf, and between Atlantic localities (pooled) and Gulf localities (pooled). Allele frequencies in all comparison groups were highly correlated (*ρ* > 0.95) and significantly different from zero, violating the assumption of uncorrelated allele frequencies implicit in approaches estimating parameters such as *ФST* (Song et al. 2006). That both *θI* and *θII* were significantly different than zero suggests that the pattern of genetic heterogeneity that exists today within the Atlantic, within the Gulf, and between the two likely reflects patterns that have existed historically. Estimates of *θI*, however, were more than an order of magnitude greater than estimates of *θII*. Song et al. (2006) found that large estimates of *θI* may reflect a tendency for estimators of *θII* to overestimate homogenizing effects of long-term gene flow, particularly if allele frequencies between populations are highly correlated, suggesting that values of *θII* estimated here could be underestimates. Regardless, the non-zero estimates of *θI* and *θII* are consistent with genetic heterogeneity of red snapper within the Atlantic, within the Gulf, and between the two.

Estimates of the effective number of breeders (*Nb*) for all but one of the localities sampled had infinite upper bounds, suggesting a uniformly large *Nb* across the localities sampled (Waples and Do 2010). However, estimates of *Nb* generated from adult samples are difficult to interpret because they are influenced by the effective number of breeders (*Nb*) that generated each cohort in a sample (Waples 2005). Red snapper can live for over 50 years (Wilson and Nieland 2001) and mature sexually between age two and four (Schirripa and Legault 1997; Fitzhugh et al. 2004). Almost all fish sampled here were adults, meaning that fish in our samples from different cohorts could have shared a parent or parents, severely compromising estimation of *Nb*.

Estimates of average, long-term genetic effective size (*NeLT*) among localities ranged from 826 to 2,111 but did not differ significantly from one another; estimates of *NeLT* for localities from the Atlantic (pooled) and localities from the Gulf (pooled), respectively, were 3,930 and 4,114, respectively. The estimate of *NeLT* for all eight localities (pooled) was 6,267. The sum of the estimates of *NeLT* for the five localities in the Atlantic was 6,450, considerably larger that the global estimate (3,930) for the region. This is consistent with a ‘propagule pool’ model (Waples 2010) where migrants come primarily from a nearby subpopulation or stock and where a metapopulation is subdivided into groups with different demographic rates (e.g., average survival and/or reproduction) (Waples 2010). The sum of the estimates of *NeLT* for the three localities in the eastern Gulf was 4,414, close to the global estimate (4,114) for that region, perhaps suggesting fewer demographic differences among the three eastern Gulf localities. The sum of the estimates of *NeLT* for the two regions (8,005) is larger than the global estimate for all eight localities (6,267), suggesting that the two regions differ demographically. These comparisons also are consistent with genetic and demographic heterogeneity among localities within the two regions, particularly within the Atlantic, and between the two regions.

Estimates of average, long-term migration rates (*m*) between the two regions were 0.0033 (Gulf into the Atlantic) and 0.0021 (Atlantic into the Gulf) and did not differ significantly from one another. The parameter *m* is defined as the proportion of individuals in a subpopulation that are migrants from an outside subpopulation, and is most often expressed as the parameter *mNe*, the effective number of migrants entering a subpopulation each generation (Mills and Allendorf 1996). Because the estimates of *NeLT* and of *m* for the two regions did not differ, we used an average estimate of *NeLT* (4,022) and of *m* (0.0027) to generate a long-term *mNe* estimate of 10.86 which, theoretically, is the effective number of migrants moving in either direction from one region to the other. Estimates of *NeLT* represent a weighted harmonic mean of effective size (*Ne*) over a period of 4*N*e generations, with greater weight on more recent generations and on smaller values of *Ne* (Beerli 2009; Hare et al. 2011). A final point to note is that the estimate of *m* (0.33%) between the two regions is considerably less than the 10% rate, suggested for contemporaneous migration (Waples 2010), beyond which populations are not considered to be demographically independent.

To summarize, there is evidence that genetic and demographic heterogeneity occurs among red snapper across the geographic region surveyed and that the species in U.S. waters is not distributed spatially as a single, panmictic population (stock). The heterogeneity may reflect the metapopulation structure previously hypothesized for red snapper in the northern Gulf (Pruett et al. 2005; Saillant and Gold 2006) and reinforced by the study of Saillant et al. (2010) of age 0 fish. The genetic signal, however, is weak and precludes straightforward definition of geographic boundaries of individual subpopulations or stocks. This is fairly typical for marine fishes with large population densities and high dispersal capability assayed with selectively neutral genetic markers such as microsatellites (Portnoy and Gold 2012). More robust genetic approaches (e.g., RADseq) that utilize next-generation sequencing and allow genome-wide surveys of variation in single-nucleotide polymorphisms (Davey et al. 2011) is the next logical step in assessing population structure, genetic demography, and connectivity of red snapper (and other species) across its (their) range in U.S. waters. These new approaches have the capability to identify genetic markers (genome regions) that affect fitness on local scales and to quantify allele frequencies at these markers across geographic space (Nielsen et al. 2009; Allendorf et al. 2010).

**VI. Tables**

Table 1. Estimates of pairwise *FST* (microsatellites, upper diagonal) and *Φ*ST (mtDNA, lower diagonal) between all eight sample localities.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | NC | SC | GA | DA | ML | SA | MG | PC |
| NC | 0 | 0.001 | 0.001 | 0.001 | 0 | 0 | 0.002 | -0.001 |
| SC | 0.046 | 0 | -0.001 | -0.001 | 0 | -0.001 | 0 | 0.001 |
| GA | -0.023 | 0.036 | 0 | 0.001 | -0.001 | -0.001 | 0 | 0.002 |
| DA | 0.005 | -0.018 | -0.004 | 0 | 0 | 0.002 | 0 | 0.002 |
| ML | -0.024 | 0.02 | -0.036 | -0.076 | 0 | 0 | 0 | -0.001 |
| SA | 0.015 | 0.027 | 0.011 | -0.023 | -0.025 | 0 | 0.001 | -0.001 |
| MG | 0.012 | -0.028 | 0.018 | -0.012 | -0.087 | -0.04 | 0 | 0 |
| PC | 0.092 | 0.056 | 0.059 | -0.04 | -0.025 | -0.019 | -0.025 | 0 |
|  |  |  |  |  |  |  |  |  |

Table 2. Global estimates of *θI*, *θII*, and *ρ* estimated using the Bayesian framework in Hickory. *θI* is an estimate of FST that corresponds to allele frequency variance across evolutionary time; *θII* is a measure of contemporaneous differentiation among sample locations, and *ρ* is the average correlation of allele frequencies among sample locations.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples |  | *θI* |  |  | *θII* |  |  | *ρ* |  |
|  | 2.5% | Mode | 97.5% | 2.5% | Mode | 97.5% | 2.50% | Mean | 97.50% |
| Within Atlantic | 0.0375 | 0.0579 | 0.0641 | 0.0015 | 0.0017 | 0.0020 | 0.9552 | 0.9670 | 0.9759 |
| Within Gulf | 0.0491 | 0.0666 | 0.0861 | 0.0009 | 0.0011 | 0.0016 | 0.9750 | 0.9821 | 0.9877 |
| Gulf/Atlantic (pooled) | 0.0387 | 0.0548 | 0.0695 | 0.0007 | 0.0009 | 0.0010 | 0.9779 | 0.9843 | 0.9893 |

Table 3. Estimates and 95% confidence intervals for theta (*θ*), obtained from Migrate-n. Estimates of *NeLT* were generated from the relationship *θ* = 4*NeLTμ*.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | *θ* |  |  | *NeLT* |
|  | 2.5% | Mode | 97.5% |  | Mode |
| NC | 0.86 | 1.61 | 2.36 |  | 1,343.54 |
| SC | 0.68 | 1.37 | 2.06 |  | 1,143.26 |
| GA | 0.52 | 1.21 | 1.90 |  | 1,009.74 |
| DA | 1.06 | 1.73 | 2.42 |  | 1,443.68 |
| ML | 1.08 | 1.81 | 2.58 |  | 1,510.44 |
| SA | 1.40 | 2.53 | 3.96 |  | 2,111.28 |
| MG | 1.08 | 1.77 | 2.44 |  | 1,477.06 |
| PC | 0.40 | 0.99 | 1.54 |  | 826.15 |
| GULF | 3.84 | 4.93 | 5.90 |  | 4,114.07 |
| ATLANTIC | 3.72 | 4.71 | 6.02 |  | 3,930.48 |
| ALL | 6.56 | 7.51 | 8.48 |  | 6,267.08 |

**VII. Figures**

**Figure 1**. Approximate sampling localities for red snapper in the U.S. Atlantic and eastern Gulf of Mexico.



**Figure 2**. Minimum-spanning network of ND4 mtDNA haplotypes: gray, haplotypes found in the Atlantic; black, haplotypes found in the Gulf. Each node (small circle) represents a unique haplotype; sizes of nodes are scaled to reflect the relative frequency of each haplotype. Lengths of lines connecting haplotypes reflect number of single-base substitutions between haplotypes; the shortest line is one base-pair substitution. Small nodes indicated by very small circles are inferred mtDNA haplotypes. Dotted lines surround putative clades found in the Atlantic.



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|  |
| --- |
| Appendix 1: Summary statistics for microsatellite and mtDNA loci at each locality. For microsatellites: *n* = number of individuals sampled, #A = number of alleles, AR = allelic richness, *HE* = expected heterozygosity, *PHW* = probability of conformance to Hardy-Weinberg expectations, and *FIS* = inbreeding coefficient. For mtDNA: *n* = number of individuals sampled, #H = number of unique haplotypes observed, *h* = haplotype diversity, *π* = nucleotide diversity, *D*\* and *F\** from Fu and Li (1993), and *FS* from Fu (1997). ‡ indicates a significant value (*P* < 0.05). |
|
|
|

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample |  | NC | SC | GA | DA | ML | SA | MG | PC |
| Microsatellite |  |  |  |  |  |  |  |  |  |
| *Lca*20 | *n* | 93 | 84 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 4 | 5 | 5 | 4 | 5 | 5 | 5 | 3 |
|  | AR | 3.80 | 4.05 | 3.72 | 3.30 | 4.25 | 4.93 | 4.03 | 2.98 |
|  | *HE* | 0.190 | 0.179 | 0.208 | 0.162 | 0.177 | 0.196 | 0.174 | 0.198 |
|  | *PHW* | 0.009 | 1.000 | 0.063 | 0.115 | 0.116 | 0.410 | 0.101 | 0.852 |
|  | *FIS* | 0.152 | -0.067 | 0.094 | 0.182 | 0.049 | 0.150 | -0.009 | -0.099 |
|  |  |  |  |  |  |  |  |  |  |
| *Lca*22 | *n* | 93 | 84 | 101 | 96 | 100 | 47 | 97 | 46 |
|  | #A | 14 | 12 | 12 | 12 | 13 | 9 | 11 | 8 |
|  | AR | 12.06 | 10.35 | 11.03 | 11.10 | 10.90 | 8.95 | 9.45 | 7.98 |
|  | *HE* | 0.751 | 0.726 | 0.727 | 0.783 | 0.725 | 0.693 | 0.752 | 0.761 |
|  | *PHW* | 0.265 | 0.629 | 0.210 | 0.077 | 0.304 | 0.032 | 0.230 | 0.661 |
|  | *FIS* | 0.055 | -0.034 | 0.087 | 0.082 | 0.062 | -0.043 | 0.040 | -0.028 |
|  |  |  |  |  |  |  |  |  |  |
| *Lca*43 | *n* | 93 | 85 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 7 | 8 | 9 | 9 | 9 | 7 | 8 | 7 |
|  | AR | 6.40 | 7.00 | 7.61 | 7.58 | 7.80 | 6.99 | 7.34 | 6.94 |
|  | *HE* | 0.491 | 0.581 | 0.539 | 0.526 | 0.583 | 0.560 | 0.560 | 0.589 |
|  | *PHW* | 0.597 | 0.127 | 0.123 | 0.486 | 0.366 | 0.745 | 0.011 | 0.196 |
|  | FIS | 0.080 | -0.013 | 0.026 | -0.029 | -0.018 | 0.033 | 0.190 | -0.107 |
|  |  |  |  |  |  |  |  |  |  |
| *Lca*64 | n | 93 | 84 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 11 | 12 | 11 | 12 | 9 | 8 | 9 | 6 |
|  | AR | 9.17 | 10.23 | 8.67 | 9.48 | 7.80 | 7.81 | 7.62 | 5.98 |
|  | *HE* | 0.789 | 0.791 | 0.782 | 0.801 | 0.774 | 0.713 | 0.770 | 0.736 |
|  | *PHW* | 0.075 | 0.792 | 0.880 | 0.953 | 0.808 | 0.468 | 0.177 | 0.384 |
|  | *FIS* | -0.077 | 0.007 | 0.013 | -0.032 | -0.036 | 0.065 | 0.023 | -0.094 |
|  |  |  |  |  |  |  |  |  |  |
| *Lca*91 | n | 93 | 85 | 101 | 98 | 101 | 47 | 97 | 46 |
|  | #A | 6 | 7 | 6 | 7 | 6 | 6 | 7 | 4 |
|  | AR | 5.19 | 5.89 | 5.65 | 5.91 | 5.17 | 5.96 | 5.60 | 3.98 |
|  | *HE* | 0.577 | 0.565 | 0.613 | 0.579 | 0.573 | 0.627 | 0.562 | 0.557 |
|  | *PHW* | 0.310 | 0.966 | 0.252 | 0.982 | 0.049 | 0.012 | 0.514 | 0.824 |
|  | *FIS* | -0.025 | 0.042 | 0.046 | -0.041 | 0.118 | 0.049 | 0.028 | 0.103 |
|  |  |  |  |  |  |  |  |  |  |
| *Lca*107 | *n* | 93 | 84 | 98 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 9 | 8 | 9 | 10 | 10 | 9 | 10 | 10 |
|  | AR | 8.46 | 7.98 | 8.41 | 9.15 | 9.22 | 8.93 | 9.36 | 9.96 |
|  | *HE* | 0.776 | 0.801 | 0.781 | 0.814 | 0.781 | 0.775 | 0.800 | 0.767 |
|  | *PHW* | 0.145 | 0.714 | 0.034 | 0.912 | 0.272 | 0.119 | 0.495 | 0.027 |
|  | *FIS* | 0.099 | 0.019 | 0.059 | 0.035 | 0.023 | -0.021 | 0.021 | 0.065 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*55 | *n* | 93 | 84 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 7 | 5 | 6 | 4 | 7 | 4 | 6 | 4 |
|  | AR | 5.50 | 4.06 | 5.27 | 3.45 | 5.19 | 3.93 | 4.60 | 3.98 |
|  | *HE* | 0.271 | 0.265 | 0.253 | 0.256 | 0.297 | 0.193 | 0.251 | 0.255 |
|  | *PHW* | 0.100 | 0.272 | 0.753 | 0.426 | 0.942 | 1.000 | 0.867 | 0.611 |
|  | *FIS* | 0.166 | -0.032 | -0.057 | 0.082 | -0.034 | -0.077 | -0.110 | 0.062 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*137 | *n* | 93 | 85 | 101 | 98 | 99 | 47 | 97 | 46 |
|  | #A | 13 | 12 | 11 | 11 | 12 | 12 | 11 | 8 |
|  | AR | 10.24 | 9.78 | 9.09 | 9.34 | 9.63 | 11.74 | 8.83 | 7.96 |
|  | *HE* | 0.732 | 0.680 | 0.721 | 0.676 | 0.720 | 0.694 | 0.682 | 0.676 |
|  | *PHW* | 0.013 | 0.613 | 0.130 | 0.513 | 0.032 | 0.714 | 0.054 | 0.102 |
|  | *FIS* | 0.105 | 0.049 | 0.066 | 0.019 | 0.031 | 0.049 | 0.063 | 0.131 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*221 | *n* | 93 | 84 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 12 | 12 | 12 | 13 | 15 | 13 | 16 | 11 |
|  | AR | 10.67 | 10.23 | 10.78 | 10.93 | 11.93 | 12.68 | 12.54 | 10.91 |
|  | *HE* | 0.766 | 0.772 | 0.800 | 0.767 | 0.803 | 0.807 | 0.797 | 0.771 |
|  | *PHW* | 0.366 | 0.360 | 0.522 | 0.185 | 0.532 | 0.625 | 0.440 | 0.388 |
|  | *FIS* | 0.032 | 0.060 | 0.022 | 0.082 | 0.075 | -0.007 | 0.056 | 0.069 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*240 | *n* | 92 | 81 | 101 | 98 | 100 | 47 | 97 | 46 |
|  | #A | 18 | 18 | 18 | 19 | 18 | 18 | 20 | 18 |
|  | AR | 17.03 | 16.79 | 15.90 | 16.61 | 16.88 | 17.83 | 17.13 | 17.87 |
|  | *HE* | 0.902 | 0.901 | 0.889 | 0.899 | 0.892 | 0.902 | 0.907 | 0.875 |
|  | *PHW* | 0.269 | 0.298 | 0.983 | 0.681 | 0.850 | 0.926 | 0.022 | 0.042 |
|  | *FIS* | -0.037 | 0.054 | -0.002 | -0.022 | -0.009 | -0.038 | 0.034 | 0.056 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*248 | *n* | 93 | 84 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 18 | 17 | 20 | 23 | 18 | 14 | 19 | 16 |
|  | AR | 15.30 | 14.29 | 15.50 | 16.60 | 14.64 | 13.80 | 16.05 | 15.89 |
|  | *HE* | 0.872 | 0.868 | 0.888 | 0.865 | 0.877 | 0.880 | 0.869 | 0.879 |
|  | *PHW* | 0.644 | 0.337 | 0.319 | 0.952 | 0.407 | 0.403 | 0.906 | 0.551 |
|  | *FIS* | 0.001 | -0.056 | 0.052 | -0.050 | -0.005 | 0.029 | 0.015 | 0.035 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*260 | *n* | 93 | 85 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 5 | 3 | 5 | 5 | 3 | 4 | 3 | 3 |
|  | AR | 3.93 | 3.00 | 4.52 | 4.17 | 3.00 | 3.94 | 3.00 | 3.00 |
|  | *HE* | 0.305 | 0.397 | 0.398 | 0.439 | 0.394 | 0.379 | 0.357 | 0.393 |
|  | *PHW* | 0.064 | 0.218 | 0.309 | 0.017 | 0.814 | 1.000 | 1.000 | 0.400 |
|  | *FIS* | 0.118 | -0.187 | -0.019 | -0.047 | -0.029 | -0.044 | -0.011 | -0.050 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*275 | *n* | 93 | 85 | 101 | 98 | 101 | 48 | 97 | 45 |
|  | #A | 6 | 8 | 6 | 7 | 10 | 5 | 7 | 5 |
|  | AR | 5.34 | 6.64 | 5.05 | 5.75 | 7.33 | 4.94 | 5.36 | 5.00 |
|  | *HE* | 0.609 | 0.579 | 0.564 | 0.610 | 0.632 | 0.603 | 0.595 | 0.608 |
|  | *PHW* | 0.837 | 0.511 | 0.392 | 0.272 | 0.930 | 0.849 | 0.539 | 0.446 |
|  | *FIS* | -0.095 | -0.057 | 0.016 | -0.121 | -0.050 | -0.002 | 0.099 | 0.050 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*282 | n | 93 | 85 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 12 | 12 | 11 | 11 | 11 | 11 | 11 | 13 |
|  | AR | 9.561 | 10.688 | 9.188 | 9.787 | 9.677 | 10.681 | 10.176 | 12.912 |
|  | *HE* | 0.599 | 0.66 | 0.636 | 0.64 | 0.684 | 0.565 | 0.655 | 0.693 |
|  | *PHW* | 0.270 | 0.443 | 0.399 | 0.895 | 0.261 | 0.630 | 0.594 | 0.006 |
|  | *FIS* | 0.049 | 0.091 | 0.082 | -0.004 | 0.073 | 0.079 | -0.022 | 0.027 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*328 | *n* | 92 | 84 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 3 | 4 | 4 | 4 | 6 | 3 | 4 | 3 |
|  | AR | 3.00 | 3.98 | 3.45 | 3.63 | 4.58 | 3.00 | 3.83 | 3.00 |
|  | *HE* | 0.547 | 0.567 | 0.568 | 0.531 | 0.559 | 0.516 | 0.546 | 0.563 |
|  | *PHW* | 0.243 | 0.487 | 0.106 | 0.856 | 0.930 | 0.592 | 0.702 | 0.842 |
|  | *FIS* | -0.173 | -0.008 | 0.128 | 0.039 | -0.010 | 0.153 | -0.075 | -0.042 |
|  |  |  |  |  |  |  |  |  |  |
| *Prs*333 | n | 93 | 85 | 101 | 98 | 101 | 46 | 97 | 46 |
|  | #A | 5 | 5 | 7 | 5 | 7 | 5 | 6 | 6 |
|  | AR | 4.85 | 4.09 | 5.08 | 3.76 | 5.53 | 4.98 | 5.01 | 5.96 |
|  | *HE* | 0.382 | 0.277 | 0.336 | 0.361 | 0.395 | 0.401 | 0.330 | 0.414 |
|  | *PHW* | 0.104 | 0.700 | 0.925 | 1.000 | 0.361 | 0.424 | 0.604 | 0.305 |
|  | *FIS* | -0.040 | -0.148 | -0.032 | -0.019 | 0.073 | 0.078 | 0.000 | 0.212 |
|  |  |  |  |  |  |  |  |  |  |
| *Ra*6 | *n* | 92 | 85 | 101 | 98 | 101 | 48 | 97 | 46 |
|  | #A | 7 | 7 | 7 | 6 | 7 | 7 | 7 | 7 |
|  | AR | 6.44 | 5.95 | 5.72 | 5.41 | 6.02 | 6.88 | 6.03 | 6.96 |
|  | *HE* | 0.392 | 0.401 | 0.384 | 0.461 | 0.355 | 0.506 | 0.384 | 0.409 |
|  | *PHW* | 0.202 | 0.134 | 0.325 | 0.994 | 0.467 | 0.049 | 0.126 | 0.958 |
|  | *FIS* | 0.085 | 0.120 | -0.032 | -0.018 | -0.003 | 0.218 | 0.140 | -0.169 |
|  |  |  |  |  |  |  |  |  |  |
| mtDNA |  |  |  |  |  |  |  |  |  |
| ND4 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
|  | #H | 8 | 11 | 7 | 8 | 11 | 10 | 7 | 6 |
|  | *h* | 0.758 | 0.884 | 0.784 | 0.805 | 0.905 | 0.842 | 0.711 | 0.658 |
|  | *π* | 0.091 | 0.128 | 0.106 | 0.066 | 0.185 | 0.095 | 0.050 | 0.051 |
|  | *D\** | -2.392 | -2.666‡ | -1.441 | -1.719 | -1.211 | -2.232 | -2.616‡ | -2.258 |
|  | *F\** | -2.534 | -2.889‡ | -1.582 | -1.887 | -1.472 | -2.390 | -2.713‡ | -2.333 |
|  | *FS* | -3.376‡ | -6.037‡ | -1.413 | -3.277‡ | -4.584‡ | -5.039‡ | -3.054‡ | -2.446‡ |

|  |
| --- |
| Appendix 2: Spatial distribution of mitochondrial (ND4) haplotypes. |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Haplotype | NC | SC | GA | DA | ML | SA | MG | PC |
| #1 | 1 |  |  |  | 2 |  |  |  |
| #2 | 9 | 6 | 8 | 7 | 4 | 6 | 10 | 11 |
| #3 | 1 |  |  |  |  |  |  |  |
| #4 | 5 | 4 | 5 | 6 | 5 | 6 | 5 | 5 |
| #5 | 1 |  |  |  |  |  |  |  |
| #6 | 1 | 1 |  | 1 |  | 1 |  |  |
| #7 | 1 |  |  | 1 | 1 |  |  | 1 |
| #8 | 1 |  |  |  | 1 |  |  |  |
| #9 |  | 1 |  |  |  |  |  |  |
| #10 |  | 1 |  |  |  |  |  |  |
| #11 |  | 2 |  |  |  |  |  |  |
| #12 |  | 1 |  |  |  |  |  |  |
| #13 |  | 1 | 1 |  |  | 1 |  |  |
| #14 |  | 1 |  | 1 |  |  |  |  |
| #15 |  | 1 |  |  |  |  |  |  |
| #16 |  | 1 |  |  |  |  |  | 1 |
| #17 |  |  | 3 |  | 2 |  |  |  |
| #18 |  |  | 1 |  |  |  |  |  |
| #19 |  |  | 1 |  |  |  |  |  |
| #20 |  |  | 1 |  | 1 |  |  |  |
| #21 |  |  |  | 2 |  |  |  |  |
| #22 |  |  |  | 1 |  |  |  |  |
| #23 |  |  |  | 1 |  |  |  |  |
| #24 |  |  |  |  | 1 |  |  |  |
| #25 |  |  |  |  | 1 |  |  |  |
| #26 |  |  |  |  | 1 |  |  |  |
| #27 |  |  |  |  | 1 |  |  |  |
| #28 |  |  |  |  |  | 1 |  |  |
| #29 |  |  |  |  |  | 1 |  |  |
| #30 |  |  |  |  |  | 1 |  |  |
| #31 |  |  |  |  |  | 1 |  |  |
| #32 |  |  |  |  |  | 1 |  |  |
| #33 |  |  |  |  |  | 1 | 1 |  |
| #34 |  |  |  |  |  |  | 1 |  |
| #35 |  |  |  |  |  |  | 1 |  |
| #36 |  |  |  |  |  |  | 1 |  |
| #37 |  |  |  |  |  |  | 1 |  |
| #38 |  |  |  |  |  |  |  | 1 |
| #39 |  |  |  |  |  |  |  | 1 |
|  |  |  |  |  |  |  |  |  |