Finding Geographic Population Structure in Marine Fish Species with High Gene Flow

Encontrar Estructura de la Población Geográfica de Especies de Peces Marinos con Alto Flujo Génico

Trouver Structure de la Population Géographique des Espèces de Poissons Marins au Flux de Gènes a Haute

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ABSTRACT

Management of highly exploited marine resources necessitates a rigorous definition of geographic boundaries that separate stocks because different stocks (populations or subpopulations) may possess local adaptations that lead to differences in important life-history parameters such as growth, fecundity, and disease resistance. Failure to recognize locally-adapted stocks potentially can result in extirpation and irretrievable loss of genetic resources. Identifying separate stocks, based on genetic data, is problematic for marine species with high dispersal capability, particularly when selectively neutral genetic markers are employed. The issue is that homogeneity in such markers may not necessarily reflect homogeneity in genes affecting life-history and/or fitness traits. Moreover, historical events, *e.g.*, population expansion or decline, often leads to violations of equilibrium assumptions inherit in traditional population genetics models. When 'traditional' F_{ST}-based approaches are combined with spatial and demographic analyses, important aspects of cryptic population structure may be revealed. A review of stock-structure assessment in exploited species of snappers (Lutjanidae) in the Gulf of Mexico and Caribbean Sea demonstrates that even for species with similar life histories, patterns of population structure vary and require robust analytical methodologies to detect fine-scale differences.

KEY WORDS: Stock structure, genetics, snappers

INTRODUCTION

Accurate delineation of stock structure is a critical issue for management of exploited marine fisheries because individual stocks may be independent demographically, meaning they may respond differently to fishing pressure and/or other environmental perturbations (Utter and Ryman 1993, Carvalho and Hauser 1994). This is because aspects of local demography and life history, (*e.g.*, birth rate, time to maturity, natural mortality) may have a greater effect on population dynamics within a stock than do migrants from neighboring stocks (Jennings et al. 2001). Failure to recognize demographically independent units thus can potentially lead to over-exploitation, loss of genetic diversity, and localized extirpation (Begg et al.1999, Hilborn et al. 2003) of one or more of the independent units. While a variety of approaches (e. g., morphometrics, tag-recapture, otolith microchemistry) have proven useful for stock identification (Ihssen et al. 1981, Pawson and Jennings 1996), they all seek to identify units that are self-sustaining (Begg and Waldman 1999) and thus directly or indirectly asses the degree of connectivity or genetic migration.

Genetic methods to assess stock structure in marine fisheries have invariably utilized selectively neutral genetic markers such as microsatellites and identified genetic heterogeneity and the degree of migration by using the fixation index F_{ST} (or analogoes measures) to assess levels of allelic/genotypic divergence attributable to genetic drift (Ward 2002, Ward and Grewe 1994). Fixation indices essentially quantify the amount of genetic variance that can be explained by population structure (Holsinger and Weir 2009). The principle behind the approach for nuclear-encoded sequences such as microsatellites is based on the relationship between the fixation index and the effective number of migrants (N_em), where $F_{ST} \sim 1/(4Nm_e+1)$, N_e is the genetic effective population size (hereafter effective size), and m is the migration rate per generation (Dobzhansky and Wright 1941, Wright 1943). As shown in Figure 1, F_{ST} values are inversely related to migration rates but the relationship is not linear. There also is an inverse effect of N_e on the magnitude of divergence due to genetic drift. The F_{ST} approach does have advantages over other methods of stock assessment. First, molecular characters do not change with environmental variables and/or ontogeny, both of which may bias stock assessment based on morphology. Second, because stocks may contain important localized genetic resources and loss of these resources may affect long term sustainability, genetic methods can simultaneously evaluate stock structure and within-stock genetic diversity (Begg et al. 1999, Carvalho and Hauser 1994). A disadvantage to the F_{ST} approach is that because levels of divergence in selectively neutral markers stem largely from genetic drift, F_{ST} values increase slowly relative to demographic processes in stocks with relatively large N_e. Consequently, stocks may differ demographically yet not differ significantly in allele or genotype distributions at selectively neutral loci.

The interpretation of non- or marginally significant results in an F_{ST}-based framework can be problematic because failure to detect significant genetic heterogeneity does not necessarily indicate a single, well-mixed stock (Pawson and Jennings 1996). This is especially true for many marine species which often feature a number of life-history characteristics that reduce the power of such analyses. For example, census sizes, and generally effective sizes, tend to be large in marine

species (*e.g.*, Gomez-Uchida and Banks 2006, Poulsen et al. 2006) and long periods of time are necessary for genetic drift to result in detectable divergence between reproductively isolated groups (Hare et al. 2011). In addition, even fairly low levels of gene flow between demographically independent groups can erode signals of genetic divergence (Waples 1998). The latter is a major limitation to F_{ST}-based analyses in exploited marine fishes that have buoyant eggs and larval periods with high dispersal potential and adults that are capable of long-distance movements. Finally, populations of many marine fishes tend to fluctuate in size either naturally or due to exploitation (Grant and Bowen 1998), leading to a violation of a central assumption behind F_{ST}-based analyses, i.e., that populations are in migration-drift equilibrium (Waples 1998).

In this paper, we compare studies from our laboratory where geographic population structure was assessed in exploited snapper species (Lutjanidae) in either the Gulf of Mexico (hereafter Gulf) or the Caribbean Sea (hereafter Caribbean). These studies shared common goals: to assess the number of stocks present within the sampled region, to estimate the degree of connectivity across the region, and to quantify extant genetic diversity. In three of the species, the F_{ST} approach or its equivalent either produced equivocal or non-significant results, necessitating secondary approaches to assess stock structure. The secondary approaches were threefold. The first involved assessing genetic divergence in a hierarchical framework and then using spatial autocorrelation analysis (Smouse and Peakall 1999) among age zero fish sampled across relatively short distances within a subregion; the second involved generating estimates of average, long-term, genetic-effective population size (a demographic parameter); while the third involved an edge-detection approach used in landscape genetic analysis (Manel et al. 2003). These analytical approaches make no explicit assumptions about equilibrium conditions and are particularly useful when there is no *a priori* hypothesis regarding the nature or scale of population structure. Complete presentations of all methods and analytical procedures may be found in each of the seminal papers noted below.

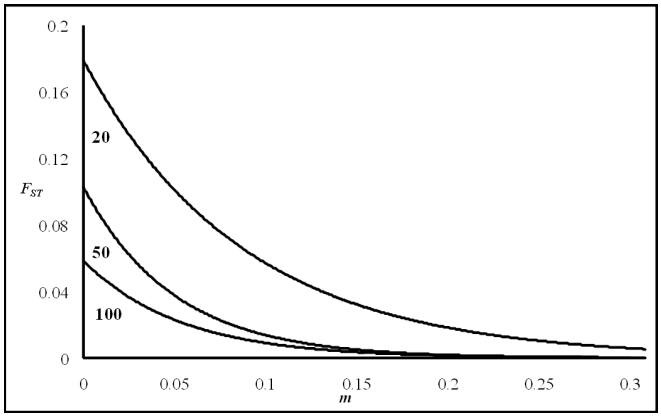

Figure 1. The relationship between the fixation index F_{ST} and the migration rate (m) for two populations with genetic effective sizes (N_e) of 20, 50, and 100. Data are based on simulations, using EASYPOP v.1.7 (Balloux 2000) and where pairwise F_{ST} values were estimated using GENEPOP V.4.0 (Raymond & Rousset 1995; Rousset 2008). The two populations were completely separated for 10 generations; F_{ST} values were estimated using 15 microsatellite loci.

RESULTS AND DISCUSSION

The first study (Gold et al. 2009) involved gray snappers, *Lutjanus griseus*, and serves as an example where a fixation index-based approach detected population structure. Gray snappers are distributed throughout the Gulf and have become an important component of U.S. snapper fisheries (Fischer et al. 2005). We genotyped individuals acquired from five localities (n = 26-42) in the Gulf (two in Texas, one in Louisiana, one along the west coast of Florida, and one in the Florida Keys) and one locality (n = 30) along the east coast of Florida at 13 nuclear-encoded microsatellites. A simulated analysis of molecular variance (SAMOVA; Dupanloup et al. 2002) indicated three distinct groups among six sample localities : one in the northwestern Gulf (the two samples from Texas), one in the north-central and northeastern Gulf (the samples from Louisiana, the west coast of Florida, and the Florida Keys), and one from the east coast of Florida. The among-groups component of molecular variance differed significantly from zero (Φ_{CT} = 0.007, p = 0.020). The pattern of genetic divergence observed between the two samples from Texas and the three samples to the east fit well with the absence of suitable larval and juvenile habitat along the northeastern Texas coast (Handley et al. 2007) and the well-publicized Gulf hypoxic zone (Rabalais et al. 1999) that extends from western Louisiana to the northeastern Texas coast. A final point to note is that the estimate of the fixation index (Φ_{CT}) indicates that only 0.7% of the total genetic variance is distributed among the three groups. This is not at all atypical of estimates of fixation indices for large, exploited marine fishes with the potential for extended spatial movement of both larvae and adults.

The second study (Saillant et al. 2010) involved red snappers, *Lutjanus campechanus*, one of the most economically important, exploited marine fishes in U.S. waters of the Gulf (Adams et. Al. 2004, Wilson and Nieland 2001). The existence of multiple stocks of red snapper in the Gulf waters had been examined extensively through studies of life history, demography, and genetic diversity. The genetic studies (Gold et al. 1997, 2001, Pruett et al. 2005, Saillant and Gold, 2006) generally indicated homogeneity in the distribution of both nuclear and mitochondrial genetic variants. Studies by Woods et al. (2003), Fischer et al. (2004), and Saillant and Gold (2006), however, indicated there were differences across the region in both life history and effective size. Saillant et al. (2010) hypothesized that stable demographic assemblages might exist but on a fine

spatial scale and that non-equilibrium conditions related to overexploitation and fluctuations in census size potentially might be obscuring signals of genetic divergence across the region. To evaluate this possibility, they (*Ibid*) sampled age zero fish in each of two years from multiple locations in each of five sub-regions: three off the Texas coast, one off the Louisiana coast, and one off the Mississippi/Alabama coast. Samples were obtained from multiple tows in each sub-region and sample sizes for each cohort in each sub-region ranged from 102 to 110 fish. The average length (± SE) of a tow was 3.27 ± 0.06 km, the number of tows per sub-region varied between 2 and 22 (average 10.2), and the average distance between tows within sub-regions was 52 km. Genotypes at 18 microsatellites were acquired from all fish and analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to partition molecular variance according to two hierarchical models: one that assessed variance components attributable to sub-region and to cohorts within regions, and one that assessed variance components attributable to sub-region and to among-tows within sub-regions. Neither spatial (among sub-regions) nor temporal (between cohorts within sub-regions) heterogeneity was detected under the first model. Spatial heterogeneity among sub-regions also was not detected under the second model; however, the component of molecular variance allocated to among-tows within sub-regions was highly significant (p = 0.009) in one cohort and close to an order of magnitude greater than the component allocated to among sub-regions in the other cohort. Spatial genetic variation also was assessed via spatial autocorrelation analysis (Smouse and Peakall 1999). The autocorrelation coefficient (r) differed significantly from zero in distances classes up to 150 km (Figure 2); identical results were obtained whether the two cohorts were analyzed separately or pooled. These results were consistent with the hypothesis that spatial genetic structuring among young-of-the-year red snapper in the Gulf occurs at small geographic scales and that recruitment is essentially local and within a 50 – 150 km range. The results also underscore the importance of maintaining healthy local spawning populations of red snapper in all regions across the Gulf. In addition, the results fit well with a metapopulation stock-structure model of partially connected genetic units (Gold and Saillant 2007).

The third and fourth studies involved mutton (*Lutjanus analis*) and yellowtail (*Ocyurus chrysurus*) snappers (Carson et al. 2011 and Saillant et al. 2012, respectively) sampled from localities offshore of three islands (Puerto Rico, St. Thomas, and St. Croix) in the Caribbean and one sample from the Florida Keys. The primary emphasis in both studies was assessment of population structure of each species in waters of the U.S. Caribbean as both species comprise important commercial fisheries in that region (Matos-Caraballo 2000, Matos-Caraballo et al. 2004, 2006). For mutton snappers, 498 individuals (total) were sampled from the west and east coasts of Puerto Rico, the southern coast of St. Thomas, the southwest coast of St. Croix, and in the Florida Keys south of the city of Marathon; sample sizes per locality ranged from 93 – 118. The locality off the west coast of Puerto Rico is near several marine protected areas (MPAs) and a mutton snapper spawning aggregation off the southwest coast. The locality off the coast of St. Thomas is near several MPAs and the locality off the coast of St. Croix is a seasonally protected mutton snapper spawning aggregation area. The locality in the Florida Keys is near a now annually protected mutton snapper spawning aggregation in the Dry Tortugas. Genotypes at 16 microsatellites were obtained from all individuals. Exact tests of homogeneity of both microsatellite allele and genotype distributions among localities were non-significant (p = 0.225, alleles; p = 0.288, genotypes), and the among-localities component of molecular variance (all microsatellites combined), estimated by AMOVA did not differ significantly from zero (Φ_{ST} = –0.0001, p = 0.644). Pair-wise exact tests (between samples) also were non-significant. At face value, these results suggest that mutton snappers over the range sampled comprise a single, large panmictic population with sufficient migration (connectivity) between localities to negate divergence in microsatellite allele or genotype distribution. Estimates of average, long-term migration rates (m_{LT}), however, indicated differences in connectivity among the localities in the Caribbean. Moreover, the larger estimates of m_{LT} (0.0053 between St. Thomas and the east coast of Puerto Rico, and 0.0054 between St. Thomas and St. Croix) were at the lower end of the range of migration rates (0.2 – 10%) where sufficiently large populations can react independently to demographic perturbations yet not be discriminated by selectively neutral genetic markers (Hastings 1993). We then generated estimates of average, long-term effective size (N_{eLT}), a parameter that reflects the effects of historical demographic processes (Luikart et al. 2010, Hare et al. 2011). The estimates of average long-term effective size varied significantly among the localities sampled (Table 1), with the lowest and highest effective size found in the samples from St. Croix (N_{eLT} = 341) and the Florida Keys (N_{eLT} = 1066), respectively. The differences in N_{eLT} indicate possible demographic independence among the localities in long-term population dynamics. Factors promoting differences in demographic parameters such as N_{eLT} are difficult to assess, but likely relate in some way to variation in reproductive success of either or both sexes, census size, habitat quality, and/or mortality (Saillant and Gold 2006, Charlesworth 2009). These results indicate that mutton snapper across the region sampled may be subdivided into demographic stocks that differ in aspects that impact N_{eLT} and hence may respond differently to exploitation. The differences among these 'stocks' could be both genetic and environmental.

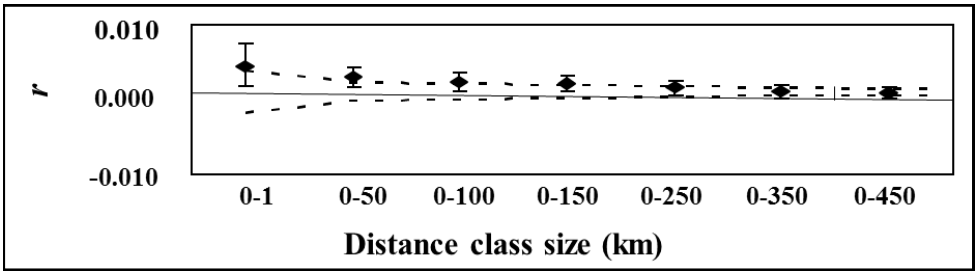

Figure 2. Autocorrelation as a function of geographic distance: Abscissa, distance class; ordinate, spatial autocorrelation (*r*).

For yellowtail snappers, a total of 511 individuals were sampled, with sample sizes per locality ranging from 86 – 109. Genotypes at 16 microsatellites were obtained from each individual. Exact tests over all microsatellites revealed significant heterogeneity in allele and genotype distributions among the five samples. However, only one pairwise exact test between localities (St. Croix versus the Florida Keys: F_{ST} = 0.0034, $p < 0.05$) was significant following Bonferroni correction (Rice 1987), and SAMOVA, using microsatellite data, failed to resolve significant groupings of localities. The among-groups component of molecular variance (Φ_{CT}) from SAMOVA was 0.002 and did not differ significantly from zero. Spatial autocorrelation analysis, alternatively, detected a significant, positive autocorrelation when all five sample localities were included in the analysis, but not when only the four sample localities from the Caribbean were used. This result indicated that there is insufficient gene flow in yellowtail snapper to maintain correlations among genotypes between the Florida Keys and localities in the U.S. Caribbean. We then employed an edge-detection approach used in landscape genetic analysis to detect occurrence of barriers to gene flow. Briefly, we used a method (Manni et al. 2004) that employs a Delauney triangulation to construct a geometric representation of sample localities that defines which localities are nearest neighbors and then estimated genetic distance between localities, using Weir and Cockerham's (1984) θ metric. A modified version of Monmonier's maximum-difference algorithm was then used to identify continuous edges (boundaries) where genetic differences between samples from adjacent localities are largest.

Support for inferred barriers (boundaries) was assessed by bootstrapping (1000 bootstrapped matrices of pairwise θ values) by resampling within sampling localities genotypes at the16 microsatellites, with replacement. Results of the analysis (Table 2) indicate highly restricted gene flow (> 95% bootstrap support for inferred barriers) between St. Croix and the west coast of Puerto Rico and between the east and west coasts of Puerto Rico. Less restrictive gene flow (71.9% bootstrap support) also was inferred between St. Thomas and the east coast of Puerto Rico. These results indicate there are differences in connectivity among yellowtail snappers at the four localities in the U.S Caribbean.

Table 1. Estimates of average, long-term effective size (N_{eLT}) and lower and upper 95% confidence intervals for five samples of mutton snapper.

LOCALITY	N_{eLT}	LOWER 95% CI	UPPER 95% CI
St. Croix	341	314	372
St. Thomas	828	766	896
Puerto Rico - East	828	766	896
Puerto Rico - West	646	607	689
Florida Keys	1066	987	1155

Table 2. Bootstrap values (based on 1000 bootstrapped datasets) reflecting the number of times an edge was defined as a primary barrier to gene flow between localities where yellowtail snappers were sampled..

EDGE (BARRIER)	BOOTSTRAP PROPORTION
St. Croix to St. Thomas	431
St. Croix to Puerto Rico - East	990
St. Croix to Puerto Rico - West	990
St. Thomas to Puerto Rico - East	719
Puerto Rico – East to Puerto Rico - West	851
Puerto Rico – West to Florida Keys	825

CONCLUSIONS

The various studies discussed here involved the use of secondary approaches and analyses to assess genetic stock structure of heavily exploited snapper species in the Gulf of Mexico and Caribbean Sea. In each case, results of the secondary analyses revealed subtle indications of genetic or demographic differences that were not readily detected by using an F_{ST}-based approach. The failure of F_{ST} approaches to detect genetic heterogeneity is due most likely to several factors, including large population (census) sizes (resulting in very slow divergence in selectively neutral markers), variable gene flow (connectivity), and non-equilibrium conditions stemming from natural processes (and perhaps more importantly) from exploitation. However, failure of F_{ST}-based approaches to detect genetic heterogeneity is not a condem-

nation of their use. This type of approach is extremely valuable as F_{ST} values that differ significantly from zero are an unambiguous indication of stock structure (Ward and Grewe 1994). It also is important to note that while the secondary analyses used in our studies may be free from many of the assumptions of F_{ST}-based analyses, they are largely descriptive in nature and inferences regarding patterns of population or stock structure based on these analyses do not necessarily involve the rigorous hypothesis testing inherit in traditional F_{ST}-based analyses. Finally, it is important to note that stock-structure assessment is not performed in a vacuum. Aspects of local oceanography and the biology or life history of the species in question should be considered to support or refute patterns of stock structure obtained from any type of stock-structure analysis. As an example, our studies of yellowtail snappers (above) and a recent study of epinephaline groupers (Portnoy et al. 2013) have indicated reduced gene flow between St. Croix and other localities in the U.S. Caribbean and between the east and west coasts of Puerto Rico. St. Croix is on a different geological platform than St. Thomas and there is a deep trench between them (Rogers et al. 2008) that potentially restricts adult movement. In addition, local surface current regimes run west and west-southwest through the Anegada Passage (Roberts 1997, Johns et al. 2002) which could restrict larval exchange between St Croix and both St. Thomas and the east coast of Puerto Rico. The apparent reduced connectivity between the east and west coasts of Puerto Rico could be due to the west-to-east, near-shore counter-current than runs along the southern shelf of Puerto Rico (Roberts 1997). Ultimately, the strength of using secondary approaches is that they present an array of independent methodologies that can be used to assess population structure in species that have the potential for high gene flow.

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LITERATURE CITED

Adams, C.M., E. Hernandez, and J.C. Cato. 2004. The economic significance of the Gulf of Mexico related to population, income, employment, minerals, fisheries and shipping. *Ocean & Coastal Management* **47**(11-12):565-580.

Balloux, F. 2000. EASYPOP (version 1.7): A computer program for population genetics simulations. *Journal of Heredity* **92**(3):301-302.

Begg, G.A. and J.R. Waldman. 1999. An holistic approach to fish stock identification. *Fisheries Research* **43**(1-3):35-44.

Begg, G.A., K.D. Friedland, and J.B. Pearce. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research* **43**(1-3):1-8.

Carson, E.W., E. Saillant, M.A. Renshaw, N.J. Cummings, and J.R. Gold. 2011. Population structure, long-term connectivity, and effective size of mutton snapper (*Lutjanus analis*) in the Caribbean Sea and Florida Keys. *Fisheries Bulletin* **109**(4):416-428.

Carvalho, G.R. and L. Hauser. 1994. Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries* **4**(3): 326-350.

Charlesworth, B. 2009. Effective population size and patterns of molecular evolution and variation. *Nature Reviews in Genetics* **10**:195-205.

Dobzhansky, T. and S. Wright. 1941. Genetics of natural populations. V. Relations between mutation rate and accumulation of lethals in populations of *Drosophila pseudoobscura*. *Genetics* **26**(1):23-51.

Dupanloup, I., S. Schneider, and L. Excoffier. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* **11**(12):2571-2581.

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.

Fischer, A.J., M.S. Baker, and C.A. Wilson. 2004. Red snapper, *Lutjanus campechanus*, demographic structure in the northern Gulf of Mexico based on spatial patterns in growth rates and morphometrics. *Fishery Bulletin* **102**(4):593-603.

Fischer, A.J., M.S. Baker, C.A. Wilson, and D.L. Nieland. 2005. Age, growth, mortality, and radiometric age validation of gray snapper (*Lutjanus griseus*) from Louisiana. *Fishery Bulletin* **103**(2):307-319.

Gold, J.R. and E. Saillant. 2007. Population structure of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico. Pages 201-216 in: W. Patterson, J. Cowan, G. Fitzhugh, and D. Nieland, (eds.) *Red Snapper Ecology and Fisheries in the U.S. Gulf of Mexico*. American Fisheries Society Symposium 60.

Gold, J.R., F. Sun, and L.R. Richardson. 1997. Population structure of red snapper from the Gulf of Mexico as inferred from the analysis of mitochondrial DNA. *Transactions of the American Fisheries Society* **126**(3):386-396.

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

Gold, J.R., Saillant, E., Ebelt, N.D. and S. Lem. 2009. Conservation genetics of gray snapper (*Lutjanus griseus*) in U.S. waters of the northern Gulf of Mexico and western Atlantic Ocean. *Copeia* **2009** (2):277-286.

Gomez-Uchida, D. and M.A. Banks. 2006. Estimation of effective population size for the long-lived darkblotched rockfish *Sebastes crameri*. *Journal of Heredity* **97**(6):603-606.

Grant, W.A.S. and B.W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* **89** (5):415-426.

Handley, L., D. Altsman, and R. DeMay (eds.). 2007. Seagrass status and trends in the northern Gulf of Mexico: 1940 – 2002. U.S. Geological Survey Scientific Investigations Report 2006-5287 and U.S. Environmental Protection Agency 855-R-04-003. http://pubs.usgs.gov/sir/2006/5287/pdf/CoverandContents.pdf.

Hare, M.P., L. Nunney, M.K. Schwartz, D.E. Ruzzante, M. Burford, R.S. Waples, K. Ruegg, and F. Palstra. 2011. Understanding and estimating effective population size for practical application in marine species management. *Conservation Biology* **25**(3):438-449.

Hastings, A. 1993. Complex interactions between dispersal and dynamics: lessons from coupled logistic equations. *Ecology* **74**:1362-1372.

Hilborn, R., T.P. Quinn, D.E. Schindler, and D.E. Rogers. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Science* **100**(11):6564-6568.

Holsinger, K.E. and B.S. Weir. 2009. Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. *Nature Reviews in Genetics* **10**(9):639-650.

Ihssen, P.E., H.E. Booke, J.M. Casselman, J.M. McGlade, N.R. Payne, and F.M. Utter. 1981. Stock identification: materials and methods. *Canadian Journal of Fisheries and Aquatic Sciences* **38**(12):1838-1855.

Jennings, S., M.J. Kaiser, and J.D. Reynolds. 2001. *Marine Fisheries Ecology*. Blackwell Science Ltd, Oxford, England. 417 pp.

Johns, W., T. Townsend, D. Fratantoni, and W. Wilson. 2002. On the Atlantic inflow to the Caribbean Sea. *Deep-Sea Research Part I: Oceanographic Research Papers* **49**(2):211-243.

Luikart, G., N. Ryman, D.A. Tallmon, F.J. Schwartz, and F.W. Allendorf. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-base approaches. *Conservation Genetics* **11**(2):355-373.

Manel S, M. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution* **18**:189-197.

Manni, F., E. Guérard, and E. Heyer. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology* **76**(2): 173-190.

Matos-Caraballo, D. 2000. Overview of Puerto Rico's small-scale fisheries statistics: 1994 – 1997. *Proceedings of the Gulf and Caribbean Fisheries Institute* **51**:215-231.

Matos-Caraballo, D., M. Cartagena-Haddock, and N. Peña-Alvarado. 2004. Historical landings and biostatistical CFSP data analysis for five important species. Portrait of the fishery of mutton snapper *Lutjanus analis* in Puerto Rico during 1988 – 2001. [Available from Dept. Nat. Environ. Res. Fish. Res. Lab., Mayaguez, Puerto Rico.]

Matos-Caraballo, D., M. Cartagena-Haddock, and N. Peña-Alvarado. 2006. Portrait of the fishery of mutton snapper *Lutjanus analis* in Puerto Rico during 1988 – 2001. *Proceedings of the Gulf and Caribbean Fisheries Institute* **57**:327-342.

Pawson, M.G. and S. Jennings. 1996. A critique of methods for stock identification in marine capture fisheries. *Fisheries Research* **25**(3-4):203-217.

Pruett, C. L., E. Saillant, and J.R. Gold. 2005. Historical population demography of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico based on analysis of sequences of mitochondrial DNA. *Marine Biology* **147**:593-602.

Portnoy, D.S., C.M. Hollenbeck, M.A. Renshaw, N.J. Cummings, and J.R. Gold. [2013]. Does mating behaviour affect connectivity in marine fishes? Comparative population genetics of two protogynous groupers (Family Serranidae). *Molecular Ecology*. In press.

Poulsen, N.A., E.E. Nielsen, M.H. Schieruo, V. Loeschcke, and P. Gronkjaer. 2006. Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Molecular Ecology* **15**:321-331.

Rabalais, N.N., R.E. Turner, D. Justic, Q. Dortch, and W.J. Wiseman. 1999. Characterization of hypoxia: topic I report for the integrated assessment on hypoxia in the Gulf of Mexico. NOAA Coastal Ocean Program Decision Analysis Series No. 15. NOAA Coastal Ocean Program, Silver Spring, Maryland. http://www.cop.noaa.gov/pubs/das/das15.pdf.

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

Roberts, C.M. 1997. Connectivity and management of Caribbean coral reefs. *Science* **278**(5342):1454-1456.

Saillant, E. and J.R. Gold. 2006. Population structure and variance effective size of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. *Fishery Bulletin US* **104**:136-148.

Saillant, E., S.C. Bradfield, and J.R. Gold. 2010. Genetic variation and spatial autocorrelation among young-of-the-year red snapper *(Lutjanus campechanus*) in the northern Gulf of Mexico. *ICES Journal of Marine Science* **67**(6):1240-1250.

Saillant, E., M.A. Renshaw, N.J. Cummings, and J.R. Gold. 2012. Conservation genetics and management of yellowtail snapper (*Ocyurus chrysurus*) in the U.S. Caribbean and South Florida. *Fisheries Management and Ecology* **19**(4):301-312.

Smouse, P.E. and R. Peakall. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* **82** (5):561-573.

Utter, F.E. and N. Ryman. 1993. Genetic markers and mixed stock fisheries. *Fisheries* **18**(8):11-21.

Waples, R.S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89**(5):438-450.

Ward, R.D. 2000. Genetics in fisheries management. *Hydrobiologia* **420** (1):191-201.

Ward, R.D. and P.M. Grewe. 1994. Appraisal of molecular genetic techniques in fisheries. *Reviews in Fish Biology and Fisheries* **4**(3): 300-325.

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

Wilson, C.A. and D.L. Nieland. 2001. Age and growth of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana. *Fishery Bulletin* **99**(4):653-664.

Woods, M.K., A.J. Fischer, J.H. Cowan, and D.L. Nieland. 2003. Size and age at maturity of female red snapper *Lutjanus campechanus* in the northern Gulf of Mexico. *Proceedings of the Gulf and Caribbean Fisheries Institute* **54**:526–537.

Wright, S. 1943. Isolation by distance. *Genetics* **28**(2):114-138.