Historical population demography of red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico based on analysis of sequences of mitochondrial DNA

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SEDAR74-RD68

March 2021

Marine Biology (2005) 147: 593–602
DOI 10.1007/s00227-005-1615-8

RESEARCH ARTICLE

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Received: 30 April 2004 / Accepted: 1 March 2005 / Published online: 5 May 2005

Abstract We evaluated stock structure and demographic (population) history of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico (Gulf) via analysis of mitochondrial (mt)DNA sequences from 360 individuals sampled from four cohorts (year classes) at three localities across the northern Gulf. Exact tests of genetic homogeneity and analysis of molecular variance both among cohorts within localities and among localities were non-significant. Nested clade analysis provided evidence of different temporal episodes of both range expansion and restricted gene flow due to isolation by distance. A mismatch distribution of pairwise differences among mtDNA haplotypes and a maximum-likelihood coalescence analysis indicated a population expansion phase that dated to the Pleistocene and probably represents (re)colonization of the continental shelf following glacial retreat. The spatial distribution of red snapper in the northern Gulf appears to have a complex history that likely reflects glacial advance/retreat, habitat availability and suitability, and hydrology. Habitat availability/suitability and hydrology may partially restrict gene flow among present-day red snapper in the northern Gulf and give rise to a metapopulation structure with variable demographic connectivity. This type of population structure may be difficult to detect with commonly used, selectively neutral genetic markers.

Communicated by P.W. Sammarco, Chauvin

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Introduction

The Gulf red snapper (*Lutjanus campechanus*) is a highly exploited marine fish found primarily along the continental shelf of the Gulf of Mexico (Hoese and Moore 1977; Allen 1985) and subjected to both recreational and commercial fishing (Goodyear and Phares 1990). Red snapper abundance in the northern Gulf of Mexico (Gulf) has decreased by almost 90% since the 1970s (Goodyear and Phares 1990), leading to intensive management beginning in the early 1990s (Christman 1997). A central question regarding conservation and management of red snapper resources in the northern Gulf is whether multiple management units (stocks) exist within the fishery. The question is of importance because separate management of regional stocks, should they exist, is necessary to avoid regional over-exploitation and maintain potentially adaptive genetic variation (Carvalho and Hauser 1995; Hauser and Ward 1998).

Prior genetic studies on adult red snapper sampled across the northern Gulf have generally revealed homogeneity among localities, consistent with the existence of a single stock (Camper et al. 1993; Gold et al. 1997; 2001; Garber et al. 2004; but see Bortone and Chapman 1995). The genetic data, however, are not fully consistent with studies of post-juvenile life history and with most tag-and-recapture experiments that indicate adult red snapper are sedentary and exhibit high site fidelity (Fable 1980; Szedlmayer and Shipp 1994; Szedlmayer 1997; but see Patterson et al. 2001). Two hypotheses forwarded to account for these seemingly disparate observations are (1) gene flow, perhaps occurring from hydrodynamic transport of pelagic eggs and larvae across the northern Gulf (Goodyear 1995), is sufficient to preclude genetic divergence, or (2) gene flow across the northern Gulf is limited but there has been insufficient time for semi-isolated lineages to completely sort into monophyletic assemblages (Gold and Richardson 1998; Gold et al. 2001). A third hypothesis is (3) that gene flow/isolation among assemblages or lineages

of Gulf red snapper is a dynamic process that varies in intensity and duration through both time and space. Under this hypothesis, gene flow over the short term could be fairly restricted geographically, while over the long term be more extensive and largely a function of passive and more-or-less random (in terms of strength and duration) movement of individuals at one of any life-history stages. Different assemblages (stocks) might thus arise in the short term but appear homogeneous over the long term.

Distinguishing among these hypotheses is problematic in that only the hypothesis of 'gene flow' can be falsified rigorously with genetic data and deployment of allele (or genotype) distribution-based statistical tests. This is especially true if gene flow varies in intensity and duration and is demographically insignificant in the short term but sufficient in the long term to preclude genetic divergence. We sought to evaluate these hypotheses, especially the latter, by using a nested phylogeographic (clade) analysis (Templeton 1998) of mitochondrial (mt)DNA sequences. The nested clade approach utilizes evolutionary genealogical information to evaluate the spatial and temporal distribution of genetic variation and often can detect cryptic geographical associations or patterns that are not readily identifiable using traditional population genetic measures. Nested clade analysis can also discriminate between the effects of restricted but recurrent gene flow and historical events such as range expansion and/or population fragmentation (Templeton et al. 1995; Templeton 1998). In addition, we employed both mismatch-distribution analysis and a maximum-likelihood coalescence analysis of mtDNA sequences to further evaluate historical population demography of red snapper in the northern Gulf.

Materials and methods

A total of 30 individual red snapper from each of four cohorts (year classes) were sampled at each of three localities (Fig. 1) in the northern Gulf of Mexico ($n = 120$ per locality; 360 total). Adult individuals belonging to the 1995 and 1997 cohorts were sampled between 1999 and 2001 by angling 25–30 miles offshore of Port Aransas (TX), Port Fourchon (LA), and Dauphin Island (AL). Heart and spleen tissues were frozen in liquid nitrogen and returned to College Station where they were stored at −80°C. Otoliths were removed and individual fish aged by otolith-increment analysis (Wilson and Nieland 2001). Young red snapper of the year (age 0) were procured in the fall of 1999 and 2000 during demersal trawl surveys carried out by the National Marine Fisheries Service (NMFS). Tissue removal and storage was the same as for adult fish. Heart tissue from four Pacific red snapper, *L. peru*, used as outgroup in phylogenetic analysis, was kindly provided by A. Buentello (Centro de Investigaciones Biológicas del Noroeste Mar Bermejo, La Paz, Mexico).

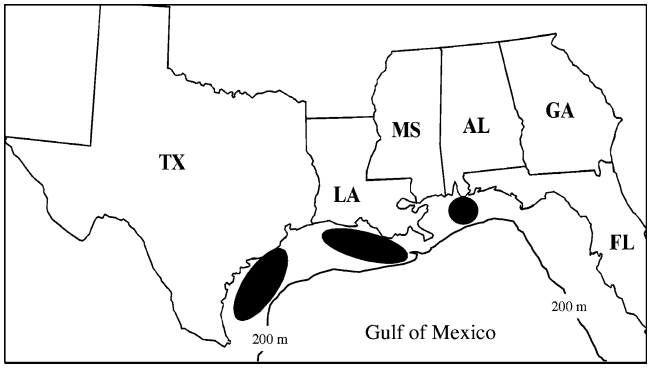

Fig. 1 Collection localities (*black circles*) of Gulf red snapper (*Lutjanus* c *ampechanus*) from waters offshore of Dauphin Island (Alabama), Port Fourchon (Louisiana), and Port Aransas (Texas)

Total genomic DNA was extracted using a phenol/chloroform method (Sambrook et al. 1989). A 590 base pair (bp) fragment of the mitochondrially encoded NADH dehydrogenase subunit 4 gene (ND-4) was amplified using standard polymerase chain reaction (PCR) protocols (Palumbi 1996a). Sequencing reactions were performed using the Big dye terminators (Applied Biosystems, Foster City, CA) and primers ND4LB (Bielawski and Gold 2002) and SnapHND4 (5'-GTGGGCTTTAGGGAGTCAGAG-3') for each DNA template. Cycle-sequenced products were electrophoresed on an automated sequencer (ABI 377, Applied Biosystems, Foster City, CA). Sequences were aligned and protein coding was verified using SEQUENCHER (v 4.1, Gene Codes, Ann Arbor, MI). The computer program MACCLADE 4.0 (Maddison and Maddison 2000) was used to identify individual mtDNA haplotypes. Sequences of all haplotypes were deposited in GENBANK. Accession numbers for the 60 haplotypes of *L. campechanus* are AY600304–AY600318, AY600320–AY600357, and AY600359–AY600365; accession numbers for 4 haplotypes of *L. peru* are AY600366–AY600369.

Number of mtDNA haplotypes, haplotype frequencies, nucleon diversity (after Nei 1987), and nucleotide diversity were obtained using the software package ARLEQUIN (Schneider et al. 2000). Homogeneity of mtDNA haplotype distributions among cohorts within regions and among regions (cohorts pooled) was assessed via exact tests based on a Markov-chain procedure (1,000 dememorizations, 10,000 steps in Markov chain) and AMOVA, as implemented in ARLEQUIN. For the latter, significance of Φ_{ST} (among localities) and Φ_{SC} (among cohorts within localities) was assessed by permutation (10,000 replicates).

Neighbor-joining, maximum parsimony, and maximum-likelihood trees of red snapper mtDNA haplotypes were generated to help resolve relationships inferred from nested clade analysis and to determine which clades were interior/ancestral. Identification of interior/ancestral clades is critical for determining geographic distance(s) between tip and interior clades in the highest nested level (the total cladogram nested together) and

for subsequent contingency test of the entire structure (Templeton and Sing 1993; Templeton et al. 1995). Trees were generated using PAUP 4.0b10 (Swofford 2001) and all trees were rooted with homologous ND-4 sequences of the closely related species *L. peru* (Pacific red snapper). The most appropriate models and parameter estimates for maximum-likelihood and neighbor-joining trees were determined using MODELTEST 3.06 (Posada and Crandall 1998). The most appropriate model was the general time reversible model with among-site rate variation approximated using the proportion of invariable sites (GTR + I).

A 95% parsimony network of mtDNA haplotypes was constructed using the program TCS 1.13 (Clement et al. 2000). Branches within the network were nested into clades according to the guidelines described in Templeton et al. (1987) and Templeton and Sing (1993). This procedure involves grouping haplotypes that are separated by a single mutation (base substitution) into one-step clades, then the one-step clades that differ by a single mutation into two-step clades. This process continues until the entire tree is nested together into a single clade (Templeton et al. 1995). The nested structure obtained was then input manually into the program GEODIS 2.0 (Posada et al. 2000), along with a geographic matrix that corresponded to straight-line distances in kilometers between the sample localities (Fig. 1) for nested clade analysis. A permutational contingency analysis was used to test the null hypothesis of random geographical distribution of clades. We used the inference key found at the GEODIS 2.0 website (http://darwin.uvigo.es/software/geodis.html) to identify processes that led to significant phylogenetic and geographical associations.

Demographic history based on mtDNA sequence variation was also examined via mismatch-distribution analysis (Rogers and Harpending 1992) and the maximum-likelihood coalescent approach of Kuhner et al. (1998). We first tested neutrality of mtDNA sequence variants via Fu's (1997) F_S statistic and Fu and Li's (1993) D^* and F^* statistics, as implemented in ARLEQUIN and the DNASP package (Rozas et al. 2003), respectively. Significance of F_S was assessed by 10,000 randomizations; significance of D^* and F^* was assessed using 10,000 coalescent simulations based on the observed number of segregating sites in each sample. Mismatch-distribution analysis (Rogers and Harpending 1992) employed the sum-of-squared-difference statistic (SSD) to test conformance of the observed (mismatch) distribution to that expected under population expansion (Rogers 1995). Significance of SSD was assessed by 10,000 parametric bootstrap replicates, as implemented in ARLEQUIN.

The maximum-likelihood coalescent approach, as implemented in the program FLUCTUATE v.1.4 available at http://evolution.genetics.washington.edu/lamarc/fluctuate.html, was used to estimate population growth rate and effective size. FLUCTUATE utilizes a model of a single population that has been expanding (or declining) exponentially and provides estimates of the parameters Θ and g. Θ is defined as $4n_1\mu$ where n_1 is the 'current' effective population size, μ is the neutral mutation rate per site; g is the exponential growth rate of the population and is positive if the population is expanding and negative if the population is declining. We used Watterson's (1975) segregating sites estimate to obtain the initial estimate of Θ for each run, and a transition/transversion ratio of eight based on estimates from MODELTEST 3.06 (Posada and Crandall 1998). One hundred short chains (1,000 steps per chain with a sampling increment of 100) followed by 10 long chains (500,000 steps per chain with a sampling increment of 500) were employed to ensure convergence of the Markov Chain Monte Carlo. Estimates of time (in generations) since a 'population' differed from the current effective size were determined by using the formula $t_s = (\ln(n_t/n_1))/(g\mu)$, where n_1 and n_t are the effective population sizes at present (n_1) and t generations (n_t) ago, respectively. Estimates of μ employed were 1.0 and 1.5% per MY and were based on molecular-clock calibrations of the mitochondrial COI ($1.2\%/MY^{-1}$) and NADH-2 and ATPase-6 ($1.3\%/MY^{-1}$) genes developed for several geminate species pairs of fishes (Bermingham et al. 1997).

Results

A total of 60 unique mtDNA haplotypes (Table 1) were found among the 360 red snapper ND-4 sequences obtained. Eleven of the haplotypes were found at all sample localities, while another 11 were shared between two of the localities. The number of 'private' haplotypes (those found only at one locality) was fairly uniform: Alabama (12), Louisiana (10), and Texas (16). A total of 46 base substitutions were found among the 60 haplotypes. Of these, 33 were synonymous substitutions occurring at either the first or third codon position. Of the 13 non-synonymous changes, eight occurred at the first codon position, four at the second, and one at the third. Nucleon and nucleotide diversities (mean ± SE) were similar among localities: Alabama (0.793 ± 0.028; 0.003 ± 0.002), Louisiana (0.770 ± 0.030; 0.002 ± 0.002), and Texas (0.797 ± 0.028; 0.003 ± 0.002). Results of exact tests of homogeneity of haplotype distributions among cohorts at each locality and among localities (cohorts within localities pooled) were non-significant ($P > 0.05$). Results from AMOVA also were non-significant ($\Phi_{ST} = -0.002$, $P = 0.422$; $\Phi_{SC} = 0.003$, $P = 0.278$). Consequently, all mtDNA haplotypes were pooled for all subsequent analyses.

Neighbor-joining (distance-based), maximum parsimony, and maximum-likelihood trees had similar topologies (Fig. 2); haplotypes found in clade 2-1 (identified from nested clade analysis of a 95% parsimony network, see Fig. 3 and below) were the most basal or ancestral, with haplotypes found in nested clades 2-2, 2-3, and 2-4 being more derived (Figs. 2, 3).

Table 1 Distribution of red snapper mtDNA haplotypes from three localities in the northern Gulf of Mexico: *AL* (Alabama), *LA* (Louisiana), *TX* (Texas)

Haplotype	AL	LA	TX	Haplotype	AL	LA	TX
A1	42	48	43	E4	0	1	2
A2	1	0	0	E5	0	1	2
A3	1	0	0	E6	0	0	1
A4	1	1	1	E7	1	0	0
A5	0	1	0	E8	0	1	0
A6	0	3	1	F1	2	1	0
A7	1	2	1	F2	0	1	0
A8	0	0	1	F3	1	0	0
A9	3	0	0	G1	1	2	1
A10	1	0	0	G2	0	1	0
A11	0	1	1	H1	0	1	0
A12	0	0	1	H2	1	0	1
A13	0	0	1	I1	0	1	0
A14	0	0	2	I2	0	1	0
A15	2	1	3	J1	5	4	3
A16	1	0	0	J2	2	0	0
A17	0	0	1	J3	0	0	1
A18	0	1	0	J4	1	2	2
B1	3	1	2	J5	0	1	0
B2	0	0	1	J6	0	0	1
B3	0	0	1	K1	0	0	1
C1	1	0	0	K2	2	0	1
C2	0	0	1	L1	1	1	0
D1	1	1	0	L2	0	0	1
D2	1	1	1	M1	1	0	0
D3	1	0	0	M2	2	0	3
D4	0	1	0	N1	1	5	3
E1	35	32	33	N2	0	0	1
E2	3	1	0	N3	0	0	1
E3	1	0	0	N4	0	1	0

Clade 2-1 also was found on the interior of the nested structure based on a 95% parsimony network (Fig. 3), further supporting the hypothesis that haplotypes in clade 2-1 are ancestral. Nested structures based on the 95% parsimony network revealed three nesting levels: 1-step, 2-step, and the entire network (Fig. 3). There were no significant geographical associations for any of the nested levels ($P > 0.05$) when an exact contingency analysis was used; however, in geographic distance analysis (where geographic distances among samples were incorporated) significant associations ($P < 0.05$) were identified for clades A and F at the 1-step level and for clades 2-3 and 2-4 at the 2-step level (Fig. 3). Contiguous range expansion or short-distance dispersal across an expanding population front were inferred to characterize associations among haplotypes within clades A at the 1-step level and for both clades (2-3 and 2-4) at the 2-step level (Table 2, Fig. 4). Significant geographical associations due to restricted gene flow caused by isolation by distance were identified for 1-step clade F and for the entire cladogram (Fig. 3, Table 2).

Significant geographic associations of clades at different nesting levels are hypothesized to correspond to events and processes that occurred at different time periods (Templeton 1998). Thus, there appears to have been both range expansion and restricted gene flow at both recent and more distant time periods. Examination of the spatial distribution of haplotypes within individual clades further outlines the potential complexity and repeated occurrence of these temporal/spatial events. All four 2-step clades, for example, contain haplotypes found at all three localities, indicating range expansion. However, only one individual from the Texas locality was found in 2-step clade 2-2, suggesting restricted gene flow. Moreover, within each of the 2-step clades, there are several 1-step clades (e.g., A, J, L, and N) that contain haplotypes at all three localities (indicating range expansion), whereas there are a number of 1-step clades that contain either no haplotypes or a very low frequency of haplotypes from one of the three localities (indicating restricted gene flow). Examples include clades I (no haplotypes from the Alabama locality), clades B, C, K, and M (no or very few haplotypes from the Louisiana locality), and clades F, 2-2, and D (no or very few haplotypes from the Texas locality). Minimally, these results indicate a continuous, historical pattern of range expansion and restricted gene flow.

Fu's F_S and Fu and Li's D^* and F^* statistics (all samples pooled) differed significantly from 0 ($F_S = -27.7$, $P = 0.00$; $D^* = = -2.947$, $P < 0.05$; $F^* = -3.182$, $P < 0.05$), consistent with a significant departure from neutrality. The mismatch distribution of pairwise differences between red snapper mtDNA haplotypes was unimodal and did not differ significantly from the distribution expected under population expansion (SSD = 0.0012, $P = 0.08$). The time at which such demographic expansion could have occurred was estimated by using the relationship $\tau = 2ut$, where τ is the crest of a unimodal mismatch distribution, u is the mutation rate per generation of the DNA region under study, and t is the time in generations (Rogers and Harpending 1992). The estimates of τ (1.491, with 99 confidence intervals of 0.806 and 1.762) were obtained from ARLEQUIN. The parameter u was estimated as $m_T\mu$ where μ is the mutation rate per nucleotide and m_T is the number (590) of nucleotides assayed. Values of μ were set to 1.0 and 1.5% MY^{-1}, bounding the molecular-clock estimates developed for three mtDNA protein-coding genes of other lutjanids (Bermingham et al. 1997). Generation time for red snapper was assumed to be similar to that of the lutjanid species (*L. apodus* and *L. argentiventris*) examined by Bermingham et al. (1997). Estimated dates since population expansion were 126,356 years bp (99% CI of 68,305–149,322) and 84,237 years bp (99% CI of 45,537–99,548) based on the 'clock' estimates of 1.0 and 1.5% sequence divergence, respectively. These dates fall well within the Pleistocene epoch (10,000 years–1.6 million years bp). FLUCTUATE runs yielded a positive growth rate (g) of 2,653.51 (SD = 78.52) consistent with an expanding population. Estimates of Θ ($\pm$SD) were 0.134 ± 0.006. The growth parameter corresponds to moderate growth, where population size would have been 10% of current size between 152,000 and 154,000 years ago. These dates are concordant with dates of expansion estimated from the mismatch distribution.

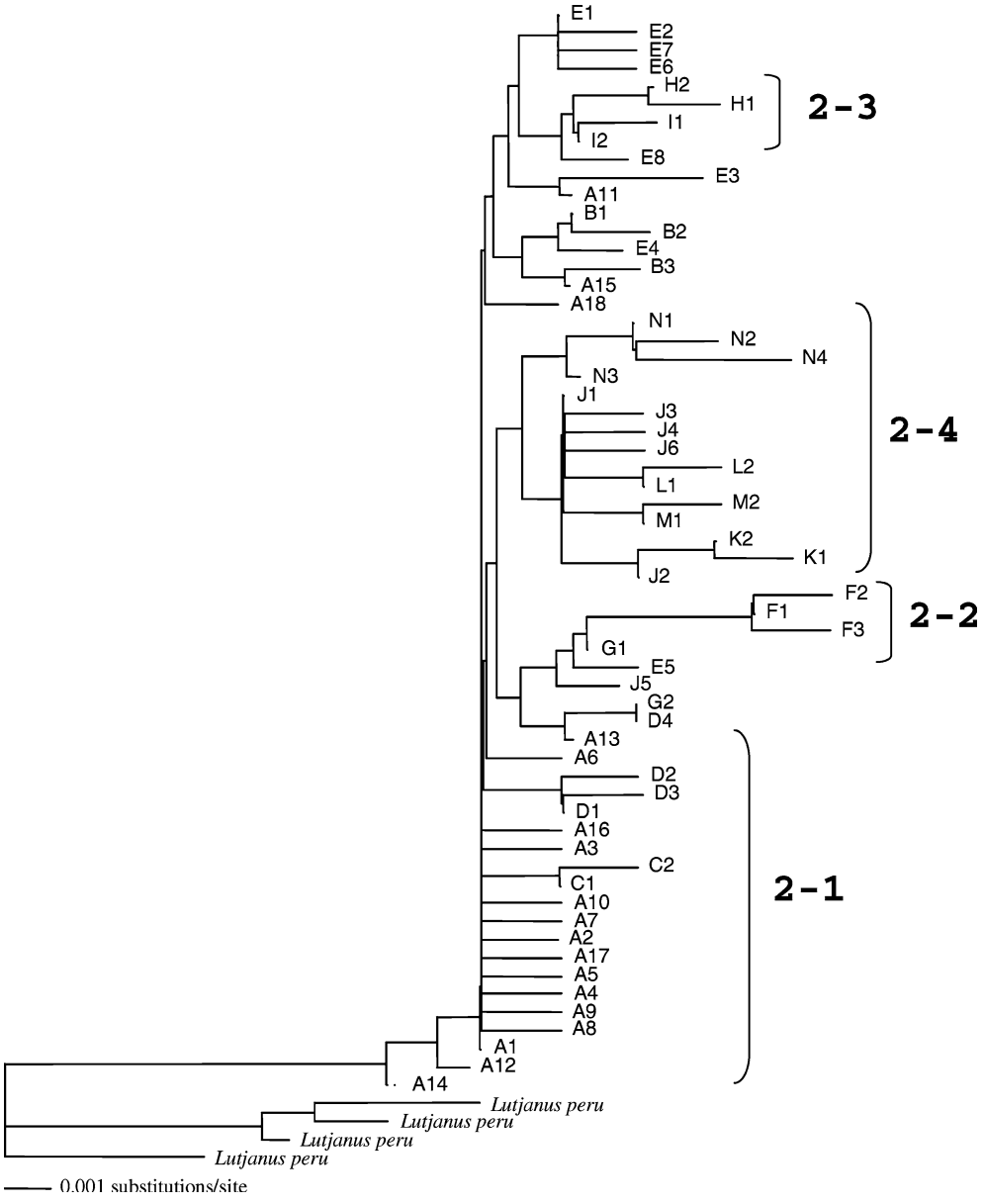

Fig. 2 Neighbor-joining tree of mtDNA haplotypes from Gulf red snapper (*Lutjanus campechanus*). The tree was rooted with mtDNA haplotypes of Pacific red snapper (*Lutjanus peru*). *Bracketed* haplotypes refer to two-step clades identified in nested clade analysis. Haplotypes and their frequencies in each locality are given in Table 1

Discussion

The temporal and spatial homogeneity of mtDNA haplotype distributions revealed by exact tests and AMOVA is consistent with most prior genetic studies (Camper et al. 1993; Gold et al. 1997, 2001; Garber et al. 2004) and with the hypothesis that a single, unit stock (population) of red snapper occurs in the northern Gulf of Mexico (Gulf). Because genetic homogeneity typically implies sufficient gene flow (migration) to offset genetic divergence, it has generally been hypothesized (Gold and Richardson 1998; Patterson et al. 2001) that extensive movement of red snapper likely occurs at one or more life-history stages. It also has been hypothesized that the observed genetic homogeneity in red snapper may reflect past (historical) rather than present-day population structure (Gold and Richardson 1998; Gold et al. 2001). Discrete populations or stocks could exist today yet have been in sufficient genetic contact in the recent (evolutionary) past to remain indistinguishable in haplotype frequencies.

Both hypotheses have caveats. The single-stock hypothesis assumes that gene flow at one or more life-history stages is sufficiently pervasive to offset genetic divergence. However, there is little direct evidence for extensive movement of red snapper across the northern Gulf. Red snapper do have highly pelagic eggs and larvae (Leis 1987), but neither egg type nor length of larval life is necessarily an effective predictor of gene flow in structure-associated marine fishes (Shulman and Bermingham 1995), and flow models coupled with mortality estimates (Cowen et al. 2000) indicate that larval exchange rates of marine species may be seriously

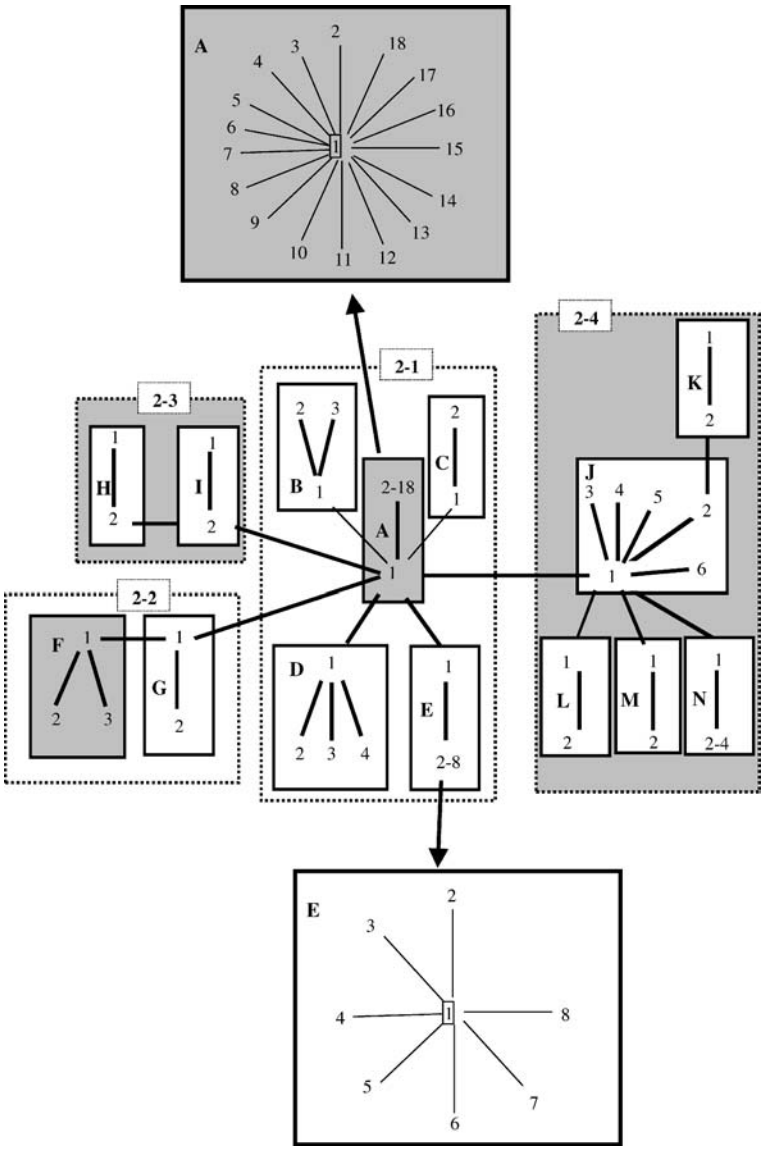

Fig. 3 Parsimony network used for nested clade analysis. *Numbers* correspond to individual haplotypes (Table 1). *Letters within boxes surrounded by a solid line* correspond to the one-step clade that encompasses those particular haplotypes. *Dashed lines* surround two-step clades, labeled with numbers that correspond to that particular clade (e.g., 2-1, 2-2, etc.). *Solid lines* between haplotypes correspond to single base-pair mutations. *Shaded boxes* indicate significant geographical associations within a clade. One-step clades A and E are also shown in *expanded boxes* to reveal the relationships of haplotypes within these clades that are separated by single base-pair mutations

overestimated. In addition, movement of red snapper across the continental shelf in the northern Gulf should be more-or-less linear and follow a model where the probability of gene flow is greater between proximal localities than between more distal ones. This should lead to an isolation-by-distance effect where haplotype frequencies are positively correlated between proximal localities (and negatively correlated between distal ones) and/or where genetic distance and geographic distance between localities are correlated. However, the correlations expected for an isolation-by-distance effect or between genetic and geographic distance in common mtDNA haplotypes have not been found (Gold et al. 1997), nor are there significant spatial differences across the northern Gulf in allele or genotype distributions at multiple, nuclear-encoded microsatellites (Gold et al. 2001; Saillant and Gold, unpublished results). The caveat to the second hypothesis (observed genetic homogeneity reflects historical, not present-day patterns) is simply that it represents a null and can only be rejected when (if) significant heterogeneity is detected.

Results of nested clade analysis of red snapper mtDNA haplotypes indicate a historical pattern of recurring range expansion and restricted gene flow. Range expansion due to short-distance dispersal across an expanding front was inferred for at least two of the 2-step clades and for one 1-step clade; while restricted gene flow was inferred at the highest nesting level (the entire cladogram) and within one of the lower-level clades (including 1-step clades) nested within it. A number of 1-step clades contained few or no haplotypes from each of the three localities, indicating that all three may have been isolated at various times. The exact timing of these events is problematic. Because a given clade is assumed to be as old (or older) than the lower-level clades nested within it (Templeton 1998), the earliest 'event' detected by these data would appear to be one of restricted gene flow (inferred from the highest nesting level).

Table 2 Results of nested clade analysis of geographic distance

Clades	Inference chain	Inference[a]
Clade A	1N, 2N, 11Y, 12N	Contiguous range expansion/ short-distance dispersal
Clade F	1N, 2N, 11N, 17Y, 4N	Restricted gene flow with isolation by distance
Clade 2-3	1N, 2N, 11Y, 12N	Contiguous range expansion
Clade 2-4	1N, 2N, 11Y, 12N	Contiguous range expansion
Total cladogram	1N, 2Y, 3N, 4N	Restricted gene flow with isolation by distance

[a]Inference key may be found at http://darwin.uvigo.es/software/geodis.html

Subsequent range expansion and additional periods of restricted gene flow could then have occurred until present times. Given that the two most divergent haplotypes in the dataset differed by nine base-pair substitutions (~1.5%), and assuming a rate for lutjanid (protein-coding) mtDNA of 1.0–1.5% sequence divergence per 10^6 years (Bermingham et al. 1997), the temporal events indicated by nested clade analysis would appear to have taken place within the last million or so years, i.e., during the Pleistocene epoch. Results from mismatch-distribution and maximum-likelihood coalescent analysis are consistent with this timing in that both indicated population expansion well within the Pleistocene period. However, mutations that give rise to base-pair substitutions (including those that identify nesting clades) do not necessarily occur at fixed time intervals but rather randomly across time. What this means essentially is that clades, even those at the same nesting level, could have arisen several thousand years apart.

It seems intuitive that glacial advance/retreat played a significant role in shaping red snapper distribution in the northern Gulf. Several glacial/interglacial periods occurred in North America (Shackleton and Opdyke 1973; Williams et al. 1998), and undoubtedly impacted marine species via altered sea-levels (Williams et al. 1998). During the last glacial maximum (occurring ~18,000 years ago), for example, sea levels in the northern Gulf were as much as 130 m lower than they are today (Rezak et al. 1985), meaning that little habitat would have been available for continental-shelf spawners such as red snappers (Allen 1985). The spatial/temporal patterns indicated by nested clade analysis are thus consistent with effects that might be expected with glacial advance/retreat. Population expansion could occur during glacial retreat and opening of continental-shelf habitat; while restriction of gene flow could occur either during glacial advance (if red snapper retreated to spatially different glacial refuges) or retreat, if (re)colonization of the continental shelf occurred at different times at spatially different localities.

A critical question relative to present-day population structure of red snapper in the northern Gulf is whether the patterns of population expansion and restricted gene flow indicated by nested clade analysis are totally a function of glacial advance/retreat or whether at least some of the patterns stem from factors that might be

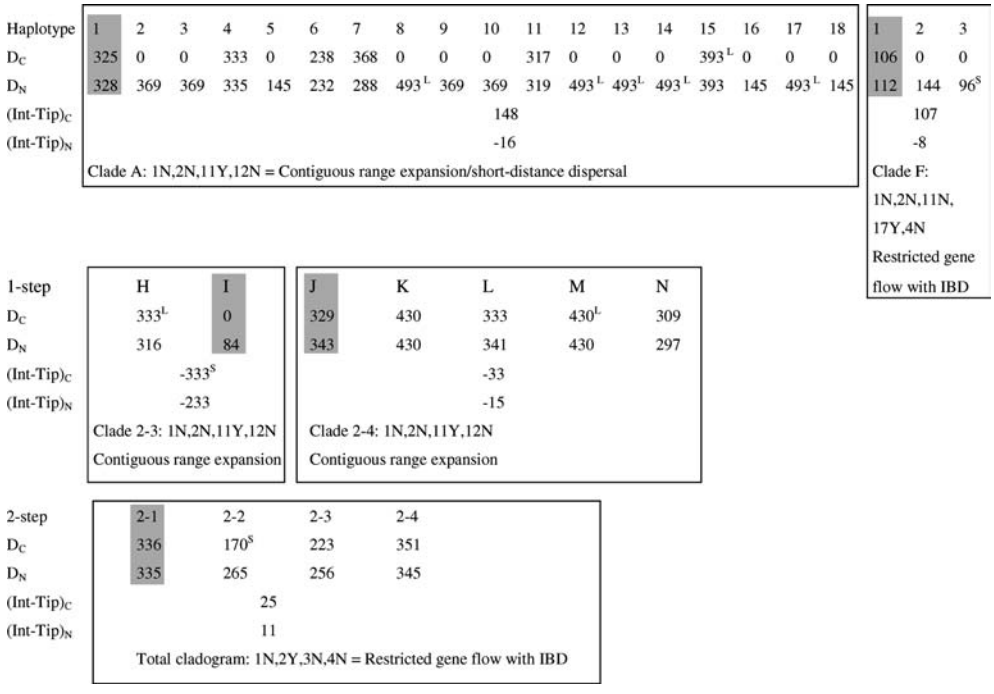

Fig. 4 Results of the nested clade distance analysis of Gulf red snappers. Only clades with significant geographical associations ($P < 0.05$) are shown. Under the haplotype or clade listed are the clade (D_C) and nested clade (D_N) distances. Under these values the interior versus tip clade and nested clade distances are also provided. Haplotypes or clades that are internal are *shaded gray*. A *superscript S* indicates a significantly small distance measure and a *superscript L* indicates a significantly large number distance measure. At the *bottom of each box* is the inference chain for that clade. The inference key may be found at http://darwin.uvigo.es/software/geodis.html

operative today. Mechanisms that could potentially impede gene flow in pelagic marine species include, among others, natural selection and/or physical processes such as varying oceanic currents and circulation patterns (Palumbi 1994, 1996b). Both could impact gene flow among present-day red snapper. The continental shelf along the northern Gulf is neither physically nor ecologically homogeneous, with salient differences in shelf width, depth, sediments, and declivity between the eastern and western Gulf (Rezak et al. 1985). There also are major differences in salinity, caused primarily by freshwater outflow from large river systems such as the Mississippi and Atchafalaya rivers in the northcentral Gulf (Rezak et al. 1985; Morey et al. 2003). Gene flow across the northern Gulf might also be affected by prevailing circulation patterns. Recoveries of experimental 'drifters' from releases west of a line from the Mississippi Delta to the Yucatan Peninsula occur almost exclusively in waters offshore of Texas, while recoveries from releases east of this line occur primarily in waters offshore of western Florida (Parker et al. 1979). In addition, nearshore current patterns in the western and northcentral Gulf are 'upcoast' or west–east during the summer, while during the rest of the year the flow is 'downcoast' or east–west (Li et al. 1997). Finally, there is the recent presence during the summer months of a major hypoxic zone that extends out along the continental shelf from the Mississippi Delta westward to the Louisiana border with Texas (Rabalais et al. 1999; Ferber 2001). The size of the hypoxic zone varies from year to year, but measured $\sim$19,000 km^2 in 1999 with dissolved oxygen levels in bottom waters as low as 2 mg/l (Rowe and Chapman 2002).

The results of nested clade analysis and the ecological and hydrographic differences across the northern Gulf are compatible with the notion that semi-isolated assemblages of red snapper occur in the northern Gulf. Because many of the hydrographic parameters, including those that impact ecological parameters, likely vary over long periods of time, such semi-isolated assemblages might exist over the short term, yet over the long term comprise a larger metapopulation tied together by periodic dispersal. This model of metapopulation structure differs from that of Levins (1969, 1970) with its emphasis on extinction and recolonization, and more closely follows the model proposed by Kritzer and Sale (2004) where a metapopulation is viewed as a network of partially closed populations that can influence one another's demographics via intermittent gene flow. Such a model, where discrete local populations are self-replenishing but where non-trivial demographic influences from other populations occurs periodically, may be a common situation in marine systems (Kritzer and Sale 2004) and clearly would impact critical fishery resource parameters such as population size, age structure, and recruitment.

An important and potentially testable prediction of the Kritzer and Sale (2004) model would be asynchrony in local population demographics but homogeneity in allele frequencies at selectively neutral genetic markers (such as used here and in previous studies of red snapper). Recently documented differences in growth rates (Fischer et al. 2004), size and age of females at maturity (Woods et al. 2003), and effective population size (Saillant and Gold 2002, unpublished results) among red snapper at the three localities investigated here clearly indicate different local population dynamics coincident with homogeneity in both mtDNA haplotype frequencies (this paper) and nuclear-encoded microsatellites (Saillant and Gold 2002; unpublished data).

The above considerations indicate that the present-day spatial distribution of red snapper assemblages in the northern Gulf has a complex history. Glacial advance/retreat, habitat availability and suitability, and hydrology are factors that could impact present-day population structure but might be difficult to detect with neutral genetic markers if periodic gene flow occurs. Studies that assess variation in genetic markers affecting traits under selection would seem a next logical step, as would precise estimates of rates of exchange among assemblages.

Acknowledgements Work was supported in part by an award from the Gulf and South Atlantic Fisheries Development Foundation (Project 70-04-20000/11824), the National Marine Fisheries Service (Marfin Project 87-FF-0426), and the Texas Agricultural Experiment Station Project H-6703). Views expressed in the paper are those of the authors and do not necessarily reflect views of the sponsors. This paper is no. 40 in the series 'Genetic Studies in Marine Fishes' and contribution no. 130 of the Center for Biosystematics and Biodiversity at Texas A&M University.

References

Allen GR (1985) Snappers of the world. FAO Species Catalogue, vol. 6. Food and Agriculture Organization of the United Nations, Rome

Bermingham E, McCafferty SS, Martin AP (1997) Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In: Kocher TD, Stepien CA (eds) Molecular systematics of fishes. Academic, San Diego, CA, pp 113–128

Bielawski J P, Gold JR (2002) Mutation patterns of mitochondrial H- and L-strand DNA in closely related cyprinid fishes. Genetics 161:1589–1597

Bortone SA, Chapman RW (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 no. NA17FF0379-03. SE Regional Office, National Marine Fisheries Service, St. Petersburg, FL

Camper JD, Barber RC, Richardson LR, Gold JR (1993) Mitochondrial DNA variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Mol Mar Biol Biotechnol 3:154–161

Carvalho GR, Hauser L (1995) Molecular genetics and the stock concept in fisheries. In: Carvalho, GR, Pitcher TJ (eds) Molecular genetics in fisheries. Chapman and Hall, London, pp 55–80

Christman MC (1997) Peer review of red snapper (*Lutjanus campechanus*) research and management in the Gulf of Mexico: statistics review. Office of Science and Technology, NOAA, Silver Spring, MD

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1660

Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000). Connectivity of marine populations: open or closed? Science 287:857–859

Fable WA (1980) Tagging studies of red snapper (*Lutjanus campechanus*) and vermilion snapper (*Rhomboplites aurorubens*) off the south Texas coast. Contrib Mar Sci 23:115–121

Ferber D (2001) Keeping the Stygian waters at bay. Science 291:968–973

Fischer AJ, Baker, MS Jr, Wilson CA (2004) Red snapper, *Lutjanus campechanus*, demographic structure in the northern Gulf of Mexico based on spatial patterns in growth rates and morphometrics. Fish Bull 102:593–603

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics 147:915–925

Fu Y-X, Li W-H (1993) Statistical tests of neutral mutations. Genetics 133:693–709

Garber, AF, Tringali MD, Stuck KC (2004) Population structure and variation in red snapper (*Lutjanus campechanus*) from the Gulf of Mexico and Atlantic Coast of Florida as determined from mitochondrial DNA control region sequence. Mar Biotechnol 6:175–185

Gold JR, Richardson LR (1998) Genetic homogeneity among geographic samples of snappers and groupers: evidence of continuous gene flow? Proc Gulf Caribbean Res Inst 50:709–726

Gold JR, Sun F, Richardson LR (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

Gold JR, Pak E, Richardson LR (2001) Microsatellite variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Mar Biotechnol 3:293–304

Goodyear CP (1995) Red snapper stocks in U.S. waters of the Gulf of Mexico. National Marine Fisheries Service, SE Fisheries Center, Miami Laboratory, CRD 95/96–05, Miami, FL

Goodyear CP, Phares P (1990) Status of red snapper stocks of the Gulf of Mexico: report for 1990. National Marine Fisheries Service, SE Fisheries Center, Miami Laboratory, CRD 89/90–05, Miami, FL.

Hauser L, Ward RD (1998) Population identification in pelagic fish: the limits of molecular markers. In: Carvalho GR (ed) Advances in molecular ecology. IOS, Amsterdam, pp 2191–2242

Hoese HD, Moore RH (1977) Fishes of the Gulf of Mexico. Texas A&M University Press, College Station, TX

Kritzer JP, Sale PF (2004) Metapopulation ecology in the sea: from Levin's model to marine ecology and fisheries science. Fish Fisheries 5:131–140

Kuhner MK, Yamato J, Felsenstein J (1998) Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149:429–434

Leis JM (1987) Review of the early life history of tropical groupers (Serranidae) and snappers (Lutjanidae). In: Polovina JJ, Ralston S (eds) Tropical groupers and snappers: biology and fisheries management. Westview, Boulder, CO, pp 189–237

Levins R (1969) Some demographic and genetic consequences of environmental heterogeneity for biological control. Bull Entomol Soc Am 15:237–240

Levins R (1970) Extinction. In: Desternhaber M (ed) Some mathematical problems in biology. Am Math Soc, Providence, RI, pp 77–107

Li Y, Nowlin WD Jr, Reid RO (1997) Mean hydrographic fields and their interannual variability over the Texas–Louisiana continental shelf in spring, summer, and fall. J Geophys Res 102:1027–1049

Maddison DR, Maddison WP (2000) MACCLADE 4: analysis of phylogeny and character evolution, ver 4.0. Sinauer, Sunderland, Mass.

Morey SL, Schroeder WL, O'Brien JJ, Zavala-Hidalgo. J (2003) The annual cycle of riverine influence in the eastern Gulf of Mexico. Geophys Res Lett 30: OCE 5.1–5.4

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Palumbi SR (1994) Reproductive isolation, genetic divergence, and speciation in the sea. Annu Rev Ecol Syst 25:547–572

Palumbi SR (1996a) Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics. Sinauer, Sunderland, MA, pp 205–247

Palumbi SR (1996b) Macrospatial genetic structure and speciation in marine taxa with high dispersal abilities. In: Ferraris F, Palumbi SR (eds) Molecular zoology: advances, strategies and protocols. Wiley, New York, pp 101–117

Parker RP, Morrison JM, Nowlin WD Jr (1979) Surface drift data from the Caribbean Sea and Gulf of Mexico, 1975–1978. ONR no. N00014-75-C-0537, Department of Oceanography, Technical Report no. 79-8-T, Texas A&M University, College Station, TX

Patterson WF, Watterson JC, Shipp RL, Cowan JH Jr (2001) Movement of tagged red snapper in the northern Gulf of Mexico. Trans Am Fish Soc 130:533–545

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818

Posada D, Crandall KA, Templeton AR (2000) GEODIS: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol Ecol 9:487–488

Rabalais NN, Turner RE, Justic D, Dortch Q, Wiseman WJ (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, MD

Rezak R, Bright TJ, McGrail DW (1985) Reefs and banks of the northwestern Gulf of Mexico. Wiley, New York

Rogers AR (1995) Genetic evidence for a Pleistocene population expansion. Evolution 49:608–615

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pair-wise genetic differences. Mol Biol Evol 9:552–569

Rowe GT, Chapman P (2002) Continental shelf hypoxia: some nagging questions. Gulf Mexico Sci 2002:153–160

Rozas J, Sánchez-Delbarrio JC, Messeguer X, Rozas R (2003) DNASP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Saillant E, Gold JR (2002) Genetic studies of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. Proc Gulf Caribbean Res Inst 55:811–820

Sambrook JE, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN ver. 2000: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva

Shackleton NJ, Opdyke ND (1973) Oxygen isotope and paleomagnetic stratigraphy of equatorial Pacific core V28-V238: oxygen isotope temperatures and ice volumes on a 10^5 and 10^3 year scale. Quat Res 3:39–55

Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49:897–910

Swofford DL (2001) PAUP* 4.0b: phylogenetic analysis using parsimony. Sinauer, Sunderland, MA

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 ST, Shipp RL (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

Templeton AR (1998) Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history. Mol Ecol 7:381–397

Templeton AR, Sing CF (1993) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. Genetics 134:659–669

Templeton AR, Boerwinkle E, Sing CF (1987) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. Genetics 117:343–351

Templeton AR, Routman E, Phillips CA (1995) Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140:767–782

Watterson G (1975) On the number of segregating sites in genetical models without recombination. Theoret Pop Biol 7:256–276

Williams M, Dunkerley D, De Deckker P, Kershaw P, Chappell J (1998) Quaternary environments, 2nd edn. Arnold, London

Wilson CA, Nieland DL (2001) Age and growth of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana. Fish Bull 99:653–664

Woods MK, Fischer AJ, Cowan JH Jr, Nieland DL (2003) Size and age at maturity of female red snapper *Lutjanus campechanus* in the northern Gulf of Mexico. Proc Gulf Caribbean Res Inst 54:526–537