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# A Molecular Approach to Stock Identification and Recruitment Patterns in Red Snapper (*Lutjanus campechanus*)

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## Abstract

The genetic structure of red snapper has been investigated by amplifying several regions of the mitochondrial DNA molecule via the polymerase chain reaction. The amplified regions were digested with four base restriction enzymes and the resulting fragments separated by polycrylamide gel electrophoresis. Two conserved regions of the molecule, cytochrome b and the 16 rRNA genes, exhibited only one variant in 40 surveyed individuals. The D-loop region of the molecule which is known to be variable in some species was invariant in red snapper. Additional primer sets are being developed to amplify the NADH-I region which has been highly polymorphic in other species. Given the limited variation found to date in red snappers, it is doubtful that sufficient variation will be found in the NADH-I region to identify discrete stocks. An alternative approach using microsatellites is being pursued in hopes of finding more polymorphic markers.

## Resumen

*La estructura genética del pargo del Golfo ha sido investigada mediante la amplificación de varias regiones de la molécula de DNA mitocondrial a través de la reacción en cadena de la polimerasa. Las regiones amplificadas fueron digeridas con cuatro enzimas de restricción base y los fragmentos resultantes separados por electroforesis en gel de policrilamida. Dos regiones conservadas de la molécula, citocromo b y los 16 genes de RNAr, mostraron solamente una variante en 40 individuos analizados. La región con "giro a la derecha" de la molécula, la cual se sabe que es variable en algunas especies, fué invariable en el pargo del Golfo. De manera adicional están desarrollándose algunas pruebas para amplificar la región NADH-1 la cual ha sido altamente polimórfica en otras especies. Debido a la poca variación encontrada hasta ahora en el pargo del Golfo, es dudoso que suficiente variación sea encontrada en al región NADH-1 como para identificar stocks discretos. Una aproximación alternativa usando microsatélites está siendo implementada con la esperanza de encontrar más marcadores genéticos polimórficos.*


## Introduction

Conservation and management of living marine resources require in-depth knowledge of the organisms' life history, including such parameters as mortality, fecundity, habitat preferences, population size, etc. In marine species, these parameters are often difficult to assess, because the organisms are free to move over a wide geographic area and many of them produce pelagic eggs permitting long distance dispersal. This complicates estimates of life history parameters as the data must be taken from a single population or stock in order to be reliable. To overcome this problem, genetic analyses of stock structure have become increasingly important tools for fisheries management (cf. Powers et al. 1990).

Red snappers (*Lutjanus campechanus*) are found from the southeastern US, throughout the Gulf of Mexico and Caribbean and southward to Brazil (Bortone 1986) (see Collins et al.; Cuellar et al., this vol.). It is one of the most sought-after demersal fishes in the Gulf of Mexico, for both commercial and recreational fisheries (Moran 1988). The alpha taxonomy of the species is relatively well known, except that some debate still exists over the status of southern populations, which have been considered as a distinct species, *L. purpureus* (cf. Bortone 1986; Allen 1987). In US waters in the subtropical Western Atlantic and Gulf of Mexico, *L. campechanus* is currently managed as part of the reef fish management plans (SAFMC/NMFS 1983; GMFMC 1990) due to declines in abundance over the past two decades. The larvae of this species are thought to be widely dispersed by oceanic currents, leading to the belief that a single stock exists within the Gulf of Mexico and perhaps throughout the US portion of the range.

Previous efforts to define stocks of red snapper within the Gulf of Mexico (Camper et al. 1993) found no evidence supporting stock separation using traditional methods to assess genetic variation in mitochondrial DNA (mtDNA). Camper et al. (1993) found 29 haplotypes in 86 specimens, but only two haplotypes were found in more than two specimens and the remaining haplotypes were usually found only once. Camper et al. (1993) reported levels of interpopulation diversity in this species as relatively low compared to other marine species. It is possible that the absence of stock structure within the Gulf of Mexico reported by Camper et al. (1993) may result from the limited variation identified by their technical approach. In this report, we assess genetic variation in mtDNA from red snapper using the polymerase chain reaction to amplify four regions of the molecule and four base restriction digests of the amplified product. Studies on four alosid species using this approach found much more variation than traditional methods using

six base digestion of the total mtDNA molecule (Chapman et al. 1994). Our goal is to identify the region(s) of the molecule that reveal sufficient variation to discriminate stocks, should they exist.

## Materials and Methods

Samples were collected from the Gulf of Mexico near Panama City, Florida, Orange Beach, Alabama and Freeport, Texas, and by sampling commercial catches. Liver samples were preserved in 95% ethanol and blood in 1% SDS and returned to the laboratory. Liver samples were dried, digested with Proteinase K and phenol-chloroform extracted. Nucleic acids were then collected by ethanol precipitation. The samples were rehydrated in 100 ml of sterile water and 1-15 ml were used in PCR reactions depending upon the DNA content.

Amplification of the 12s-16s rRNA (bases 1109-2510 on the human genome), cytochrome b-D-loop (15560-16498), 6-ATPase (8566-9139), NADH dehydrogenase I (2525-4410) and 16s rRNA (2500-3058) regions followed the protocol of Kocher et al. (1989). Oligonucleotide sequences for the primers are given in Meyer (1993). Unincorporated nucleotides and Taq polymerase were removed from the amplified products by either phenol chloroform extraction and ethanol precipitation or by Magic PCR preps (Promega Biotechnology). The products were then digested by a battery of four base restriction enzymes and the resulting fragments separated on polyacrylamide gels. The gels were stained with ethidium bromide and photographed under UV light following Chapman and Powers (1984). Molecular weight estimates of the restriction fragments were made using multiple regression procedures in Lotus 1-2-3.

Restriction fragment patterns were assigned a letter designation for each enzyme. The first pattern encountered was assigned the letter and subsequent variants were assigned B, C, etc. Composite haplotypes for each individual were composed of these letter designations arranging the restriction enzymes in alphabetical order. The frequency distributions of composite haplotypes in each population were then compared using the Roff and Bentzen (1989) chi-square test.

## Results

Of the five primer sets attempted in this survey only the ND 1 (2525-4410) set failed to amplify *L. campechanus* mtDNAs. This primer set has functioned well in partially purified mtDNAs from a variety of species. The remaining primer sets functioned well, generating consistent amplifications from either blood or liver derived DNA.

Molecular weight estimates for the restriction fragments obtained by digesting the 12s-16s regions of *L. campechanus* mtDNA are presented in Table 1. In general these estimates total about 1 600 bases and are consistent with the estimate of the total amplified region obtained from agarose gel electrophoresis. The exceptions to this are estimates obtained from Alu I and Hpa II digests which were less than those obtained for the other restriction enzymes. In the Alu I digests, several low molecular weight bands were noted in the photographs, but these bands were too faint to score reliably. The single variant pattern seen in this region of the mtDNA was generated by a Cfo I digest (Table 1) and this pattern was seen in only one individual. No variant haplotypes were seen in the forty individuals amplified for the cyt b-D-loop or 6 ATPase regions (Table 2).

Unlike the other regions amplified in this study the 16s rRNA segment revealed substantial variation using four base digestions. Of the five restriction enzymes employed, four showed two variants each (Table 3). Composite haplotypes based upon these variants (Table 4) for 52 individuals revealed a total of ten dinstinct lineages. Five of these

**Table 1. Estimated molecular weights of fragments obtained by digestion with the indicated endonuclease for the 1109-2500 (12s to 16s rRNA) region of *Lutjanus campechanus* mtDNA. [*Pesos moleculares estimados de los fragmentos obtenidos por digestión con la endonucleasa especificada para la 1109-2500 (12s a 16s rRNA) región del DNA mitocondrial de* Lutjanus campechanus.]**

| Alu | Cfo I | | Hae III | Msp | Rsa | Hpa II |
|---|---|---|---|---|---|---|
| A | A | B | A | A | A | A |
| 335 | 1 254 | 581 | 750 | 581 | 539 | 480 |
| 229 | 410 | 410 | 410 | 383 | 390 | 250 |
| 164 | - | 324 | 229 | 347 | 351 | 203 |
| 141 | - | 320 | 121 | 263 | 272 | 115 |
| 131 | - | - | 103 | 116 | 126 | 80 |
| 112 | - | - | - | - | 73 | 54 |
| - | - | - | - | - | - | 43 |
| - | - | - | - | - | - | 37 |
| 1 112 | 1 664 | 1 635 | 1 613 | 1 690 | 1 751 | 1 262 |

**Table 2. Composite haplotypes for three regions of the mtDNA molecule taken from 40 red snappers. [*Haplotipos compuestos para tres regiones de la molécula de DNA-mitocondrial tomada de 40 individuos de pargo del Golfo.*]**

| Region | N |
|---|---|
| 1109-2500 (12s-16s rRNA) | |
| AAAAAA | 39 |
| ABAAAA | 1 |
| 15560-16498 (cyt b-D-loop) | |
| AAAAAA | 40 |
| 8566-9139 (6-ATPase) | |
| AAAAAA | 40 |

**Table 3. Molecular weight estimates for fragments obtained by digesting the 16s rRNA portion of *Lutjanus campechanus* mtDNA molecule with the indicated enzyme. All estimates are accurate to within 5%. [*Pesos moleculares estimados para fragmentos obtenidos por la digestión de la región 16s rRNA de la molécula de DNA-mitocondrial de* Lutjanus campechanus, *con la enzima indicada. Todas las estimaciones son precisas a un nivel 95%.*]**

| Alu I | | Cfo I | Hae III | | Rsa I | | Taq I | |
|---|---|---|---|---|---|---|---|---|
| A | B | A | A | B | A | B | A | B |
| 213 | 241 | 361 | 220 | 410 | 610 | 349 | 628 | 519 |
| 164 | 213 | 138 | 184 | 122 | - | 201 | - | 90 |
| 140 | 140 | 115 | 122 | 88 | - | 60 | - | - |
| 90 | - | - | 88 | - | - | - | - | - |

**Table 4. Composite haplotypes found in the 16s rRNA portion (2 500-3 058) of *Lutjanus campechanus* mtDNA. Composite haplotypes are based upon patterns produced by the restriction enzymes from Table 3 in alphabetical order. [*Composición de haplotipos encontrados en la región 16s rRNA (2500-3058) de DNA mitocondrial de* Lutjanus campechanus. *La composición de haplotipos está basada en los patrones producidos por las enzimas de restricción de la tabla 3, en orden alfabético.*]**

| Haplotype | Orange Beach Alabama | Freeport Texas | Panama City Florida | Total |
|---|---|---|---|---|
| AAAAA | 4 | - | - | 4 |
| AAAAB | 8 | - | 12 | 20 |
| AABAA | 1 | - | 1 | 2 |
| AABAB | 1 | - | - | 1 |
| BAABC | 1 | - | - | 1 |
| BAABB | 2 | - | - | 2 |
| BAABA | 2 | - | - | 2 |
| BABBB | - | 5 | - | 5 |
| BAAAB | 6 | - | 2 | 8 |
| BAAAA | 8 | - | - | 8 |

haplotypes are common (i.e., found in more than two individuals), three haplotypes are represented by two individuals and the rest were seen only once.

Geographically the distribution of mtDNA haplotypes clearly distinguish the Texas population as different from Panama City, Florida or Orange Beach, Alabama populations. All five of the Texas specimens shared a common haplotype that was not observed at the other locations. The chi-square tests also differentiated Orange Beach from Panama City ($p<0.001$).

## Discussion

The results of this study are important to our understanding of mtDNA evolution and to the population structure of red snapper. These issues will be discussed in some detail. The data should, however, be viewed with some reservations as the sample sizes are somewhat restricted.

The vast majority of data on mtDNA variation within and among species is based upon six base digestion of the entire molecule (cf. Meyer 1993). In most of these studies, restriction site losses and gains are not mapped to specific regions of the molecule and we cannot judge the relative rates of change among various regions. Despite this limitation some generalities have emerged from sequencing studies of portions of this genome. Meyer (1993) compared mtDNA sequences of cod and frog and reported that the 16s rRNA portion was among the most conserved regions. In addition, it is generally accepted that the D-loop region is subject to higher rates of change than most other segments. In some species, the D-loop appears to be constrained and may evolve at a pace consistent with the rest of the mitochondrial genome (Shedlock et al. 1992). In the present study, our limited survey of variation in the D-loop indicates a rate of evolution very much slower than the 16s rRNA. In addition, our results are not consistent with the conclusion that the 16s rRNA is one of the more conserved elements of *L. campechanus* mtDNAs. This should not be taken as a challenge to the general conclusion regarding rates of evolution in this region. Meyer (1993) compared only 207 bases of this region in frog and cod, where our study surveyed variation in approximatly 610 bases. It is possible that we found variation in a part of the region that was not assessed by Meyer (1993). In addition, we have no doubts about the conservation of this region in some species. We found no restriction site differences in a comparison of dusky and hammerhead sharks (two different families). This information was one of the reasons we did not examine the 16s rRNA region until recently. If the region is so highly conserved that one cannot differentiate families, there is little chance of finding intraspecific variation.

The geographic distribution of mtDNA haplotypes found in the 16s rRNA region clearly distinguishes populations of *L. campechanus* in the northern Gulf of Mexico. The data contrast markedly from those of Camper et al. (1993) where no evidence of population subdivision was observed. We believe that the difference is due to the distribution of mtDNA haplotypes in the two data bases. Most of the haplotypes reported by Camper et al. (1993) were represented by more than two individuals, permitting greater statistical power. In addition, the geographic distribution of common haplotypes was highly structured. All five of the BABBB haplotypes were found in the Texas specimens. These results should be viewed as provisional due to the limited number of individuals in the Texas specimens. Should further analyses of Texas specimens support the data presented here, a substantial revision of current plans for this species are in order.

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