Studies in Conservation Genetics of Tarpon (*Megalops atlanticus*) - IV. Population Structure among Gulf of Mexico Collection Sites Inferred from Variation in Restriction Site Polymorphisms of Tarpon Mitochondrial DNA

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ABSTRACT

Tarpon once supported valuable recreational fisheries in Texas waters. Beginning in the late 1940s catch rates began to decline, leading to reductions in tarpon-related activities including abandonment of lucrative tournaments. Desires to restore these valuable fisheries in Texas waters have prompted proposals for management interventions, including culture and stocking of hatchery-reared tarpon. Texas Parks and Wildlife Department Coastal Fisheries Division policies require examinations of the genetic consequences of enhancement programs prior to implementation of any stockings. In order to determine stock structure and estimate gene flow in the Gulf of Mexico, tarpon were collected from sites distributed between the Bay of Campeche in the southern Gulf and the Florida Keys in the eastern Gulf. Four mitochondrial DNA (mtDNA) fragments were amplified using the polymerase chain reaction. Each fragment was then digested with a suite of restriction enzymes and the resulting fragment patterns (RFLPs) were analyzed to infer population structure and gene flow.

KEY WORDS: *Megalops atlanticus*, conservation genetics, mitochondrial DNA

Estructura de los Sitios de la Colección en el Golfo de México deducidos por la Variación en Polimorfismos del Ditio de la Restricción de la DNA Mitochondrial de Sábalo

El Sábalo mantuvo una pesquería recreativa de mucho valor económico en las aguas de Texas. A inicios de los años 40 la tasa de captura declino, provocando que las actividades relacionadas con la pesca del sábalo se redujeran incluyendo el

abandono total de los torneos deportivos. El deseo de restaurar esa valiosa pesquería en aguas de Texas ha motivado plantear medidas de manejo, que incluyen el cultivo y la reintroducción de sábalos provenientes de poblaciones artificiales. El Departamento de Parques y Vida Silvestre, División de Pesquerías Costeras requiere conocer las consecuencias genéticas de los programas de mejoramiento antes de implementar cualquier actividad de reintroducción. Para determinar la estructura genética y estimar el flujo genético en el Golfo de México, se colectó sábalos de diferentes localidades distribuidas entre la Bahía de Campeche en el Sur del Golfo hasta Florida al Este del Golfo. Cuatro fragmentos de ADN mitocondrial (ADNm) fueron amplificados usando la Reacción en Cadena de la Polimerasa (PCR). Cada fragmento fue digerido con enzimas de restricción y los patrones de restricción (RFLPs) fueron analizados para inferir la estructura y el flujo genético.

PALABRAS CLAVES: *Megalops atlanticus,* genética , ADN mitochondrial DNA

INTRODUCTION

The tarpon (*Megalops atlanticus*) was historically one of the premier game fishes of the Texas coast, supporting a valuable recreational and tournament fishery. However, the Texas tarpon fishery collapsed near the middle of the 20th Century, resulting in a loss of recreational opportunity and an economic hardship on Texas' coastal communities. The Texas recreational fishery now focuses on estuarine species such as red drum (*Sciaenops ocellatus*) and spotted seatrout (*Cynoscion nebulosus*) and offshore species such as red snapper (*Lutjanus campechanus*). The recreational fishery represents a considerable economic asset for coastal communities, having recovered from overfishing in the 1970s (McEachron and Daniels 1995). The success of this recovery has renewed interest in attempts to effect recovery of the tarpon fishery. Proposals have centered on research to better understand the biology of tarpon and to determine the causes of the tarpon's decline in the northwestern Gulf of Mexico. In addition, the use of hatchery-spawned tarpon juveniles to enhance the natural population has been proposed as a possible management tool to facilitate the recovery of tarpon in Texas waters.

Marine stockings are considered to be an important component of the recovery and maintenance of Texas's recreational fishery (McEachron et al. 1995). Well designed enhancement programs may serve as valuable tools in the management of exploited organisms (Botsford and Hobbs 1984, Daley 1993), however poorly designed or poorly executed stocking programs represent a threat to the community structure (Courtenay and Moyle 1992), genetic diversity (Allendorf and Ryman 1987, Harada 1992) and stock structure (Nelson and Soulé 1987, Evans and Willox 1991) of natural populations. Protecting the genetic integrity of a native population impacted by a stocking program requires an understanding of within-population genetic diversity and among-population genetic divergence of the target species. In

recognition of the importance of protecting the genetic integrity of managed populations, the Coastal Fisheries Division of Texas Parks and Wildlife Department (TPWD) has established a policy requiring a thorough investigation of the genetic structure of a species prior to the implementation of a stocking program. This research provides the information necessary to implement sound protocols and safeguards to ensure the protection of the genetic resources of the species.

A series of investigations into the population genetics of tarpon have been conducted by TPWD biologists and their collaborators. The present paper describes variation observed in the mitochondrial DNA (mtDNA) of tarpon collected in the Gulf of Mexico. Variation in mtDNA across the distribution of tarpon has been reported (Blandon 2002), with samples from most regions characterized as highly diverse genetically. Population structure was limited, with tarpon from the western Atlantic, the Caribbean, and the Gulf of Mexico composing a single population. Tarpon from the Bay of Guinea and from the Pacific Ocean of Panama were genetically divergent and less diverse than conspecifics inhabiting the nonperipheral part of the species' range. A separate analysis (Garcia de Leon 2002) reached a different conclusion. This study, which examined variation in allozymes and 12S mtDNA RFLP data among tarpon from the western Gulf of Mexico, found evidence of distinct genetic differentiation between tarpon collected above Matagorda Bay, Texas and those collected from Matagorda Bay south to Veracruz. The present analysis attempts to specifically examine the Gulf of Mexico data contained in Blandon et al. in greater detail with the goal of resolving the differences between the earlier analyses.

METHODS

The strategy followed in this study was that of Chow et al. (1993). Targeted regions of the mtDNA genome were PCR amplified (Saiki et al. 1988) using conserved primers (Kocher et al. 1989), then digested with restriction endonucleases to produce fragments whose number and size depended upon the presence or absence of recognition sites. PCR allows analysis of small amounts of tissue collected by minimally invasive techniques (Whitmore et al. 1992) a characteristic especially important in tarpon, which are often accessed during recreational captures requiring minimal handling and release of fish. Samples were primarily scales obtained from a variety of sampling sites (Figure 1). Most Texas samples were obtained by TPWD personnel during routine resource sampling or opportunistically following fish kills. Recreational fishermen and guides from across the distribution of the species were enlisted to collect scales from catch-and-release captures. Several samples were obtained from tournaments in Mexico, Texas, Louisiana, and Florida and from fish markets in Mexico. For perspective, a sample from Chetumal, on the Mexican Caribbean Coast, was included in some analyses. Scales were placed in 100% ethanol (diluted to 70% with DI water), refrigerated for 24 hours, then stored at room temperature until shipment to the Perry R. Bass Marine Fisheries Research Station.

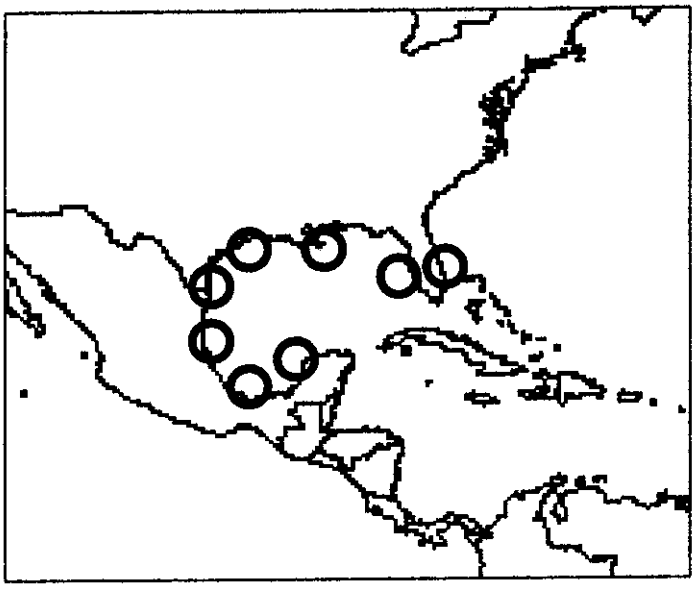

Figure 1. Tarpon collection localities in the Gulf of Mexico and Atlantic Ocean

Table 1. Genotypic diversity and percent mean nucleotide sequence diversities for seven tarpon (*Megalops atlanticus*) sampling localities

Locality	Number of individuals	Number of haplotypes	Nucleon diversity (mean +/- SD)
Florida	21	8	0.723+/-0.101
Louisiana	20	10	0.758+/-0.101
Texas – Upper	28	11	0.807+/-0.060
Texas – Lower	25	14	0.890+/-0.052
Tampico	27	12	0.849+/-0.059
Veracruz	53	17	0.731+/-0.064
Campeche	3	2	0.667+/-0.314
Total	177	43	0.776+/-0.001

Genomic DNA was recovered from scales using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Four mtDNA fragments were PCR amplified using primers designed for broad application among teleost fishes (Table 1). Reactions employed Ready-To-Go PCR beads (Amersham Pharmacia Biotech, Piscataway, NJ) in 25 µl reactions containing 1.0 µl of template DNA and 1µl of each member of the primer pair. Amplifications were performed using Techne

Genius thermalcyclers (Techne Inc., Princeton, NJ) programmed for the following profile: 35 cycles at 95°C (60 s), 48 °C (75 s), 72 °C (90 s), followed by 72 °C for 7 min, then a 4 °C hold.

A sub-set of amplification products were digested with 26 restriction endonucleases: *AciI, AluI, BfaI, BsaJI, BslI, BsoFI, BstUI, Cac8I, DdeI, Fnu4HI, HaeIII, HhaI, HinfI, MaeIII, MboI, MnlI, MseI, MspI, MwoI, NlaIII, NlaIV, RsaI, Sau96I, ScrFI, TaqI*, and *Tsp509I*. Digestion protocols provided by restriction enzyme suppliers were followed. Results were screened for polymorphism and quality of digest. Polymorphic RFLP patterns were confirmed through repeat digestions separated on 2% agarose gels. Fragment sizes were determined by comparison with pGEM molecular weight DNA markers (Promega Corporation, Madison, WI) and analyzed using the Gene Profiler 1-D Gel Analysis and Databasing Program (Scanalytics, Inc., Fairfax, VA).

Nucleotide sequence diversity within samples and divergence among sampling sites were estimated using the software package REAP (McElroy et al. 1992). Phylogeographic relationships of composite haplotypes were examined using minimum-length parsimony networks. Composite haplotypes were connected based on similarity of inferred restriction site sequences. Changes in restriction site sequence were assumed to reflect single nucleotide gain, loss, or point mutation. Exact tests were used to examine pair-wise differences in haplotype frequency (Raymond and Rousset 1995a, Goudet et al. 1996) using the statistical program ARLEQUIN (Schneider et al. 1999). Significance of exact test probabilities was estimated by resampling 1000 times. Population subdivision was estimated using the statistical program GENEPOP (Raymond and Rousset 1995b) which calculated θ (Weir and Cockerham 1984). ARLEQUIN was used to perform an analysis of molecular variance (AMOVA) generating estimates of genetic variance components (Excoffier et al. 1992). The program TREEVIEW (Page 1996) was used to generate a neighbor-joining tree generated from the matrix of interpopulational (mtDNA) nucleotide sequence divergence.

RESULTS

Intrapopulational mtDNA nucleotide-sequence diversities (Table 1) ranged from 0.667 (SD = 0.314) at Campeche to 0.890 (SD = 0.052) on the lower coast of Texas, with a value of 0.776 (SD = 0.001) for the combined samples. Campeche, with a limited sample (n = 3), had three observed composite haplotypes and Veracruz was observed to have 17 composite haplotypes. Forty-three composite haplotypes were observed across the Gulf of Mexico. The diversity of encountered nucleotide sequences is reflected in the minimum-length parsimony network (Figure 2). The composite haplotypes (H, M, N, and AL) observed to be most divergent differed from the most common composite haplotype (A) by a minimum of 3 base-pair (bp) changes. Three secondary hubs (E, F, and L; in the sense of Gold et al. 1997) were found, each related to composite haplotype A by a minimum of 1 bp. There is little apparent biogeographic correspondence among haplotype lineages.

Louisiana and Veracruz had representatives of each of the secondary hubs. The only apparent biogeographic structuring involved the absence of hub L from Tampico and the lower coast of Texas and the rarity of that hub in the upper Texas coast and in Louisiana.

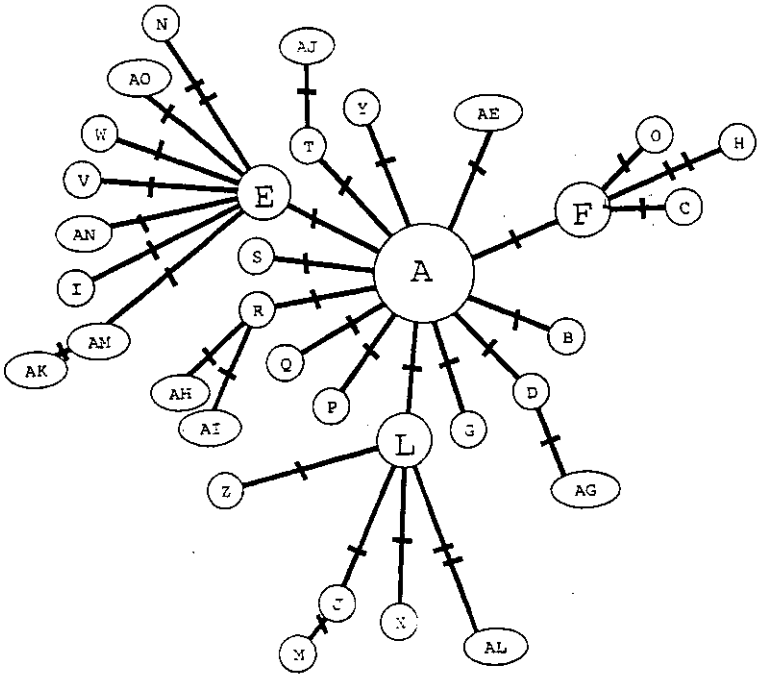

Figure 2. Minimum length parsimony network for composite haplotypes of tarpon collected in the Gulf of Mexico. Missing letters represent composite haplotypes not observed in the Gulf of Mexico

Nucleotide divergence among populations was low (Table 2). The combination of high within-region genetic diversity and low among-regions genetic divergence is reflected in the results of the AMOVA (Table 3) which found a strong preponderance of the observed variance to be attributable to within sample diversity. Estimation of a θ value of 0.007 (p = 0.665) suggests absence of biologically significant population structuring among collection regions in the Gulf of Mexico. However, pairwise examinations of among-sampling region genetic differentiation (Table 2) found differences between the northwestern Gulf of Mexico (Tampico and the Texas collections) and the southern and eastern Gulf of Mexico. Though the structure was not pronounced, a neighbor-joining tree generated from interpopulational mtDNA nucleotide sequence divergence values (Figure 3) also differentiated between the northwestern Gulf of Mexico and eastern and southern reaches of the Gulf, however distances among nodes were minimal.

Table 2. Above diagonal, nucleotide sequence divergence among sampling regions. Below diagonal, probability values (*p*) associated with pairwise exact tests of population differentiation among sampling regions. Second value represents the confidence in the probability value estimated using 1,000 resamplings.

	Florida Gulf	Louisiana	Texas Upper	Texas Lower	Tampico	Veracruz	Campeche
Florida Gulf	-	-0.0005	-0.0001	-0.0001	0.0002	-0.0003	-0.0015
Louisiana	0.892 ±0.003	-	-0.0003	-0.0002	-0.0004	-0.0003	-0.0013
Texas Upper	0.017 ±0.004	0.377 ±0.011	-	-0.0002	-0.0001	-0.0001	<0.0001
Texas Lower	0.029 ±0.005	0.438 ±0.016	0.139 ±0.010	-	0.0005	0.0005	0.0003
Tampico	0.051 ±0.009	0.918 ±0.009	0.007 ±0.003	0.026 ±0.007	-	0.0002	-0.0003
Veracruz	0.992 ±0.013	0.687 ±0.013	0.044 ±0.006	0.033 ±0.007	0.070 ±0.007	-	-0.0015
Campeche	0.284 ±0.016	0.316 ±0.012	0.037 ±0.004	0.284 ±0.010	0.162 ±0.015	0.401 ±0.021	-

Table 3. Analysis of molecular variation (AMOVA) among composite mtDNA haplotypes of tarpon from the Gulf of Mexico.

Variance component	Observed partition	
	Variance	% total
Among regions	-0.00980	-0.68
Within regions	1.45715	100.68
Total	1.44735	100.00

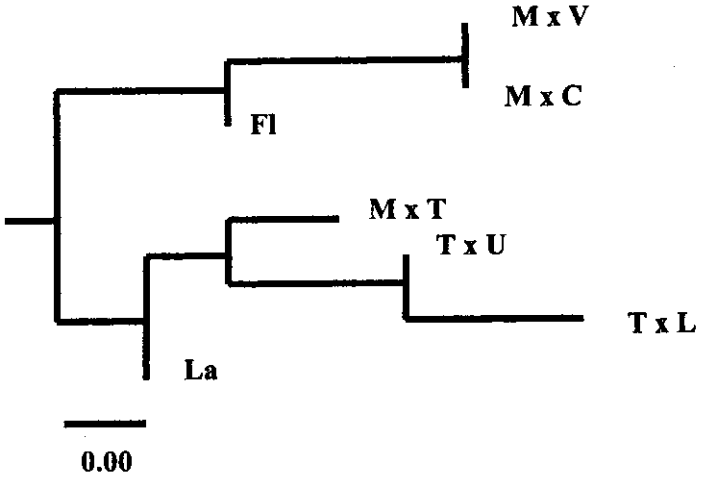

Figure 3. Neighbor-joining tree generated from matrix of interpopulational (mtDNA nucleotide sequence divergence. Bar scale below tree represents relative distance units

DISCUSSION

Gulf of Mexico tarpon are characterized by extensive within-region genetic variability and by reduced among-region genetic differentiation. The exception to the latter is the northwester Gulf, where tarpon appear to exhibit some degree of differentiation. This divergence is reminiscent of the conclusions drawn by García De León et al. (2002) which suggested a break in the frequencies of allozyme allelic distributions between the upper and lower coasts of Texas. This discontinuity is further west in the Gulf of Mexico than any differences detected in the present study, but that may be a function of different evolutionary histories of the allozyme loci

(nuclear) studied by García De León and the mtDNA fragments examined in the present study.

If tarpon from the northwestern Gulf of Mexico are genetically distinct from conspecifics in the southern and eastern Gulf some mechanism must be found to account for this differentiation. Tarpon spawn in deep offshore waters, have eggs and larvae with an extended pelagic phase, and adults are highly migratory (Zale and Merrifield 1989). This suite of life history traits would predict little or no population structuring in the absence of physical barriers to gene flow (Gyllensten 1985). It's possible the observed differentiation is attributable to chance geographic variation in a panmictic gene pool or to sampling error. The geographic cohesion of the cluster of genetically differentiated sampling regions argues against this interpretation.

If tarpon of the northwestern Gulf of Mexico are genetically differentiated, stocking strategies will have to be developed that take this structuring into account. Broodfish will need to be collected from the same region into which their offspring will be stocked. Optimally, broodfish should be collected from Texas, with possible contributions from Louisiana or northern Mexico. Certainly tarpon derived from broodfish collected in Florida or from south of Tampico should not be released in Texas. The high levels of within-sample genetic variation noted in Gulf of Mexico tarpon require that the numbers of broodfish be extensive and that broodfish turnover be frequent. It will also be necessary to insure an equal contribution from each female broodfish and an approximately equal number of male and female broodfish.

The reduced population of wild tarpon targeted for enhancement makes care in the design and implementation of culture and stocking strategies critical. The smaller the effective size of the target population the greater the potential impact, both positively and negatively, an enhancement program will have if that program is effective.

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