Col.Vol.Sci.Pap. ICCAT, 54 (2): 372-376. (2002)

GENETIC ANALYSES OF ATLANTIC NORTHERN BLUEFIN TUNA CAPTURED IN THE NORTHWEST ATLANTIC OCEAN AND THE MEDITERRANEAN SEA

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SUMMARY

In recent years, there has been considerable debate about whether the Atlantic northern bluefin tuna, Thunnus thynnus thynnus, reproduces as a panmictic unit. To address this question, we examined both mitochondrial DNA control region nucleotide sequences and nuclear gene ldhA allele frequencies in replicate samples of northern bluefin tuna from the Mediterranean Sea and the northwestern Atlantic Ocean. AMOVA analyses of both types of data revealed no significant differences between samples from the two regions. These results demonstrate the importance of analyzing multiple year classes and large sample sizes in stock structure analyses. In addition, larval samples from the Gulf of Mexico and the Mediterranean Sea were not significantly different from each other or from the other samples when control region sequences were compared. However, despite the strong evidence presented here, failure to find genetic evidence for population substructure does not constitute evidence for a single panmictic population. It is possible that multiple subpopulations do exist, and that genetic differentiation at the loci analyzed in this study has not occurred because of large population sizes and/or low levels of reproductively successful migration between the subpopulations.

RÉSUMÉ

Des débats prolongés ont porté ces dernières années sur l'existence d'une reproduction du thon rouge de l'Atlantique nord, Thynnus thynnus thynnus, en tant qu'unité panmictique. Pour répondre à cette question, nous avons examiné la séquence nucléotide de la région de contrôle de l'ADN mitochondrial et la fréquence des allèles dans des échantillons répliqués de thon rouge du nord de la Méditerranée et de l'Atlantique nord-ouest. L'analyse AMOVA des deux types de données n'a révélé aucune différence significative entre les deux régions. Ces résultats démontrent l'importance d'analyser de multiples classes d'âge et d'amples échantillons dans les analyse sur la structure du stock. En outre, les échantillons larvaires du golfe du Mexique et de la Méditerranée ne différaient pas de façon significative, entre eux ou par rapport à d'autres échantillons, au moment de comparer les séquences de la région de contrôle. Toutefois, malgré les preuves solides présentées ici, le fait de ne pas déceler de preuves génétiques de l'existence d'un sous-structure de la population ne constitue pas une preuve de l'existence d'une population panmictique unique. Il se peut qu'il y ait de multiples sous-populations, et qu'il n'ait pas été trouvé de différentiation génétique dans les loci analysés dans la présente étude à cause de la grande taille de la population et/ou du faible niveau de migration reproductrice positive entre les sous-populations.

RESUMEN

En los últimos años, se ha producido un debate intenso sobre si el atún rojo del Atlántico norte, Thunus thynnus thynnus, se reproduce como una unidad panmíctica. Para abordar este tema, examinamos tanto las secuencias de nucleótidos de la región de control del ADN mitrocondrial como las frecuencias de alelos IdhA del gen nuclear en muestras replicadas de atún rojo del norte del Mediterráneo y del Atlántico noroeste. Los análisis AMOVA de ambos tipos de datos revelaron la ausencia de diferencias significativas entre las muestras de las dos regiones. Estos resultados demuestran la importancia que reviste analizar múltiples clases de edad y realizar amplios muestreos de talla amplias en los análisis del stock. Además, las muestras de larvas del Golfo de Méjico y del Mediterráneo no presentaron diferencias significativas entre ellas o con

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otras muestras cuando se realizó una comparación de las secuencias de la región de control. Sin embargo, a pesar de la evidencia contundente presentada aquí, el hecho de no encontrar una prueba genética de existencia de una subestructura de población no constituye una evidencia concluyente de la existencia de una única población panmíctica. Es posible que existan múltiples subpoblaciones y que la diferenciación genética en el loci analizado en este estudio no se haya detectado debido al gran tamaño de la población o a los bajos niveles de migración reproductiva efectiva entre las subpoblaciones.

KEYWORDS

Atlantic bluefin tuna, Thunnus thynnus thynnus, DNA, population genetics, stock identification, depleted stocks,geographical distribution

1. INTRODUCTION

The Atlantic northern bluefin tuna are currently managed by ICCAT as two units, one located in the northwest Atlantic Ocean and the other located in the northeast Atlantic Ocean and the Mediterranean Sea. This division is based partly on the existence of two spawning areas, one in the Mediterranean Sea and a second in the Gulf of Mexico. For management purposes, it is assumed that fisheries in the western Atlantic are predominately West Atlantic fish and that fisheries in the eastern Atlantic are predominately East Atlantic fish. However, a review of the tagging data has suggested that trans-Atlantic migration may be occurring at levels sufficiently high that the Atlantic northern bluefin tuna should be considered a single population unit (National Research Council, 1994). More recent tagging studies have demonstrated that tuna tagged in the western Atlantic frequently cross into the eastern management zone (Block et al., 1998; 2001; Lutcavage et al. 1999).

One approach to test the hypothesis of a single population unit would be a genetic analysis. To stimulate genetic analyses of bluefin tuna, a workshop was convened in Charleston, SC in 1994 that resulted in a well-defined plan (Dean and Woodley, 1994; Ahlquist, 1998). The primary hypothesis to be tested was that Atlantic bluefin tuna consist of a single genetically homogenous population. We have now analyzed both the mtDNA control region and the nuclear *ldhA* locus in replicate year class samples representing 245 bluefin tuna from both the northwest Atlantic Ocean and the Mediterranean Sea. In addition, we have analyzed mtDNA control region sequences of bluefin larvae collected in the Gulf of Mexico and the Mediterranean Sea. Neither set of data provides evidence for population subdivision between the areas sampled.

2. METHODS

Samples from the western Mediterranean Sea were obtained from collections made in 1993 and 1994 (1993 year class; n = 37), and 1998 (1998 year class; n = 37). In addition, samples of mature adults samples obtained from the western Mediterranean Sea in 1997 and 1998 were divided into year classes based on an age length table (Turner, personal communication). Samples representing the 1990 (n = 30) and 1992 (n =32) year classes were chosen for further analyses. Similarly, a sample collected in the northwestern Atlantic Ocean in 1994 was divided into two size classes based on length at age. The smaller fish were 127 to 190 cm, corresponding fish that were 5 to 9 years old (n = 39), and the larger fish were 197 to 277 cm (n = 35), corresponding to fish that were greater than 10 years old. Larval samples were obtained from cruises in the Gulf of Mexico (n=36) and the Mediterranean Sea (n=32). Total DNA was extracted and PCR amplifications of the mitochondrial D-loop region were performed as described by Alvarado Bremer et al. (1996). PCR products were purified for nucleotide sequence analysis using the QIAquick PCR Purification Kit (Qiagen Inc., Chatsworth, CA) and 335 bases of nucleotide sequence were obtained using a LICOR automated DNA sequencer. Intron 6 of the ldhA gene was amplified as described by Quattro and Jones (1999). The amplified product was cleaved with *Ase* I and *Mwo* I to identify three alleles that had been identified by nucleotide sequence analyses.

For the mtDNA nucleotide sequence data, the total number of distinct haplotypes was determined using test version 4.0d61 of PAUP, written by David L. Swofford. Neighbor joining (NJ) analyses (Saitou and Nei, 1987) were performed using Tamura-Nei's distances. In cases where there were missing data or insertions and deletions (indels), these sites were ignored in the affected pairwise comparisons. In all of the mtDNA phylogenetic analyses, trees were rooted using the control region DNA sequences of Pacific northern bluefin tuna (Alvarado Bremer et al., 1997) as the outgroup. Nucleotide and haplotypic diversities were calculated as defined by Nei (1987) using Arlequin (A software for population genetic data analysis, Ver. 1.1, Schneider et al., 1997).

For both sets of data, the level of genetic population differentiation was estimated using either AMOVA (Excoffier et al., 1992) as implemented in Arlequin.

3. RESULTS AND DISCUSSION

Most mtDNA D-loop nucleotide sequences were unique with nucleotide diversities of 2.3 to 4.8% among the various samples. Haplotype diversities ranged from 0.98 to 0.99. Thus, more than 99% of the variance was observed within the samples, and no significant variation could be attributed to variation between ocean basins or among samples within an ocean basin. In pairwise comparisons, no statistically significant differences were found among the four Mediterranean samples or between the two Atlantic samples. Likewise, no significant differences were observed when the two Atlantic samples were combined and compared to the combined Mediterranean samples. Thus, analyses of mtDNA nucleotide sequence data failed to provide evidence for population subdivision of Mediterranean and northwestern Atlantic bluefin tuna. However, a divergent group of lineages that resemble albacore or Pacific bluefin mtDNA sequences (Chow and Kishino, 1995; Alvarado Bremer et al., 1997; Takeyama et al., 2001) was found at a low level (2 to 6%) in three of the four Mediterranean year class samples. These lineages were not present in the two northwestern Atlantic samples. Since these sequences occur at such a low frequency in the Mediterranean, a larger sample size would be needed to determine if they were truly absent from the Atlantic bluefin population. Since the divergent lineages did not contain an *Eco*R I site that was present in all of the other Atlantic Bluefin tuna lineages, we screened the mtDNA from an additional 193 bluefin tuna captured in the northwestern Atlantic for the presence of the rare lineages. Nucleotide sequences belonging to these rare lineages were identified in three individuals and confirmed by nucleotide sequence analysis. Thus, the rare sequences are shared among fish from both regions. Taken together, these results emphasize the importance of analyzing multiple year classes and large sample sizes in analyses of population substructure.

Since failure to find genetic differentiation could be due to sampling a mixed population in the northwestern Atlantic, we analyzed the mtDNA control region of bluefin tuna larvae captured in either the Gulf of Mexico or the Mediterranean Sea. When these larval samples were compared, no significant differences were observed. Furthermore, an AMOVA analysis that included both the adult and the larval samples failed to provide any evidence for genetic differentiation between fish from the two regions. Therefore, we conclude that there are no detectable differences in mtDNA control region nucleotide sequences between western Atlantic and Mediterranean bluefin tuna.

The mtDNA analyses were corroborated by analyses of intron 6 of the nuclear gene, *ldhA* (encoding lactate dehydrogenase). We identified three *ldhA* alleles in northern bluefin tuna from nucleotide sequence analyses of amplified products and analyzed three Mediterranean and two Atlantic samples for the presence of the alleles. An AMOVA analysis was performed to compare the two Atlantic samples to three Mediterranean year classes (1990, 1992, and 1998). None of the variance could be attributed to the between group comparison, but 7.7% of the variance was due to within group differences. No significant differences were found when the allele frequencies were compared between the two Atlantic samples, but when the three Mediterranean year classes were compared, the 1998 year class sample was significantly different from the other two samples. Since it was a one time event, the 1998 year class sample may be an example of a significant departure in allele frequencies associated with a particular year class or cohort. Alternatively, the observed variation could be due to sampling error. Either way, the *ldhA* data provide no evidence for population subdivision in bluefin tuna. However, this result again demonstrates the importance of sampling multiple year classes to avoid drawing erroneous conclusions from samples with atypical allele frequencies.

In summary, neither the mitochondrial nor the *ldhA* nuclear data provide evidence for population subdivision in Atlantic northern bluefin tuna. However, this failure to reject the null hypothesis does not prove that it is true. Population subdivision may actually exist in this species and might be detected with other genetic markers. Alternatively, historically high population levels and/or straying between populations may have prevented genetic differences from accumulating. Thus, further studies using a variety of genetic and non-genetic approaches are needed to determine whether the Atlantic bluefin population is actually subdivided.

4. ACKNOWLEDGMENTS

We thank members of the NMFS Southeast Fisheries Science Center, C. Woodley, H. Howell, G. Pharis, Ralboray Inc, L. Dukehart, D. Gallagher, C. Pla, G. De Metrio, P. Grewe, I. Oray, S. Daoud, A. Hattour, Bluewater Fishermen's Association for providing samples. Expert technical assistance was provided by A.

Ibarreche-Alvarado, S. Wasser, S. Ford, S. Leonard, N. Robinson, and S. Bhatia. We thank D. L. Swofford for providing a test version of PAUP. This study was funded in part by the Cooperative Institute for Fisheries Molecular Biology (FISHTEC) NOAA-NMFS (RT/F-1) and is listed as contribution number FISHTEC 01-07.

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Table 1. Allele frequencies at the *ldhA* locus

Sample	*Allele*		
	12	*13*	*243*
Atlantic Large	0.40	0.24	0.36
Atlantic Small	0.45	0.27	0.28
Med 90	0.44	0.31	0.26
Med 92	0.41	0.19	0.40
Med 98	0.09	0.28	0.63