GENETIC ANALYSIS OF THE STOCK STRUCTURE OF THE YELLOWFIN TUNA, Thunnus albacares, IN THE SOUTHWEST EQUATORIAL ATLANTIC OCEAN AS SUGGESTED BY MITOCHONDRIAL DNA SEQUENCES

Análise genética da estrutura do estoque populacional da albacora lage, *Thunnus albacares*, no Sudoeste Equatorial do Oceano Atlântico de acordo com sequências do DNA mitocondrial

Vera Lúcia A. Vieira[1], Ana Carla A. El-Deir[1], Manuel Antonio A. Furtado-Neto[2], Steven M. Carr[3]

RESUMO

*A pesca de atum é uma atividade crescente no nordeste do Brasil, onde a albacora lage (*Thunnus albacares, *Bonnaterre 1788) é a espécie mais abundante. A distribuição populacional de* T. albacares *no Oceano Atlântico equatorial tem sido considerada contínua no meio científico, porém a Comissão Internacional para Conservação do Atum do Atlântico (ICCAT) trabalha com a descontinuidade desta população. Por razões práticas uma hipótese de "dois estoques", separados a 30° W, tem sido usada na administração pesqueira desta espécie. No presente trabalho foi utilizada a técnica de sequenciamento de de DNA mitocondrial para investigar a variação genética no estoque de* T. albacares *do Oceano Atlântico equatorial sudoeste, e a posição genética desta espécie dentro do gênero* Thunnus. *Dentre os 401 pares de bases amplificados do gene do citocromo b, um único nucleotídeo variável foi indentificado entre 35 amostras coletadas de* T. albacares. *Este nível de divergência é muito baixo e não é suficiente para determinar a presença de dois estoques na área estudada. A monofilia do gênero* Thunnus *foi confirmada pelas análises filogenéticas e as albacoras lage do Canadá e EUA estão mais proximamente relacionadas entre si de que com a brasileira. As espécies mais próximas a* T. albacares *foram* T. atlanticus *e* T. tonggol. *Com base nos resultados obtidos neste estudo, é sugerido que as autoridades do ICCAT e IBAMA considerem apenas um estoque de* T. albacares *para fins de administração da pesca Oceano Atlântico equatorial sudoeste.*

Palavras chaves*: Genética populacional, DNA mitocondrial, administração pesqueira,* Thunnus albacares.

ABSTRACT

*Tuna fisheries is a growing industry in northeastern Brazil, where the yellowfin tuna (*Thunnus albacares, *Bonnaterre 1788) is the most abundant species. The distribution of the populational stock of* T. albacares *in the equatorial Atlantic Ocean has been considered continuous, however the International Commission for Conservation of the Atlantic Tuna (ICCAT) has worked with a discontinuous population. For practical reasons a "two stock" hypothesis has been used in most assessments and the two stocks are separated at 30° W. In the present paper, a DNA sequencing technique was used to investigate the genetic variation in the* T. albacares *stock from the southwest equatorial Atlantic Ocean off northeastern Brazil, and the phylogenetic position of this species in the genus* Thunnus. *Within the 401-bp amplified segment of the cytochrome b gene, a single variable nucleotide site was identified among the 35 sampled individuals of* T. albacares. *This level of nucleotide sequence divergence is very low and it is not enough to determine the presence of two stocks in the studied area. The monophyly of the genus* Thunnus *was supported by the phylogenetics analyses. Yellowfin tuna from Canada and the U.S.A. were more closely related to each other than to the yellowfin tuna from Brazil. The species more closely related to* T. albacares *were* T. atlanticus *and* T. tonggol. *Based on the results obtained in this study, we suggest that the fisheries authorities from the ICCAT and Brazilian Institute of Environment (IBAMA) should consider a unique stock of* T. albacares *in the southwest equatorial Atlantic Ocean for fisheries management purposes.*

Key words*: Population genetics, mitochondrial DNA, fisheries management,* Thunnus albacares.

[1] Professora do Departamento de Pesca, Universidade Federal Rural de Pernambuco, Av. Dom Manuel de Medeiros, s/n, Dois Irmãos, Recife-PE, 52.171-900, Brasil.

[2] Professor Visitante (Bolsista da CAPES) no Departamento de Engenharia de Pesca da Universidade Federal do Ceará, e Coordenador do ELACE, Campus do Pici, Fortaleza, CE 60451-970.

[3] Associate Professor, Department of Biology, Memorial University of Newfoundland, St. John´s, Newfoundland, A1B 3X9, Canadá.

INTRODUCTION

The yellowfin tuna (*Thunnus albacares*, Bonnaterre 1788) is the most abundant species of tuna in the Equatorial Atlantic Ocean (Fonteneau, 1991). Since the early seventies, it has been believed that there is a continuous populational distribution of *T. albacares* in the equatorial Atlantic Ocean associated with East-West seasonal migration.

In all the area, individuals captured by longline and purse seine fisheries CPUE (capture per unit of effort) data has been identified morphologically as belonged to the same population, which supports the hypothesis of continuous distribution first proposed by Honma & Hisada (1971) and further elaborated by Yanez & Barbieri (1980) & Fonteneau (1981).

However, this model of stock structure for yellowfin tuna was not accepted by the International Commission for Conservation of the Atlantic Tuna (ICCAT) in their reports. For practical reasons a "two stock" hypothesis has been used in most assessments (Fonteneau, 1991). According to the ICCAT's hypothesis the two stocks are separated at 30°W. Furthermore, based on morphometric parameters, Mahon & Mahon (1987) suggested that two stocks existed in the western Atlantic. These stocks would have distinct seasonality with one stock moving from the Caribbean Sea to the north coast of Brazil, and sometimes mixing with the other stock that comes from Africa.

Transatlantic recoveries of tagged adult yellowfin tuna (Bard & Scott, 1991) support the hypothesis of a single stock (Honma & Hisada, 1971; Yanez & Barbieri, 1980; Fonteneau, 1981). The ICCAT acknowledge that it would not be possible to accept the "two stock" hypothesis if Bard & Scott's (1991) results are true (Fonteneau, 1991).

A number of molecular genetic studies have been carried out on the stock structure of tuna species. Similarities between mitochondrial DNA sequences of albacore tuna (*T. alalunga*) from the Atlantic and the Pacific were reported by Graves & Dizon (1989), who did not find any restriction endonuclease sites that could distinguish the two stocks. Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA of *T. albacares* from the Pacific Ocean showed no evidence of genetic variation among individuals from distant geographic locations in the Pacific Ocean (Scoles & Graves, 1993). However, allozyme and restriction enzyme analyses have suggested that stocks of of *T. albacares* are distinct in the Atlantic, Indian, and Pacific oceans (Ward *et al*., 1997).

Bartlett & Davidson (1991) observed interspecific variation in 307-base pair (bp) segments of the mitochondrial cytochrome b gene between four species of tuna caught off the east coast of Canada: *Thunnus thynnus* (bluefin tuna), *T. obesus* (bigeye), *T. albacares* (yellowfin) and *T. alalunga* (albacore). RFLP analysis of mitochondrial DNA has shown that the bluefin tuna (*T. thynnus orientalis*) from the northern Pacific Ocean shares a larger number of restriction fragment sites with the albacore than with its Atlantic correlate (*T. thynnus thynnus*) (Chow & Inoue, 1993).

Block *et al.* (1993) presented the first molecular phylogeny for the suborder Scombroidei (mackerels, tunas, and billfishes) based on a 600-bp region of the cytochrome b gene. The monophyly of the genus *Thunnus*, including *T. albacares, T. maccoyii* and *T. thynnus*, *T. alalunga* and *T.obesus*, was supported by bootstrap results. A close relationship between *T. albacares* and a *T.maccoyii* + *T.thynnus* clade was detected in this study and confirmed by Finnerty & Block (1995). A second molecular phylogeny for tuna species of the genus *Thunnus* based on partial sequences of the cytochrome b (292-bp) and ATPase (400-bp) genes was presented by Chow & Kishino (1995). They identified a close relationship between *T. albacares* and two others species of tuna (*T. altlanticus* and *T. tonggol*) not studied by Block *et al.* (1993) or Finnerty & Block (1995).

Preliminary studies in northeastern Brazil (02°36'S–04°15'S and 32°34'W–33°45'W), where tuna fisheries is a growing industry, have shown differences in four of 19 morphometric characters between the Brazilian and the African yellowfin tunas (Neiva, 1992).

In the present paper, partial nucleotide sequences (401-bp) of the cytochrome b gene were used to investigate genetic variation in the *T. albacares* stock from the southwest equatorial Atlantic Ocean off northeastern Brazil, and the phylogenetic position of the yellowfin tuna among the species of the genus *Thunnus*.

MATERIAL AND METHODS

Samples collection

Yellowfin tuna were collected in 11 voyages of the Research Vessel (RV) "Riobaldo" from March 1993 to November 1995, in three areas between latitudes 01°00'N and 09°00'S and longitudes 29°00'W and 40°00'W in Brazilian waters (Figure 1). Muscle, liver and heart tissues of 35 animals were removed and stored at -5°C.

DNA extraction

DNA was isolated from frozen or DMSO-preserved specimens by a acid guanidium thiosulfate-phenol-chloroform extraction procedure modified from Chomczynski & Sacchi (1987). DNA was extracted

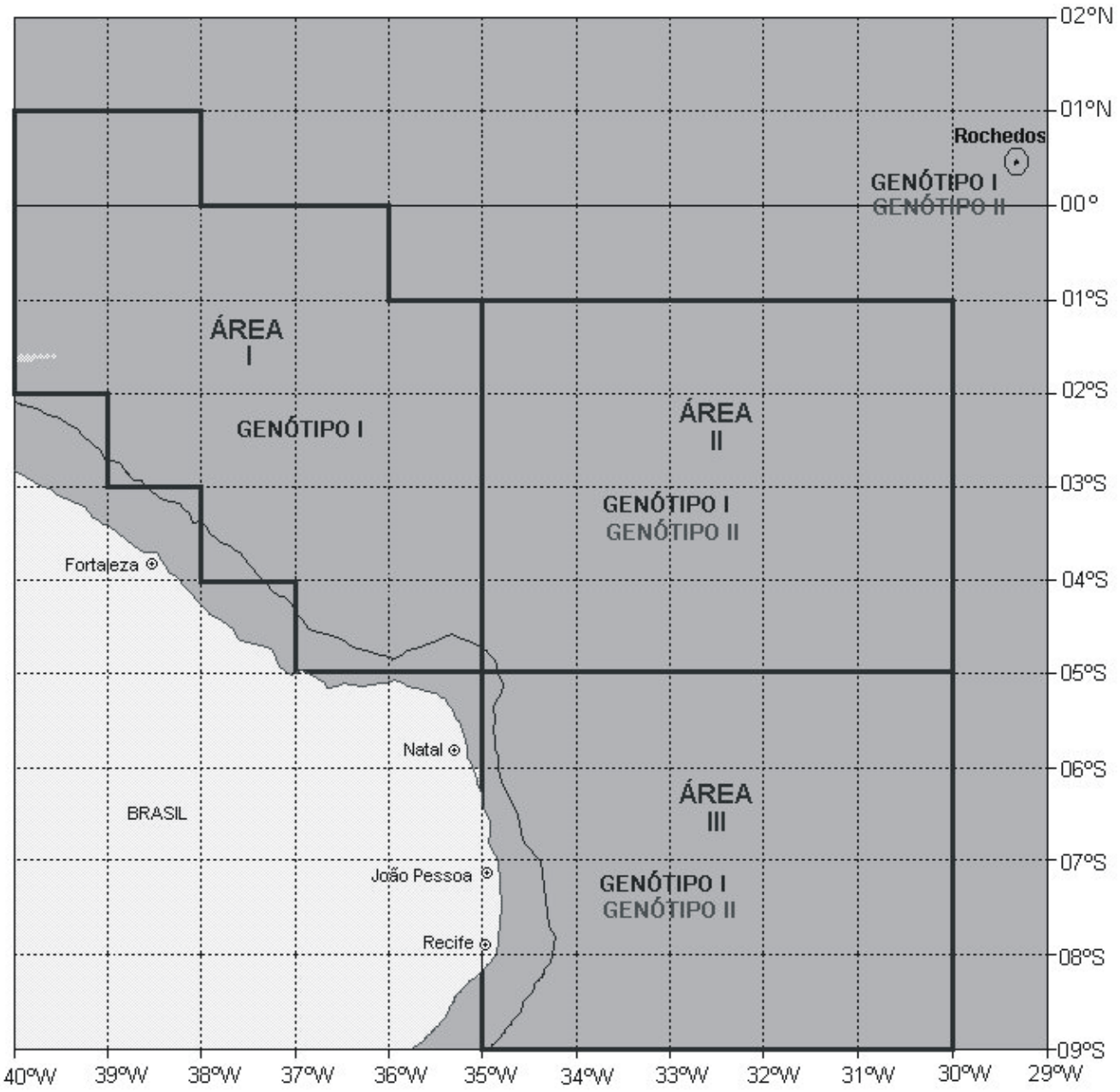

Figure 1 - Map of northern Brazil showing the areas in the southwest Equatorial Atlantic where samples of yellowfin tuna (*Thunnus albacares*) were collected, and where genotypes (genótipos) I and II where found. "Rochedos" represents the São Pedro and São Paulo Islands.

with chloroform-isoamyl alcohol, precipitated with isopropanol, washed with 75% ethanol, and resuspended in 50µl distilled water.

DNA amplification

PCR (polymerase chain reaction) was used to amplify 401-base pair sequences of the mitochondrial DNA cytochrome b gene from the collected samples. The primers used were L14724 (5'-CGAAGCTTGATATGAAAAACCATCGTTG-3') and H15149 (5'-GCCCCTCAGAATGATATTTGTCCTCA-3') (Irwin *et al*., 1991). Each amplification reaction was performed in a 100µl solution contaning: 67mM Tris-HCl (pH 9.0), 1.96 mM HCl_2, 9.94 mM β-mercaptoethanol; 2 mM of each dATP, dCTP, dGTP and dTTP; 0.4 µM of each oligonucleotide primer; 1 to 3 units of *Amplitaq* ™ DNA Polymerase (Perkin-Elmer Cetus, Mississauga, ON); and 2 µl of isolated DNA. One drop of light white mineral oil was placed in each tube to prevent evaporation. Amplification was carried out in a Perkin-Elmer Cetus TC-1 Thermal Cycler as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of 93°C for 1 minute (denaturation), 55°C for 40 seconds (annealing), 72°C for 2 minutes (extension), and a final step of 72°C for 10 minutes. Eletrophoresis of 5µl of PCR product with 1µl dye though 2% NuSieve GTG agarose (FMC Bioproducts, Rockland, ME) gel in 1.0M TBE buffer. PCR product was visualized by staining the gel with ethidium bromide and exposing to ultraviolet (UV) light on Ultraviolet Transilluminator (Ultra-Violet Products Inc., San Gabriel, CA) to check if successful amplifications were obtained.

Purification of PCR product

DNA was purified using Wizard™ Magic PCR Preps DNA Purification System (Promega Corp., Madison, WI) following the manufacturer's instructions. Purified DNA was then quantified with a DNA Fluorometer model TKO 100 (Hoefer Scientific Instruments, San Francisco, CA). Measurement of DNA concentratiom (ng/µl) were obtained using fluorochrome bisbenzimide-zole (Hoechst 33258) which binds to DNA and allows rapid quantification.

DNA Sequencing

The optimum mass of DNA solution was determined by the fluorometer readings (DNA concentration(ng/µl) = 400 / (fluorometer reading x 0.4)) and dried under reduced pressure. Each sample was then resuspended in 7.3 µl of distilled H_2O, 9.5 µl of reaction premix (Applied Biosystems PRISM™ Ready Reaction Dye Deoxy terminator Cycle Sequencing Kit) and 3.2 µl of 1mM primer. Both primers were used in separate reactions. Sequencing reactions were carried out in a Perkin-Elmer TC-1 Thermal Cycler in 25 cycles, on the following step-cycle profile: 98°C for 1 second, 50°C for 15 seconds, and 60°C for 4 minutes. Excess primers and unincorporated dye were removed by passing the reaction product through a Sephadex G-50 spin column. The eluted DNA was then dried under reduced pressure and resuspended in 5 µl of 5:1 mixture of deionized formamide and 50 mM Na_2EDTA (Sigma Chemical Co., St. Louis, MO). Sequencing of both strands of the 401 base pair region was done on an ABI 373A (Applied Biosystems, Inc., Foster City, CA) Automated DNA Sequencer. Samples were loaded into 6% polyacrylamide (19:1 Bis), 7M urea gels, and elephoresed at 32 watts constant power for 11 hours. DNA sequence data were collected using the ABI collection analysis software package version 1.0.2. Alignments of sequences were done by eye and complementary strands were compared using the Sequence Navigator DNA sequence editor version 1.0.1. (Perkin Elmer, Inc.). Alignment of sequences in a publishable format was obtained from the Eyeball Sequence Editor (ESEE) version 3.0S (Cabot & Beckenbach, 1989).

Phylogenetic Analyses

A consensus sequence of the genotypes of *T. albacares* identified by DNA sequencing was analyzed together with sequences of *T. albacares* from Canada (Bartlett & Davidson, 1991) and the United States (Block *et al*., 1993). These sequences were analyzed alongside those of another six *Thunnus* species (*T. thynnus, T. alalunga, T. atlanticus, T. maccoyii, T. obesus, T. tonggol*) and three tuna species from other genera which were used as the outgroup (*Euthynnus affinis, Katsuwonus pelamis, Auxis thazard*). Sequences of *T. atlanticus* and *T. tongoll* (Gen Bank accession numbers D63492 and D63493) were from Chow & Kishino (1995). Sequences of *T. albacares* from U.S., *T. thynnus, T, alalunga, T. maccoyii, T. obesus* (L11556-L11560) and *E. affinis, K. pelamis, A. thazard* (L11534, L11539, and L11532, respectively) were from Block *et al*. (1993). Phylogenetic analyses were performed with the PAUP [version 4.0d61] program of Swofford (1997). Maximum parsimony trees were identified with the heuristic search algorithm (tree-bisection-and-reconnection) with random addition and delayed-character-transformation optimization. Ratios of transversions to transitions of 10:1, 3:1, and 1:1 were used. Bootstrap analyses were performed by means of the heuristic search algorithm with 10 random taxon additions and the

tree-bisection-and-reconnection option in each of 1,000 replicates. Neighbor Joining (NJ) analysis was performed using the Kimura-2-parameters (Saitou & Nei 1987).

RESULTS

Within the 401-bp amplified segment of the cytochrome b gene, a single variable nucleotide site was identified among the 35 sampled individuals of *T. albacares* (Figure 2). It was a silent third position pyrimidine (C-T) transition at the 66th nucleotide position.

The most common genotype (**T.albac-01**), identified by a thymine at position 66, was found in 29 individuals, and the less common genotype (**T. albac-02**), identified by a cytosine at position 66, was observed in six individuals (Figure 2). Genotype **T. albac-01** was detected in the Areas I, II, III and "Rochedos", while Genotype **T.albac-02** was observed in the Areas II, III, and "Rochedos" (Figure 1). Three **T.albac-02** were sampled in the "Rochedos" area, two in the Area II one specimen in the Area III (Figure 1).

The consensus sequence (**T.albac-BR**), of the two genotypes differed in two positions from the 299-bp cytochrome b sequence of yellowfin tunas from the east coast of Canada (Bartlett & Davidson, 1991). It also differs in three positions from the 286-bp fragment of the cytochrome b sequence of the U.S. east coast (Block *et al*., 1993). All substitutions are third position silent changes.

The three *T. albacares* sequences (from Brazil, Canada, and U.S.) were ranked in a monophyletic group in all the maximum parsimony analyses with bootstrap values of 59, 55, and 52, respectively for transversions:transitions ratios of 10:1, 3:1 and 1:1. Another monophyletic group, constituted by *T. thynnus* and *T. maccoyii*, was observed (bootstrap values of 68, 64, 67) within a six-*Thunnus* clade (trees not shown).

Figure 3 shows the phylogenetic tree resulting from neighbor-joining analysis using distance matrices calculated by Kimura-2-parameters is shown in Figure 3. The genetic distance between the three genotypes of *T. albacares* was 0.003. Yellowfin tuna from Canada and U.S. were more closely related to each other than to the yellowfin tuna from Brazil. The sister species more closely related to *T. albacares* were *T. atlanticus* and *T. tonggol*. The monophyly of the genus *Thunnus* was supported by Bootstrap Value (BV) of 98.

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            M   A   S   L   R   K   T   H   P   L   L   K   I   13
T.albac-01 atg gca agc ctc cga aaa act cac ccg cta cta aaa atc  39
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            A   N   D   A   L   V   D   L   P   T   P   S   N   26
T.albac-01 gct aac gac gca cta gtt gac ctt cct acc ccc tct aat  78
T.albac-02 ... ... ... ... ... ... ... ... ..c ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ..y ... ... ... ...

            I   S   A   W   W   N   F   G   S   L   L   G   L   39
T.albac-01 atc tct gca tga tga aac ttt ggc tca cta ctt ggc ctt  117
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            C   L   I   S   Q   I   L   T   G   L   F   L   A   52
T.albac-01 tgc ctt att tct caa atc ctt aca gga cta ttc ctc gca  156
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            M   H   Y   T   P   D   V   E   S   A   F   A   S   65
T.albac-01 ata cac tac acc cct gat gtc gaa tca gcc ttc gcc tca  195
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            V   A   H   I   C   R   D   V   N   F   G   W   L   78
T.albac-01 gta gcc cac att tgc cga gat gtc aac ttc ggt tga ctc  234
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            I   R   N   L   H   A   N   G   A   S   F   F   F   91
T.albac-01 atc cgg aac ctc cac gca aac ggg gcc tct ttc ttc ttt  273
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            I   C   I   Y   F   H   I   G   R   G   L   Y   Y   104
T.albac-01 atc tgc atc tac ttc cac atc ggc cga gga ctt tac tac  312
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            G   S   Y   L   Y   K   E   T   W   N   I   G   V   117
T.albac-01 ggc tct tac cta tac aag gaa aca tga aac atc gga gta  351
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            V   L   L   L   L   V   M   M   T   A   F   V   G   130
T.albac-01 gta ctc cta ctc cta gtt atg atg acc gcc ttc gtt ggc  390
T.albac-02 ... ... ... ... ... ... ... ... ... ... ... ... ...
T.albac-BR ... ... ... ... ... ... ... ... ... ... ... ... ...

            Y   V   L                                           133
T.albac-01 tac gtc ctt cc                                       401
T.albac-02 ... ... ... ..
T.albac-BR ... ... ... ..
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Figure 2 - Variation in Brazilian *Thunnus albacares* mitochondrial DNA sequences within a 401-bp region of the cytochrome b mitochondrial gene. Dots represent nucleotides that are identical to genotype **T.albac-01**. The consensus sequence (*Thunnus albacares*-BR) used in the phylogenetic analysis has a total length of 286-base pairs and starts at the 133th position, and has the same length as the sequences obtained from GenBank. The top line gives the inferred amino acid sequence according to the single letter code specified by the International Union of Biochemists. Numbers at the end of the first and second line indicate the position numbers in the protein and nucleotide sequences, respectively.

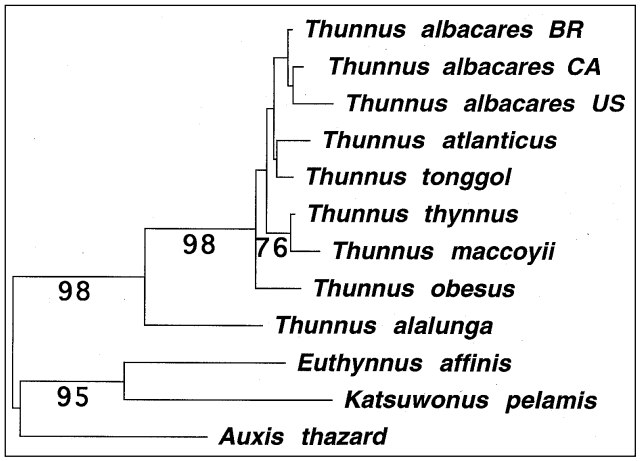

Figure 3 - Consensus bootstrap tree (1,000 replicates) of heuristic search algorithm and Neighbor Joining using Kimura-2-parameters, inferred from 286-bp mitochondrial cytochrome b sequences of 12 species of scombrids. The three non-*Thunnus* species were used as outgroup. Numbers indicate bootstrap values.

DISCUSSION

The presence of different stocks or populations in determined area is generally associated with a reasonable amount of genetic variation (Scoles & Graves, 1993; Avise, 1994). The level of nucleotide sequence divergence in yellowfin tuna from the northeastern coast of Brazil is very low. The single nucleotide difference in a 401-bp fragment of the cytochrome b gene between the genotypes **T.albac-01** and **T.albac-02** is not enough to determine the presence of two stocks in the studied area. Previous study on the Pacific yellowfin tuna also did not detect significant genetic differentiation among individuals from geographically distant locations, including samples from the Pacific and Atlantic oceans (Scoles & Graves, 1993).

There is no indication that there are distinct stocks of *T. albacares* in the area studied. The genetic homogeneity of yellowfin tuna from the southwest equatorial Atlantic suggests that the stock present in that area shares a common gene pool. In two instances both genotypes were observed in the same Areas II and III (Figure 1), which indicates that tuna with different genotypes probably travel together in the same schools.

Comparison of mtDNA sequence data (Scoles & Graves, 1993) with morphological data (Schaefer, 1991; 1992) from the same locations in the Pacific Ocean have shown that although morphometric characters and gill-raker counts differed significantly, genetic differences were not observed. Scoles & Graves (1993) suggested that the morphological variation among Pacific yellowfin tunas was the consequence of the phenotypic plasticity of this species, evidencing that morphological characters were environmentally influenced. Previous observation of greater morphological variation among yellowfin tuna from the Pacific than variation between the Atlantic and the Pacific tunas (Schaefer & Walford 1950) also supports this suggestion.

T. albacares is a migratory species (Collete & Naven, 1993) and several studies of tagged adults have demonstrated that they do make trans-Atlantic crossings (Bard & Scott, 1991) and can travel large distances between regions in the Pacific (Fink & Bayliff, 1970; Bayliff 1984; Itano & Williams, 1992). The circumtropical occurence of *T. albacares* larvae in both Atlantic and Pacific oceans (Nishikawa *et al*., 1985) suggests the existence of spawning areas throughout the tropical oceans and consequently allows gene flow between distant locations (Scoles & Graves, 1993). The results obtained in this study were in agreement with the hypothesis of gene flow in the Atlantic Ocean (Homna & Hisada, 1971), since different genotypes were observed in the same region. The neighbor joining results (Figure 3) show that the genotypes found in North Atlantic and South Atlantic are distinct.

The low frequency of occurrence of the genotype **T.albac-02** is congruent with the concept that "unusual" mitochondrial DNA genotypes do not occur in high frequencies (Slatkin, 1985). This condition has been observed in other fishes species, where high gene flow has been verified, such as marine catfishes of the family Ariidae (Avise *et al*., 1987), bluefish, *Pomatomus saltatrix* (Graves *et al*., 1992), and Greenland halibut, *Reinhardtius hippoglossoides* (Vis *et al*., 1997).

The low nucleotide variation in the mitochondrial DNA sequences of *T. albacares* from northeastern Brazil is consistent with the hypothesis that there is only a single stock of yellowfin tuna in the southwest Atlantic Ocean. The genetic homogeneity of the *T. albacares* stock occuring in the studied area suggests that the stock shares a common gene pool. It also implies that this species sustains sufficient gene flow in that area to prevent genetic variation. These results are similar to those obtained in the Pacific Ocean by Scoles & Graves (1993). Based on the results obtained in this study, we suggest that the fisheries authorities from the ICCAT and Brazilian Institute of Environment (IBAMA) should consider a unique stock of *T. albacares* in the southwest Atlantic Ocean for management purposes.

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BIBLIOGRAPHIC REFERENCES

Avise, J. C. *Molecular markers, natural history and evolution*. Chapman & Hall, New York, N.Y., 1994.

Avise, J. C., Reeb, C. A., & Saunders, N. C. Geographic population structure and species differences in mitochondrial DNA of mouthbrooding marine catfishes (Ariidae) and demersal spawning toadfishes (Batrachoididae). *Evolution*, v. 41: p. 991-1002, 1987.

Bard, F. X., & Scott, E. L. *Traversees de thons albacares marques de cotes americaines aux cotes africaines.* Document realise dans la cadre du programme YYP, ICCAT. Paris, France, 1991.

Bartlett, S. E., & Davidson, W. S. Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochome b genes. *Can. J. Fish. Aquat. Sci*., v. 48, p. 309-317, 1991.

Bayliff, W. H. Migrations of yellowfin and skipjack tuna released in the central portion of the eastern Pacific ocean, as determined by tagging experiments. *ICCAT Internal Rep*., v. 18, p. 1-107, 1984.

Block, B. A., Finnerty, J. R., Stewart, A. F. R., & Kidd, J. Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny. *Science*, v. 260, p. 210-214, 1993.

Bonnaterre, J. P. *Tableu encyclopédique et methodique des trois regnes de la nature, Ichthyologie*. Paris, France, 1788.

Cabot, E. L., & Beckenbach, A. T. Simultaneous editing of multiple nucleic acid sequences with Esee. *Comput. Appl. Bio Sci*., v. 5, p. 233-234, 1989.

Chomczynski, P., & Sacchi, N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem*., v. 162, p. 156-159, 1987.

Chow, S., & Inoue, S. Intra- and interspecific restriction fragment length polymorphism in mitochondrial genes of *Thunnus* tuna species. *Bull. Natl. Res. Inst. Far Seas Fish*., v. 30, p. 207-225, 1993.

Chow, S., & Kishino, H. Phylogenetic relationships between tuna species of the genus *Thunnus* (Scombridae: teleostei): inconsistent implications from morphology, nuclear and mitochondrial genomes. *J. Mol. Evol*., v. 41, p. 741-748, 1995.

Collette, B. B., & Nauen, C. E. FAO species catalogue. Vol 2. Scombrids of the world: an annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. *FAO Fish. Synop*., v. 125, n. 2, p. 1-137, 1983.

Fink, B. D., & Bayliff, W. H. Migrations of yellowfin and skipjack tuna in the eastern Pacific Ocean as determined by tagging experiments, 1952-1964. *Inter-Am. Trop. Tuna Comm. Bull*., v. 15, p. 1-227, 1970.

Finnerty, J. R., & Block, B. A. Evolution of the cytochrome b in the Scombroidei (Teleiostei): molecular insights into billfish (Istiophoridae and Xiphiidae) relationships. *Fisheries Bulletin*, v. 93, p. 78-96, 1995.

Fonteneau, A. *Dynamique de la population d'albacore (Thunnus albacares) de l'ocean Atlantique.* These Doctorat. Université Paris VI, France, 1981.

Fonteneau, A. Modeling a single Atlantic yellowfin stock with a mixing model. *SCRS Report*, v. 30, p. 272-285, 1991.

Graves, J. E., & Dizon, A. E. Mitochondrial DNA sequence similarity of the Atlantic and Pacific albacore tuna (*Thunnus alalunga*). *Can. J. Fish. Aquat. Sci*., v. 46, p. 870-873, 1989.

Graves, J. E., McDowell, J. R., Beardsley, A. M., & Scoles, D. R. Stock structure of the bluefish *Pomatomus saltatrix* along the mid-Atlantic coast. *Fisheries Bulletin*, v. 90, p. 469-475, 1992.

Honma, M., & Hisada, K. Structure of yellowfin tuna population in the Atlantic ocean. *Bull. Far Seas Fish. Res. Lab*., v. 4, p. 93-124, 1971.

Irwin, D. M., Kocher, T. D., & Wilson, A. C. Evolution of the cytochrome b gene of mammals. *J. Mol. Evol*., v. 32, p. 128-144, 1991.

Itano, D. G., & Williams, P. G. Analysis of yellowfin tagging data and related information collected by the Skipjack Survey and Assessment Programme. SPC *Tuna and Billfish assessment Programme (TBAP) Tech. Rep*., v. 28, p. 1-39, 1992.

Mahon, R., & Mahon, S. Seasonality and migration of pelagic fishes in the eastern Carribean. *FAO Fish. Rep*., v. 393, p. 1-270, 1987.

Neiva, M. A. *Morfometria da albacora lage, Thunnus albacares, vindos do Sudoeste Equatorial no Atlântico*. B. Sc. (Honours) Thesis, Universidade Federal Rural de Pernambuco, Recife, Brazil, 1992.

Nishikawa, Y., Homna, M., Ueyanagi, S., & Kikawa, S. Average distribution of larvae of oceanic species of scombroid fishes, 1956-1981. *Far Seas Fish. Res. Lab. S. Ser*., v. 12, p. 1-99, 1985.

Saitou, N., & Nei, M. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*., v. 4, n. 4, p. 406-425, 1987.

Schaefer, K. M. Geographic variation in morphometric characters and gill-raker counts of yellowfin tuna *Thunnus albacares* from the Pacific Ocean. *Fisheries Bulletin*, v. 83, p. 289-297, 1991.

Schaefer, K. M. An evaluation of geographic and annual variation in morphometric characters and gill-raker counts of yellowfin tuna *Thunnus albacares* from the Pacific Ocean. *Inter-Am. Trop. Tuna Comm. Bull*., v. 20, p. 133-163, 1992.

Schaefer, M. B., & Walford, L. A. Biometric comparations between yellowfin tunas (*Neothunnus*) of Angola and the Pacific coast of Central America. U.S. Fish and Wildl. Serv. *Fisheries Bulletin*. 51: 425-443, 1950.

Scoles, D. R., & Graves, J. E. Genetic analysis of the population structure of yellowfin tuna, *Thunnus albacares*, from the Pacific Ocean. *Fisheries Bulletin*, v. 91, p. 690-698, 1993.

Slatkin, M. Rare alleles as indicators of gene flow. *Evolution*, v. 39, p. 53-65, 1985.

Swofford, D. L. *PAUP: Phylogenetic Analysis Using Parsimony, versions 4.0.* Computer program, 1997.

Vis, M. L., Carr, S. M., Bowering, W. R., & Davidson, W. S. Greenland halibut (*Reinhardtius hippoglossoides*) in the North Atlantic are genetically homogeneous. *Can. J. Fish. Aquat. Sci*., v. 54, p. 1813-1821, 1997.

Ward, R. D., Elliott, N. G., Innes, B. H., Smolenski, A. J., & Grewe, P. M. Global population structure of yellowfin tuna, *Thunnus albacares*, inferred from allozyme and mitochondrial DNA variation. *Fisheries Bulletin*, v. 95, n. 3, p. 566-575, 1997.

Yanez, E., & Barbieri, M. A. Analyse de la prise par unité d'effort saisonnière et de l'evolution de l'indice gonado somatique de la peche palangriere (1956-1977) et de surface (1969-1978) du yellowfin (*Thunnus albacares*) de l'Atlantique. *Rec. Doc. Scient. ICCAT*, v. 9, n.1, p. 76-91, 1980.