

**DETERMINATION OF STOCK STRUCTURE IN BLUEFIN TUNA AT THE NMFS LABORATORY,
CHARLESTON SC**

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SUMMARY

This document outlines planned exploration of bluefin tuna stock structure using technology now available to detect genetic polymorphisms at the nucleic acid level. Adult bluefin tissues will be screened for useful polymorphisms in mitochondrial and nuclear DNA. After genetic markers have been identified using adult tissues, variability in the DNA of juvenile bluefin from geographically distinct spawning grounds will be analyzed to determine the existence of genetically discrete populations.

RESUME

Le présent document décrit dans les grandes lignes l'exploration planifiée de la structure de stock du thon rouge en utilisant la technologie qui est maintenant disponible pour détecter le polymorphisme génétique au niveau de l'acide nucléique. Des tissus de thon rouge adulte seront triés à la recherche de polymorphismes utiles du DNA mitochondrial et nucléaire. Une fois identifiés les marqueurs génétiques au moyen des tissus adultes, on analysera la variabilité du DNA des thon rouges juvéniles en provenance de zones de frai géographiquement distinctes, afin de déterminer l'existence de populations différant du point de vue génétique.

RESUMEN

Este documento esboza la exploración planificada de la estructura del stock de atún rojo, por medio de la tecnología que está ahora disponible para detectar los polimorfismos genéticos en el nivel del ácido nucleico. En los tejidos del atún rojo adulto se buscarán los polimorfismos útiles en el DNA mitocondrial y nuclear. Tras identificar los marcadores genéticos en los tejidos adultos, se analizará la variabilidad en el DNA del atún juvenil procedente de caladeros de distintos puntos geográficos, para determinar la existencia de poblaciones genéticas separadas.

INTRODUCTION

Since assessment and subsequent management of bluefin tuna stocks in the Atlantic Ocean are based upon a stock hypothesis, and the success of these management decisions may be affected by the validity of the stock hypothesis used, it is important to continue efforts to determine whether bluefin spawning in different areas of the Atlantic, Gulf of Mexico, and Mediterranean are genetically distinct.

The newest technology, which allows for detection of genetic polymorphisms at the nucleic acid level, is the most powerful to date in that it allows a tremendous expansion of the number of possible genetic markers of those previously available at the protein level. Markers commonly used are based on polymorphisms of either mitochondrial (mtDNA) or nuclear DNAs (Hallerman and Beckman, 1988; Billington and Herbert, 1991). Although it is unclear exactly which marker will be informative for bluefin stock identification, the application of this technology to the question of bluefin stock determination is promising.

METHODS

Currently, 15 adult bluefin tissue samples have been obtained from NMFS/SEFSC Charleston and Miami Laboratory archives, and from the NMFS Observer Programs. Additional samples will be obtained directly from U.S. fisheries harvests, the NMFS Observer program and other researchers as they become available. These samples will be used to identify regions in the mtDNA or genomic DNA that contain a sufficient amount of genetic variation to be informative in stock structure analyses. Bartlett and Davidson (1991) reported that the cytochrome b region of mtDNA does not contain sufficient variability for stock structure determination in bluefin tuna. Initial investigations will be made in regions of the mtDNA such as the ND1 and ND4 which are known to be more variable than the cytochrome b in some species (Chapman, personal communication). In addition, microsatellites will be isolated from a "shot-gun" genomic library and characterized for variation. Once regions of variability have been identified, analysis of larval and juvenile (not yet migrating) samples will be used to determine the putative subpopulations of Atlantic bluefin.

DNA Isolation

Genomic and/or mtDNA will be isolated from available tissue by standard proteinase K digestion, phenol/chloroform extraction procedures, followed by ethanol precipitation.

Initially, PCR analysis will be carried out with suitable mtDNA primers supplied by collaborators or synthesized commercially from sequence data available in the literature. Reactions will be carried out using the Perkin-Elmer Cetus PCR kit and a Gene Machine II thermal cycler (USA Scientific). The procedure for obtaining amplification products, single-stranded template and sequencing will be according to the procedure of Bartlett and Davidson (1991).

DNA Sequence Analysis

DNA sequencing is based on Sanger's dideoxy sequencing technique (Sanger, et al., 1977) using Sequenase version 2.0 DNA sequencing kit (United States Biochemical Corporation, Cleveland, Ohio).

Alternatively, sequencing can be sent to the University of Florida, Core DNA Sequencing Facility, Gainesville, FL, to have automated sequencing performed on an ABI 373a DNA sequencer with about 300-350 nucleotide bases provided with each sequencing run.

Literature Cited

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