RFLP ANALYSIS ON SINGLE COPY NUCLEAR GENE LOCI IN YELLOWFIN TUNA (*THUNNUS ALBACARES*) TO EXAMINE THE GENETIC DIFFERENTIATION BETWEEN THE WESTERN AND EASTERN SAMPLES FROM THE INDIAN OCEAN

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ABSTRACT

In the previous analyses in 2000, we used the mitochondrial DNA (mDNA) variation to test possible heterogeneous stocks between the western and the eastern Indian Ocean. However, mDNA was resulted to be the ineffective marker. Hence, we attempted the another marker, the restriction fragment length polymorphism (RFLP) in the nuclear gene loci for this study. It was resulted that there were no significant different between the western and the eastern samples. The genetic study by Ely et al showed that there was no statistically significant difference among samples from three Oceans of yellowfin tuna. Thus, there might be very little genetic differentiation in the global yellowfin tuna population. On the other hand, morphometric heterogeneity has been reported among local samples in the Indian Ocean, i.e., there are 2-3 morphometrically different yellowfin stocks in the Indian Ocean (Kurogane and Hiyama, 1958 and Reika Maldeniya, unpublihsed). Thus, it is concluded that there are no genetically different yellowfin stocks in the Indian Ocean, although there are a few morphometrically different yellowfin stocks.

INTRODUCTION

In the seventh expert consultation meeting on the Indian Ocean tuna in 1998, National Research Institute of Far Seas Fisheries (NRIFSF) of Japan proposed 'genetic analyses' to study the stock structure of yellowfin tuna (*Thunnus albacares*) in the Indian Ocean. Then, this study was recommended in the seventh consultation meeting and also the IOTC Scientific Committee in 1999. The preliminary analyses in 2000, used the mitochondrial DNA variation to examine the heterogeneity between the western and the eastern stock of yellowfin tuna in the Indian Ocean. Then, it was resulted that there was no statistically significant difference between samples from the western and the eastern part. This was likely due to the possibility that the mitochondrial DNA was not effective marker to test such heterogeneity (Chow et al., 2000). Thus, we re-attempted the analyses using variation in the nuclear genome.

For fish genetic study, several universal PCR primer sets which amplify intron regions of nuclear gene loci have been reported (Chow and Takeyama, 1998; Chow, 1998; Chow and Hazama, 1998). In the yellowfin tuna, two primer sets targeting second intron of S7 ribosomal protein gene (S7RP2) and fourth intron of calmodulin gene (CaM) successfully amplified single fragment in the former and three in the later (Chow, 1998; Chow and Hazama, 1998). Further, Mendelian inheritance in the yellowfin tuna was proved in S7RP (Chow et al., 2001), and minor modification of primer sequence for CaM resulted in single fragment amplification (unpublished).

We here report the results of our analysis using restriction fragment length polymorphism (RFLP) in these two nuclear gene loci for comparing the western and eastern samples of yellowfin tuna from the Indian Ocean.

SAMPLES

Table 1 lists names of Country, Agencies, contact persons that have provided yellowfin tuna tissue samples to NRIFSF. Table 1 also shows the status of sample collections. As of the end of May 2001, 1,127 sample tissues were provided from 11 countries, France, India, Iran, Japan, Maldives, Mauritius, Le Union (France), Seychelles, Spain (at

Seychelles office), Sri Lanka and Thailand. Map 1 shows number of samples collected by sub-area.

From 1,127 samples, we have chosen two local samples collected by Nippon-Maru (JAMARC), as these two were westernmost and easternmost samples and samples are collected by same investigators. The western Indian Ocean sample was collected at 1S and 49E on September 1999 and the eastern sample was at 1-3S and 91E on November 1999. All fish were juvenile caught by purse seine and relatively uniform in size. Average fork length of the western and eastern samples were 50 cm (± 4.9 SD) and 47 cm (± 2 SD), respectively.

HYPOTHESIS

We set up following two hypotheses and, in this study, we attempted to examine the first hypothesis (Box 1):

Box 1: Two hypotheses

1st Ho: There are two (west and east) stock.

2nd Ho: If the 1st Ho is accepted, there are three or more stocks.

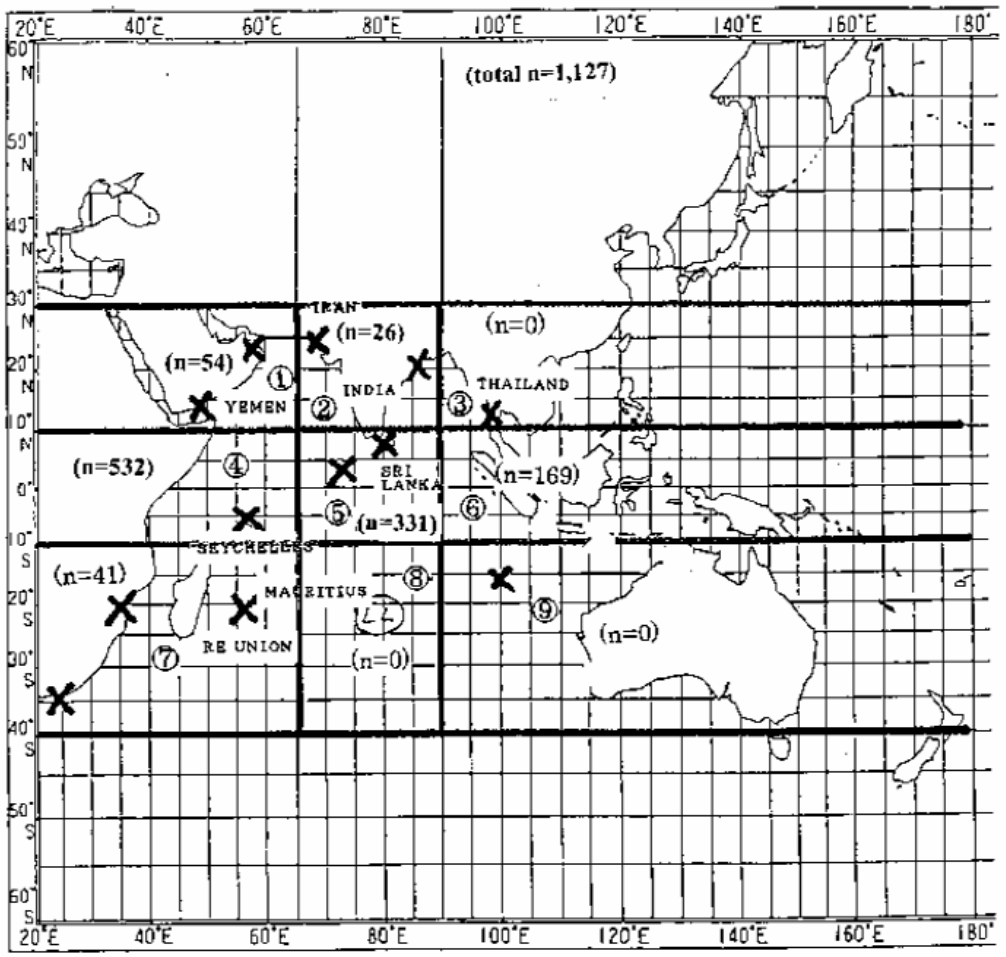
We will try the second hypothesis later, if the first hypothesis is accepted. If it is rejected, we will not analyze further because of rejection of the two stock hypotheses, which implies the single stock.

Table 1 List of country, Agency, contact persons that agreed to cooperating collection of tissue samples for the YFT stock structure by the DNA analyses and the status of the sample collections.

Country (alphabetical order)	Agency	Contact persons	Sample area number (see Map 1) and number of samples provided (as of May, 2001)
France	Comité National des Pêches Maritimes et des Elevages Marins	Michel Goujon	? n= 50(?)
India	Fisheries Survey of India	V. S. Somvanshi	? n=26
Iran	Iranian Fisheries Research Organization	Farhad Kaymaram	? n=54
Japan	Japan Marine Resources Research Center (JAMARC) through the Nippon-maru cruise	Susumu Ikame Shojiro Kurihara	? n=162 ? n=155 ? n=139
Maldives	Marine Research Section (MRS)	Zaha Waheed	? n= 81
Mauritius	Albion Fisheries Research Centre	Devanand Norungee	? n= 60
Reunion (France)	IFREMER, Delegation de la Reunion	Francois Poisson	? n= 41
Seychelles	Seychelles Fishing Authority	Rose-Marie Bargain	? n= 179 ? n= 20
Spain (at Seychelles)	Spanish Fisheries Representative in Seychelles,	Juan Jose Areso	? n= 81
Sri Lanka	National Aquatic Resources Agency (NARA),	R.D.K.D. Amarasooriya	? n= 74
Thailand	Andaman Sea Fisheries Development Center (AFDEC)	Praulai Chantawong	? n= 1 ? n= 30
Total sample size by sub-area			? n= 54 ? n= 26 ? n= 0 ? n=532 ? n=331 ? n=169 ? n= 41 ? n= 0 ? n= 0 Grand total n= 1,127

Table 1. Genotype and allele frequencies in S7RP2 and CaM loci of the western and eastern samples of yellowfin tuna in the Indian Ocean.

	Western (n=40)	Eastern (n=40)
S7RP2/*Hha* I		
AA	22	21
AB	12	13
AC	3	3
AD	0	1
BB	1	0
BC	2	1
EE	0	1
A	0.737	0.737
B	0.2	0.175
C	0.063	0.05
D	0	0.013
E	0	0.025
CaM/*Nla* III		
AA	24	19
AB'	1	6
AB	5	3
AC	7	8
AD	3	2
BB	0	1
CC	0	1
A	0.8	0.712
B	0.063	0.063
B'	0.013	0.075
C	0.087	0.125
D	0.037	0.025



Map 1: Number of tissue sample collected by sub-area.

METHODS AND RESULTS

Small piece of muscle was dissected on board, fixed with ethanol and transferred to the laboratory. Procedures for total DNA extraction from muscle tissue and PCR amplification followed by RFLP analysis are described elsewhere (Chow and Inoue, 1993; Chow et al., 2000). Condition for PCR amplification is also reported elsewhere (Chow and Inoue, 1993), but annealing temperature was set high (60?). *Hha* I digestion detected RFLP for S7RP2 locus (Chow and Hazama, 1998), and preliminary investigation revealed high RFLP in *Nla* III digestion for CaM locus. The restricted PCR products were electrophoresed in a 2.5% agarose gel (Biogel, BIO101) in 0.5 x TBE buffer, stained with EtBr and photographed. Genotypes of forty individuals from each sample were determined, and chi-square analysis was conducted using the Monte Carlo simulation of Roff and Bentzen (1989) with 1,000 randomizations of the data to test heterogeneity of genotype distributions between the two samples.

Genotype and allele frequencies in S7RP2 and CaM loci are presented in Table 1. *Hha* I and *Nla* III digestions for both S7RP2 and CaM loci detected seven genotypes with five alleles. The genotype frequency distribution and allele frequency were very similar between the western and eastern samples with no significant difference (P=0.932 in S7RP2 and P=0.317 in CaM).

5. DISCUSSION

The results together with previous mtDNA assay (Chow et al., 2000) rejected the null hypothesis that yellowfin tuna in the Indian Ocean consists of two (west and east) stock. Ely *et al* (personal communication) using mitochondrial DNA showed that there was no statistically significant difference among samples from three Oceans of yellowfin tuna. Thus, there might be very little genetic differentiation in the global yellowfin tuna population. On the other hand, morphometric heterogeneity has been reported among local samples. For example, there are 2-3 morophometrically different yellowfin stocks in the Indian Ocean (Kurogane and Hiyama, 1958 and Reika Maldeniya, unpublihsed). Thus, it is concluded that there are no genetically different yellowfin stocks in the Indian Ocean, although there are a few morphometrically different yellowfin stocks.

ACKNOWLEDGEMENT

We deeply appreciate Zaha Waheed (Marine Research Center, Maldives), V. S. Sombanshi (Fisheries Survey of India), Michel Goujon (Comité National des Pêches Maritimes et des Elevages Marins, France), Farhad Kaymaram (Iranian Fisheries Research Organization, Iran), Devanand Norungee (Albion Fisheries Research Centre, Mauritius), Francois Poisson (IFREMER, Delegation de la Reunion), Rose-Marie Bargain (Seychelles Fishing Authority), Juan Jose Areso (Spanish Fisheries Representative in Seychelles), R.D.K.D. Amarasooriya (National Aquatic Resources Agency, Sri Lanka) and Praulai Chantawong (Andaman Sea Fisheries Development Center, Thailand), who provided tissue samples to the NRIFSF.

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