A PRELIMINARY GENETIC ANALYSIS ON YELLOWFIN TUNA STOCK STRUCTURE IN THE INDIAN OCEAN USING MITOCHONDRIAL DNA VARIATION

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ABSRACT

This paper described a preliminary genetic analysis of the structure of the yellowfin stocks in the Indian Ocean. This study had been proposed by the National Research Institute of Far Seas Fisheries (NRIFSF) of Japan, and approved and recommended during the seventh Expert Consultation on the Indian Ocean and the IOTC Scientific Committee meeting in 1999. The main objective of the study was to determine whether two or more stocks of yellowfin existed in the Indian Ocean. A total of 996 tissue samples of yellowfin tunas have been received from several fishing agencies and offices, and more samples are still expected. The current study used two selected samples, taken at the westernmost and easternmost parts of the Indian Ocean. As results of the analyses, the effective makers within the mitochondrial DNA were not found, which implied two suggestions, i.e., yellowfin tuna stock in the Indian Ocean is (a) homogeneous or (b) heterogeneous, but mitochondria DNA did not contain effective makers to segregate the heterogamous stocks. As the study of Ely et al, also with mitochondria DNA showed that yellowfin tuna DNA from three Ocean did not indicate statistical significance. Therefore, other makers such as PR2 will be investigated for the next analyses using the same samples in the Indian Ocean, as well as the global samples from three Oceans, to see if there are effective markers that can prove heterogamous stocks of yellowfin tuna. Thus, the fist hypothesis (existence of the heterogamous stocks) is still un-solved at this stage and no conclusion was made regarding the stock structure in the Indian Ocean by this study.

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 in the Indian Ocean. Upon approval, this study was recommended in that consultation meeting and also the IOTC Scientific Committee in 1999. This document reports the progress of the tissue sample collections and the results of the preliminary analyses.

SAMPLE COLLECTION

Table 1 lists names of Country, Agencies, contact persons that agreed to cooperate providing yellowfin tuna tissue samples to NRIFSF. Thirteen countries agreed to cooperate for providing samples to the NRIFSF. Table 1 also shows the status of sample collections. As of the end of September 2000, 996 sample tissues were provided from eight countries, Iran, Japan, Mauritius, Le Union (France), Seychelles, Spain (at Seychelles office), Sri Lanka and Thailand. Map 1 shows number of samples collected by sub-area. Appendix A shows the instruction describing the method how to collect the tissue samples and how to make records.

METHODS AND RESULTS

In order to study genetic stock structure of yellowfin tuna (*Thunnus albacares*) in the Indian Ocean, PCR-RFLP assay on mitochondrial DNA (mtDNA) was used to investigate the genetic variation. We set up following two hypotheses. In

this study, we attempted to examine the first hypothesis (Box 1):

Box 1: Two hypotheses

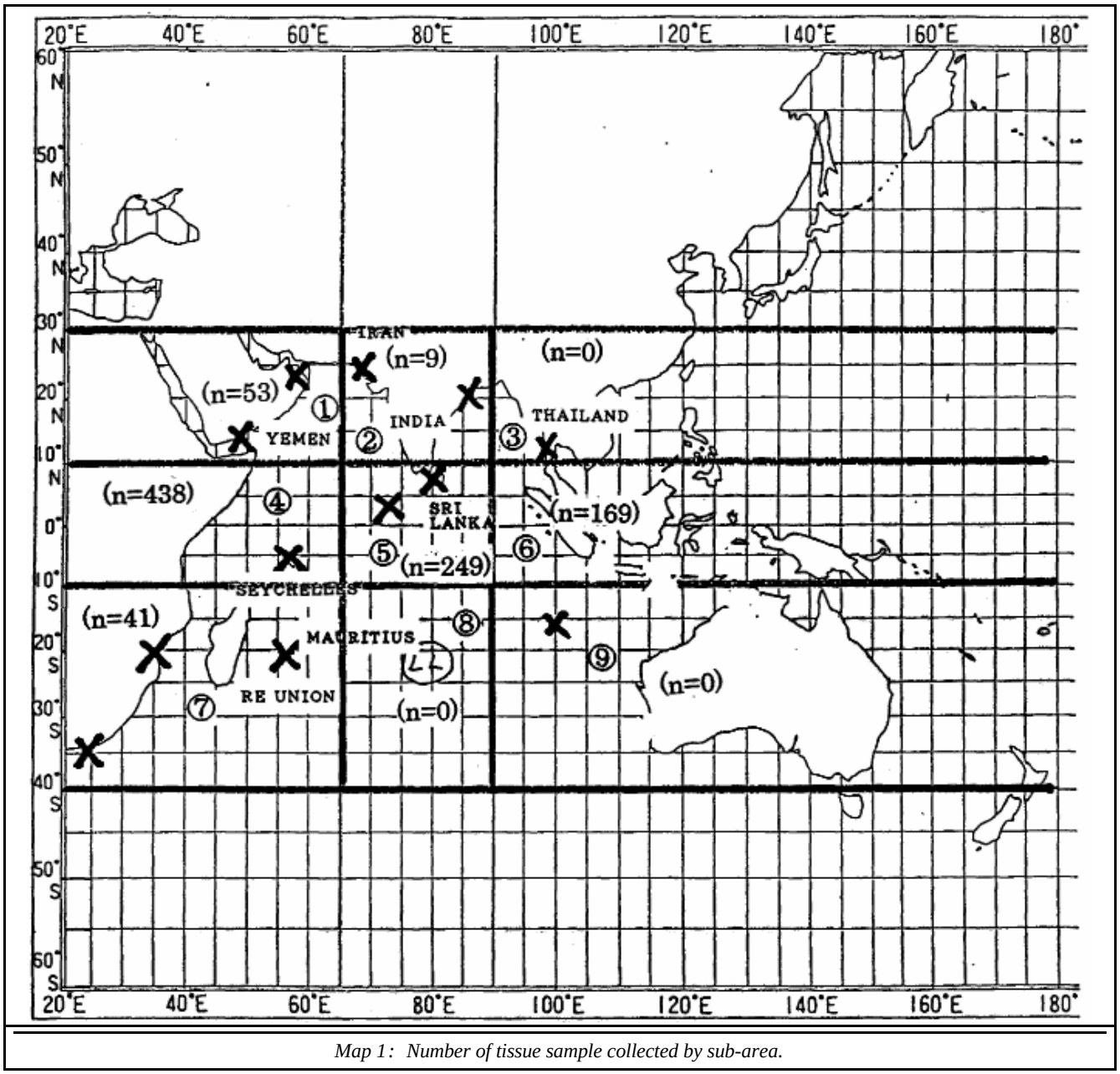
1st Ho: There are two (west and east) stock.

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.

Tale 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 September, 2000)
Australia	WA fisheries (Broome)	Lee Chan	(waiting)
	Hazcu fishing Inc.,	John Totterdell	(waiting)
	Former WA fisheries DG	Jack Robins	(waiting)
France	Comité National des Pêches Maritimes et des Elevages Marins	Michel Goujon	(waiting)
India	Fisheries Survey of India	V. S. Somvanshi	(Waiting)
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	(waiting)
Mauritius	Albion Fisheries Research Centre	Devanand Norungee	? n= 60
Re Union (France)	IFREMER, Delegation de la Reunion	Francois Poisson	? n= 41
Seychelles	Seychelles Fishing Authority	Rose-Marie Bargain	? n= 179 ? n= 20
South Africa	Marine and coastal management	Marcel Krose	(waiting)
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= 0 ? n= 0 ? n=482 ? n=250 ? n=169 ? n= 41 ? n= 0 ? n= 0 Grand total n= 996



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

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. 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). A primer set (CB3R-L and 12SAR-H) to amplify mitochondrial DNA (mtDNA) control region (D-loop) was from Palumbi *et al.* (1991). A preliminary investigation revealed very high restriction fragment length polymorphism (RFLP) in *Nla* III and *Taq* I digestions, and these two enzymes were applied to all samples. 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 frequencies in *Nla* III and *Taq* I digestions are presented in Table 1. *Nla* III and *Taq* I digestions yielded fifteen and nine genotypes, respectively, showing high genotypic diversity (0.73 and 0.77 in *Nla* III and 0.51 and 0.73 in *Taq* I digestions). The genotype frequency distribution was similar between the western and eastern samples with no significant difference (P=0.58 in *Nla* III and P=0.35 in *Taq* I). Although these two samples may be from different spawning

units considering the fish size and collection date, there was no significant heterogeneity in the genotype distributions between the samples.

The present investigation failed to detect genetic heterogeneity between the westernmost and easternmost samples in the Indian Ocean; supporting that yellowfin tuna in Indian Ocean consists of single stock. Ward *et al*. (1994), however, detected significant difference in allele frequency at *Gpi* allozyme gene locus between the western and eastern of yellowfin tuna samples in the Pacific. They also failed to detect heterogeneous distribution in mtDNA genotypes, suggesting that mtDNA variation might be inadequate gene marker for delineating genetic stock structure in the yellowfin tuna.

We are still seeking other variations not only in this mtDNA region but also in the nuclear genome, which might have better resolving power for the stock structure study. Atlantic and Pacific samples as well as other local samples in the Indian Ocean are also under investigation for genetic stock study at global level.

Table 2. Genotype frequency comparison between the two-yellowfin tuna samples in the Indian Ocean.

Enzyme	Type	West	East
Nla III	A	17	16
	B	6	8
	C	0	2
	D	10	4
	E	1	0
	F	1	2
	G	0	1
	H	0	1
	I	0	1
	J	1	2
	K	2	1
	L	1	0
	M	1	0
	N	0	1
	O	0	1
	n	40	40
Taq I	A	4	4
	B	0	3
	C	27	18
	D	3	5
	E	1	2
	F	4	6
	G	0	1
	H	0	1
	I	1	0
	n	40	40

DISCUSSION

We could not find the effective makers within the mitochondrial DNA, which implied two suggestions, i.e., yellowfin tuna stock in the Indian Ocean is (a) homogeneous or (b) heterogeneous, but mitochondria DNA did not contain effective makers to segregate the heterogamous stocks. The study of Ely *et al*, also with mitochondria DNA showed that yellowfin tuna DNA from three Ocean did not indicate statistical significance (personal communication). Therefore, we will attempt to use other makers such as PR2 and examine same samples in the Indian Ocean, as well as the global samples from three Ocean, to see if there are effective markers that can prove heterogamous stocks of yellowfin tuna. Thus, the fist hypothesis in Box 1 is still un-solved at this stage and no conclusion was made regarding the stock structure in the Indian Ocean by this study.

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APPENDIX A : INSTRUCTION TO COLLECT TISSUE SAMPLES

We require only a small amount of muscle piece (about 5 mm cubic or slice). We prefer to have samples from small size of fish not exceeding 60 cm in its fork length. The muscle tissue can be taken from anywhere of the fish body. We usually take a piece of meat from the core that the processors use to determine flesh quality. The sampling vials that we are sending have already been filled with the ethanol (70-100%). Once the tissue is in the ethanol solution, it can be kept and shipped at room temperature by normal airmail.We would like to have following information on the sampled fish are obtained (refer also to attached Figure 1 and Map 1): (a) please put sequential sample number on the cover (cap) of the vial, (b) make data sheet as appeared in the attached Figure 1. We prefer to have the data entered to Microsoft Excel and to have it in diskette, but the hand-written paper forms are also acceptable. Please include date, location (latitude and longitude in minutes level), boat name (or landing sight name), fork length, sex (if known) and remarks, (c) we would like to have 60 –81 individuals (samples) from each location. We define 9 locations (see Map 1). We will send one box containing 81 vials per location. If you think that you can cover more than 2 locations and can collect more samples in each location, let us know. We will send

additional vials, (d) we need samples within a half-year, hopefully by October, this year.

REFERENCES

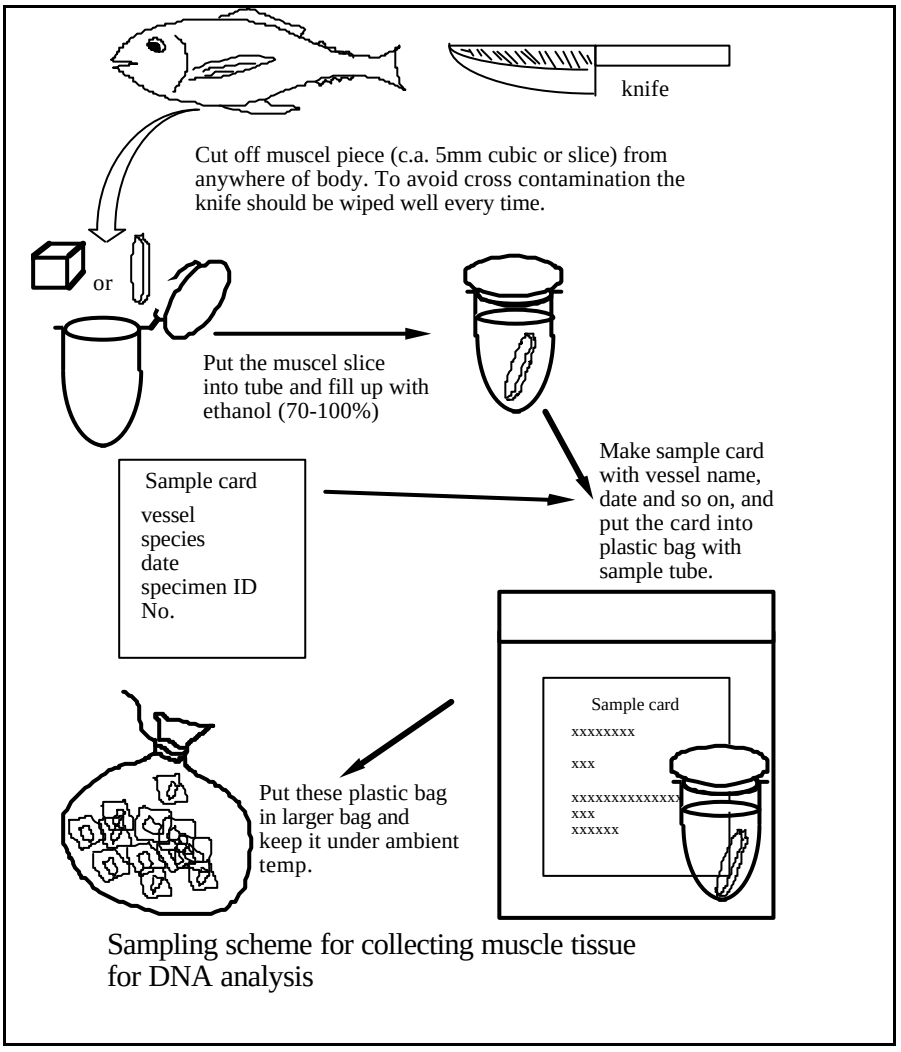
CHOW, S. AND INOUE, S. (1993). Intra-and interspecific restriction fragment length polymorphism in Mitochondrial genes of *Thunnus* tuna species. Bull. Natl. Res. Inst. Far Seas Fish. 30: 207-225.

CHOW, S., OKAMOTO, H., MIYABE, N., HIRAMATSU, K. AND BARUT, N. (2000). Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. Mol. Ecol. 9: 221-227.

PALUMBI, S., MARTIN, A., ROMANO, S., MCMILLAN, W. O., STICE, L. AND GRABOWSKI, G. (1991). *The Simple Fool's Guide to PCR, Version 2.0.* University of Hawaii, Honolulu.

ROFF, D. A. AND BENTZEN, P. (1989). The statistical analysis of mitochondrial DNA polymorphisms: X^2 and the problem of small sample sizes. Mol. Biol. Evol. 6: 539-545.

WARD, R. D., ELLIOTT, N. G., GREWE, P. M. AND SMOLENSKI, A. J. (1994). Allozyme and mitochondrial DNA variation in yellowfin tuna (*Thunnus albacares*) from the Pacific Ocean. Mar. Biol. 118: 531-539.



Sampling scheme for collecting muscle tissue for DNA analysis