Maternal Inheritance of Yellowfin Tuna (*Thunnus albacares*) in Captivity

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Abstract: Mitochondrial DNA introgression has been suggested to be responsible for the maternal consistent implications. Study on mt-DNA (mitochondrial DNA) variation in the yellowfin tuna (*Thunnus albacares*) using RFLP (restriction fragment length polymorphisms) has provided the evidence of maternal inheritance of yellowfin tuna in captivity. Eggs were collected in every spawning in 2004-2006 season. The mt-DNA genotypes of broodstock were compared with their eggs and the maternal inheritance of these females was determined from genotypes in the eggs. The result showed that six genotypes of female broodstock were observed in eggs and four of them were found to share a single female's identity and one type was shared by two females. The same genotype was observed in almost every sampling throughout the year. The female broodstocks spawned almost daily throughout the season.

Key words: Genotype, mt-DNA, maternal inheritance, yellowfin tuna.

1. Introduction

Yellowfin tuna (*T. albacares* (*Thunnus albacares*)) is a cosmopolitan fish species inhabiting tropical and subtropical waters in the Atlantic, Pacific, and Indian Oceans. Yellowfin tuna is a large pelagic fish with a common size of 150 cm [1]. Spawning occurs throughout the year in the tropical oceans, preferably near islands and coasts [2]. The Indian Ocean of Java and Bali Islands are important spawning ground of tuna species, so this area contributes greatly to the conservation of the species.

IMRAD (Institute for Mariculture Research and Development) at Gondol, Bali initiated yellowfin tuna propagation in land-based facilities under research cooperation with OFCF (Overseas Fisheries Cooperation Foundation) in Japan. Previously the similar project was started among OFCF, AITTC (Inter America Tropical Tuna Commission) in Panama

[3, 4]. The project site in Bali was established to collect scientific data of tuna species, especially yellowfin tuna (*T. albacares*).

The first spawning occurred in August 2004, and continued spawning through 2005. Strict maternal inheritance of mt-DNA (mitochondrial DNA) in vertebrates may be feasible for identifying reproductively active females. In sexually reproducing organisms, mitochondria are normally inherited exclusively from the mother. The mitochondria in fish sperm are usually destroyed by the egg cell after fertilization. In addition, most mitochondria are present at the base of the sperm's tail, which is used for propelling the sperm cells. Sometimes the tail is lost during fertilization. Usage marker mt-DNA (mitochondria DNA) is easy to understand and apply to know pattern of genetic endowment that is indigenous to female individual (maternal inheritance) [5, 6]. All the mitochondria come from the mother, therefore, any defect is passed on through the generations from parent to offsprings. This research aimed to identify maternal pattern from mt-DNA

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genotypes comparing between broodstock and their offsprings to identify females contributing to spawn. The study will have important implication not only for our understanding of mitochondrial genetics but also for increasing spawning productivity of yellowfin tuna as well.

2. Materials and Methods

2.1 Broodstock

The main broodstock tank was designed to be large enough to reduce the stress of captivity and to enhance the chances of spawning. Twenty five yellowfin tunas were maintained in the main broodstock tank (18 m in diameter, 6 m in depth with working volume of 1.526 m³). Vertical black stripes, 2.5 cm wide at 65 cm intervals, were painted on the entire interior tank wall to improve visibility of the wall. Spawning of captive yellowfin tuna occurred in 2004 in a land-based tank at IMRAD [7, 8].

Each fish was tagged with a microchip implant tag in the dorsal musculature for individual identification at stocking. A small piece of finlet from all group individuals was clipped at stocking. These tissue samples were preserved in ethanol 95%. The yellowfin tuna broodstock was fed with 50% fish (mostly scad mackerel of fork-length was 10-15 cm) and 50% squid of about 12-18 cm once a day at an average daily ration of 2%-3%. A premixed vitamin powder supplement was added at 0.5%-1.0% of the food weight.

2.2 Eggs

Spawning occurred almost daily in the broodstock tank from October 2004 to May 2006. Fertilized eggs were collected for DNA analysis from 98 spawning events at daily to two-week interval from October-November 2004, and next spawning season from August 2005 to May 2006. The eggs were collected with a net (0.8 m²) and number of eggs was counted after every spawning. Collected fertilized eggs were between 50,000-100,000.

2.3 DNA Analysis

Samples were preserved in 500 µL of ethanol 95% and analyzed in National Far Sea Fisheries Laboratory Shimidzu-Japan and Biotechnology Laboratory at IMRAD Gondol-Bali Indonesia. The egg samples and finlet were extracted using phenol chloroform [9]. The DNA from the larvae, eggs and yellowfin broodstock was rehydrated with 10-30 µL TE (Tris EDTA) buffer. The two primers CB3R-LT and 12SAR-H were used to amplify the Dloop region of mt-DNA following Palumbi et al. [10]; their sequences are 5'CACATTAAACCTGAATGATATTT-3' and 3' ATAGTGGGGTATCTAATCCCAGTT-5', respectively. The PCR (polymerase chain reaction) was carried out in 25 µL total volume with an initial denaturation at 95 °C for 3 min with followed by 30 cycles of amplification (denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 2 min) with a final extension at 72 °C for 10 min. The PCR products were directly digested by each of five restriction enzymes endonucleases (DdeI, MnlI, MspI, NlaIII and TaqI). These enzymes were selected by preliminary screening of the RFLP (restriction fragment length polymorphisms) in this DNA fragment using yellowfin individuals [11]. Digested PCR samples were electrophoreses through 2.5% agarose gel (Biogel, BIO101), followed by ethidium bromide staining and that was photographed.

3. Results and Discussion

3.1 Genotypes of Broodstock Individuals

Stocking and biological profiles of 25 broodstock yellowfin tunas were shown in Table 1. The amplified fragment size of each mt-DNA segment was estimated to be about 1,900 bp (base pair), and no apparent size difference among individuals was observed. Of the total of 25 broodstocks and 222 eggs of yellowfin tuna examined, it was found that the numbers of observed genotypes in each restriction assay using MspI, DdeI and MnlI, TaqI and NlaIII were two, three, five and eight, respectively.

Genotypes of yellowfin tuna were obtained by the five restriction analyses produced determining 20 composite genotypes, of which 10 established a single individual's identity, five were shared by two individuals (Table 2). This finding is in concordance to the result of a study in Panama Achotines Laboratory applying six restriction enzymes for mt-DNA-Dloop region [11], which showed that less than 30% of females contributed to spawning. Most of the fish in the tank appeared to participate in daily courtship and prespawning activities, which was usually a good indicator of participation in spawning in the tank. Therefore, it is likely that the number of spawning females was underestimated in this genetic

analysis due to the conservative sampling frequency and sample sizes.

3.2 Genotypes of Eggs

Four composite genotypes were detected, and these composite genotypes matched those of five broodstock during 2004-2005 female yellowfin tuna (Table 3). One composite genotype (type B10) was shared by two yellowfin tuna broodstocks (Table 4).

Maternal inheritance has a great influence on the offspring's gene expression [12, 13]. Mitochondrial genes, in contrast to genes in the nucleus, have an exclusively maternal mode of inheritance in mammals [14]. This should be a weakness of this study. Nuclear

Table 1 Stocking (tag code) and biological profile of 25 broodstock of yellowfin tuna.

No. of fish	Code No.	Date of transfer	Tag No.	Fork length at capture (cm)
1	B1	September 1, 2003	4213747E0E	51.0
2	B2	September 30, 2003	42137B0450	32.0
3	B3	October 17, 2003	42137F5578	47.0
4	B4	October 24, 2003	42137A0567	52.0
5	B5	October 30, 2003	4213601A0E	48.3
6	B6	October 30, 2003	42137A026A	55.0
7	B7	November 6, 2003	42137F0B17	51.0
8	B8	November 7, 2003	42135B0A2F	47.0
9	B9	November 7, 2003	42135A1D48	47.0
10	B10	November 14, 2003	4213683511	37.0
11	B11	December 5, 2003	421363684D	51.0
12	B12	March 19, 2004	42136E4F0C	72.0
13	B13	March 19, 2004	421370090B	72.5
14	B14	March 19, 2004	4213766F34	71.0
15	B15	March 19, 2004	421378445A	67.0
16	B16	March 19, 2004	4214012A5E	68.0
17	B17	June 7, 2004	4213591A36	60.0
18	B18	June 16, 2004	42135D6741	65.0
19	B19	August 5, 2004	42137F611F	64.0
20	B20	June 28, 2004	42137C5D4D	61.0
21	B21	April 23, 2004	4213637241	64.5
22	B22	May 6, 2005	4210312D74	70.0
23	B23	May 6, 2005	42135E332F	82.0
24	B24	May 6, 2005	42102F205B	69.0
25	B25	May 6, 2005	420B4A4F3B	74.0

Table 2 Maternal evidence from restriction profile of yellowfin tuna broodstocks and eggs observed in Dloop region mt-DNA.

Genotype No.	Haplotype in broodstock					Number of individuals	Genotype detected in egg	Sex identified
B1	B	B	B	B	A	1		
B2	B	B	B	D	A	1		
B3	E	B	A	B	C	1	EBABC	Female
B4	E	A	A	E	B	2		
B5	C	B	B	A	B	1		
B6	C	A	B	E	A	1		
B7	A	B	A	B	A	1	ABABA	Female
B8	C	A	A	D	B	2		
B9	D	B	B	C	A	1	DBBCA	Female
B10	D	A	B	E	A	2	DABEA	Female
B11	C	B	B	D	B	1		
B12	C	A	B	D	B	2		
B13	B	A	B	A	A	1	BABAA	Female
B14	B	A	B	E	A	1		
B15	C	B	B	C	A	2		

Table 3 Composite genotype of eggs of October-November 2004 to September-December 2005.

Date of spawning	Genotype No.	Restriction enzymes					Number of samples
		TaqI	Msp	MnII	Nla III	DdeI	
October 26-31, 2004	B-13	B	A	B	A	A	20
November 1-3, 2004	B-13	B	A	B	A	A	20
September 1-6, 2005	B-7	E	B	A	B	A	10
September 7-8, 2005	B-7	E	B	A	B	A	10
	B-3	E	B	A	B	C	1
September 12-21, 2005	B-7	E	B	A	B	A	10
	B-3	E	B	A	B	C	5
September 24-30, 2005	B-7	E	B	A	B	A	10
October 1-26, 2005	B-7	E	B	A	B	A	10
October 27-30, 2005	B-7	E	B	A	B	A	11
	B-3	E	A	B	E	C	5
November 1-3, 2005	B-7	E	B	A	B	A	10
November 4, 2005	B-3	E	B	A	B	C	5
November 7, 2005	B-3	E	B	A	B	C	10
	B-7	E	B	A	B	A	10
November 8-9, 2005	B-3	E	B	A	B	C	10
	B-3	E	B	A	B	C	5
November 10-13, 2005	B-7	E	B	A	B	A	10
November 14, 2005	B-7	E	B	A	B	A	10
November 15-20, 2005	B-3	E	B	A	B	C	5
November 21-30, 2005	B-7	E	B	A	B	A	5
	B-10	D	A	B	E	A	2
December 1-15, 2005	B-7	E	B	A	B	A	10
	B-10	D	A	B	E	A	6
December 16-24, 2005	B-3	E	B	A	B	C	12

Table 4 Maternal pattern detected in eggs and homologous female yellowfin tuna broodstocks.

Haplotype No.	Number of fish	Female broodstock predicted	Genotype detected in egg
B-3	1	EBABC	EBABC
B-7	1	ABABA	ABABA
B-9	1	DBBCA	DBBCA
B-10	2	DABEA	DABEA
B13	1	BABAA	BABAA

DNA should give more information on the inheritance including Mendelian inheritance of yellowfin tuna, as evidenced in previous publications [6, 15]. Furthermore, the study of spawning should be confirmed using histological approach. Fresh postovulatory follicles in the gonads of fully mature females are convincing evidences of recently spawned fishes. Study on the histological analysis of the gonads of wild yellowfin tuna has indicated that females apparently can spawn on near-daily intervals [2]. The result of this study shows that captive yellowfin spawns daily support the finding that reproductively active yellowfin females spawn every 1.27-1.52 days on average [2]. To proof the mitochondrial maternal inheritance, the sequences of broodstocks and eggs should be compared.

In the future, the analyses of maternal inheritance would be conducted using sequencing method of mitochondrial DNA fragments. Targeting highly polymorphic gene fragments are more accurate, especially in the processing of large numbers of tuna samples [16].

4. Conclusions

Twenty haplotypes were detected from broodstock of yellowfin tuna during experiment and six of them were female. Female yellowfin tuna broodstock can spawn every day and several females contributed to spawning in a single day. Furthermore, research is needed to determine the clear mechanism involved in maternal inheritance. In addition, paternal inheritance in selective breeding would be more elucidated using nuclear DNA analysis on polymorphic gene loci.

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