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# Genetic monitoring for spawning ecology of captive yellowfin tuna (*Thunnus albacares*) using mitochondrial DNA variation

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**Abstract**

Mitochondrial DNA genotypes of captive broodstock of yellowfin tuna (*Thunnus albacares*) were compared with those of their offspring in order to monitor spawning frequency and periodicity. Among 38 broodstock individuals, 27 genotypes were observed, 18 of which established a single individual's identity. Spawned eggs and hatched larvae were collected on 48 sampling days over a period of 1 year. Among 538 eggs and larvae analyzed, 10 genotypes were observed; eight of them established a single female's identity, and two types were shared by two females. The spawning profiles of these females were determined by observing the occurrence of these genotypes in the offspring. Based on the dates when genotypes first occurred and on growth trajectories estimated for individual fish, the size of a female at first spawning was estimated to be 12–28 kg and 75–112 cm. Usually, multiple females spawned on a given date. The same genotypes were observed on almost any sampling day throughout the year. The results indicated that some individual females were capable of spawning almost daily for extended periods of time as long as they remained in the appropriate range of water temperatures and had sufficient food.


*Keywords:* Yellowfin tuna; Mitochondrial DNA; Spawning profile; Genetic monitoring

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## 1. Introduction

Molecular genetic analysis is useful in identifying individuals within a group of fish. Strict maternal inheritance of mitochondrial DNA (mtDNA) in vertebrates may be feasible for identifying reproductively active females, if the molecule being analyzed contains enough variation to discriminate among individuals. The mtDNA *D-loop* region in tunas of the genus *Thunnus* is highly polymorphic (Alvarado Bremer et al., 1998, 1999; Chow et al., 2000), and this variation can be detected using conventional PCR-RFLP analysis in several tuna and billfish species (Chow et al., 1997, 2000; Chow and Takeyama, 2000).

Rearing of large tuna species such as yellowfin tuna (*Thunnus albacares*) and northern bluefin tuna (*Thunnus thynnus orientalis*) in floating ocean net pens or land based tanks and subsequent spawning in captivity has been pioneered by scientists at Kinki University (Harada et al., 1980, 1985), the Japan Sea Farming Association (JASFA) (Masuma, 1992) and the Inter-American Tropical Tuna Commission (IATTC) (Scholey et al., 2001; Wexler et al., in press). Large-scale production of early-juvenile tunas is now possible, as well as the release of juveniles for stock enhancement. Approximately 12,000 juvenile bluefin tuna (ca. 50 mm in total length (TL)) were produced at the Amami Station of JASFA in 1998 (S. Masuma, personal communication). In 1998 and 1999, small numbers of bluefin tuna juveniles (100 and 30 individuals, respectively) (ca. 30 cm TL) produced by JASFA Amami Station were experimentally released for a tagging experiment (S. Masuma, personal communication).

If there are large-scale releases of juvenile tunas, the genetic impact of the released individuals on natural populations would be of concern. Recently, there have been strong recommendations put forth for tracing the fate of released fish by genetic markers (FAO, 1992). Therefore, it would be helpful to develop reliable genetic markers and to understand the genetic variability of reared and released tuna.

A feasibility study to rear and spawn yellowfin tuna in land-based facilities was initiated at the IATTC Achotines Laboratory in Los Santos Province, Republic of Panama, in 1994 (Scholey et al., 2001; Wexler et al., in press). Wild yellowfin tuna juveniles (approximately 2–6 kg in weight) were captured by hook and line in near-shore waters, transferred to the laboratory and reared in land-based tanks. In October 1996, the broodstock fish began to spawn in the largest tank (17 m in diameter, 6 m in depth), and spawning has continued almost daily over the last 6 years (Scholey et al., 2001; Wexler et al., in press). In this study, mtDNA genotypes were compared between broodstock and their offspring in order to identify spawning females and to monitor the spawning frequency and periodicity of individual females.

## 2. Materials and methods

### 2.1. Broodstock

Regular spawning of captive yellowfin tuna has occurred since 1996 in a land-based tank at the IATTC Achotines Laboratory in the Republic of Panama (Scholey et al.,

2001; Wexler et al., in press). Five yellowfin tuna (group A) had been maintained in the main broodstock tank (17 m in diameter, 6 m in depth) since 1996, and 33 additional juveniles (group B) captured after 1998 were added during 1999 and 2000. Stocking and biological profiles of 38 broodstock individuals are shown in Table 1.

Table 1
Stocking and biological profiles of 38 broodstock yellowfin tuna

| No. of fish | Group | Code no. | Date transferred to main broodstock tank | At capture | | At death | | Sex |
|---|---|---|---|---|---|---|---|---|
| | | | | Date | Body weight (kg) | Date | Body weight (kg) | |
| 5 | A | 1 | from June to September 1996 | 4 June 1996 | 2.30 | 29 March 2000 | 87.50 | F |
| | | 2 | | 8 June 1996 | 3.60 | 16 November 1999 | 80.50 | F |
| | | 3 | | 15 July 1996 | 3.50 | 7 February 2000 | 58.00 | M |
| | | 4 | | 10 June 1996 | 4.25 | 27 October 2000 | 95.60 | F |
| | | 5 | | 15 July 1996 | 4.45 | 8 April 2001 | 61.40 | F |
| 14 | B | 1 | 17 August 1999 | 4 August 1998 | 3.20 | 24 March 2000 | 30.00 | F |
| | | 2 | | 14 October 1998 | 2.95 | 28 June 2001 | 21.60 | M |
| | | 3 | | 15 December 1998 | 4.05 | 9 October 1999 | 17.50 | M |
| | | 4 | | 15 March 1999 | 3.35 | 28 February 2000 | 28.80 | F |
| | | 5 | | 15 March 1999 | 3.05 | 28 November 2000 | 38.50 | F |
| | | 6 | | 31 March 1999 | 4.80 | 11 December 2000 | 50.95 | M |
| | | 7 | | 6 April 1999 | 4.60 | 14 April 2000 | 19.25 | M |
| | | 8 | | 7 April 1999 | 3.55 | 23 July 2001 | 28.30 | M |
| | | 9 | | 28 April 1998 | 5.30 | 29 December 2001 | 57.65 | F |
| | | 10 | | 28 April 1999 | 5.90 | 25 March 2001 | 41.05 | F |
| | | 11 | | 28 April 1999 | 5.15 | 3 March 2001 | 37.45 | F |
| | | 12 | | 29 April 1999 | 4.70 | 26 August 2000 | 32.25 | F |
| | | 13 | | 29 April 1999 | 5.10 | | | |
| | | 14 | | 30 April 1999 | 6.00 | 9 December 1999 | 20.45 | M |
| 6 | B | 15 | 8 February 2000 | 22 October 1999 | 5.45 | 3 August 2000 | 23.50 | F |
| | | 16 | | 28 October 1999 | 4.05 | 18 January 2001 | 20.65 | F |
| | | 17 | | 6 December 1999 | 3.30 | 31 January 2002 | 44.50 | M |
| | | 18 | | 17 December 1999 | 3.05 | | | |
| | | 19 | | 24 January 2000 | 3.45 | 22 February 2001 | 29.05 | F |
| | | 20 | | 24 January 2000 | 3.30 | 17 March 2001 | 22.85 | M |
| 12 | B | 21 | 10 May 2000 | 25 January 2000 | 2.95 | 27 January 2002 | 42.50 | M |
| | | 22 | | 25 January 2000 | 2.70 | | | |
| | | 23 | | 25 January 2000 | 2.65 | 23 October 2002 | 83.35 | M |
| | | 24 | | 25 January 2000 | 2.85 | 14 October 2000 | 11.60 | M |
| | | 25 | | 4 February 2000 | 2.85 | 5 May 2001 | 20.45 | M |
| | | 26 | | 4 February 2000 | 3.15 | 5 February 2002 | 27.80 | F |
| | | 27 | | 4 February 2000 | 2.95 | 12 August 2001 | 30.05 | M |
| | | 28 | | 4 February 2000 | 3.25 | | | |
| | | 29 | | 4 February 2000 | 2.95 | 7 October 2000 | 19.60 | M |
| | | 30 | | 4 February 2000 | 3.10 | 25 May 2001 | 16.20 | M |
| | | 31 | | 4 February 2000 | 2.95 | 15 May 2000 | 5.80 | F |
| | | 32 | | 4 February 2000 | 3.25 | 7 December 2000 | 9.00 | F |
| 1 | B | 33 | 24 Jun 2000 | 22 June 2000 | 7.10 | | | |

Each fish was tagged with a microchip implant tag in the dorsal musculature for individual identification at stocking. A small piece of fin from all group B individuals was clipped at stocking, and muscle tissue samples of group A individuals were obtained as they died by the end of April 2001. These tissue samples were preserved in ethanol.

The yellowfin broodstock were fed a diet of 50% fish (mostly Pacific thread herring or anchovy; ca. 10–15 cm) and 50% squid (ca. 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 and larvae

Spawning occurred almost daily in the main broodstock tank during most months from 1997 through 2000. Fertilized eggs and hatched larvae were collected for DNA analysis from 48 spawning events at daily to 3-week intervals from August 1999 through August 2000. The eggs were collected with a net (0.8-m square mouth opening × 1.2-m length) secured along the tank wall just below the surface of the water and near the tank sump. The number of eggs was counted after every spawning event, and some eggs (usually 50,000–100,000) were transferred to rearing tanks where hatching occurred within 24 h. Eggs (7–23) or newly hatched larvae were sampled on a given sampling day and preserved in ethanol. Only posterior body tissue of larval specimens was used for the analysis to avoid contamination from digestive organs.

### 2.3. DNA analysis

Preserved tissue samples were digested in 500 μl of TEK buffer (50 mM Tris, 10 mM EDTA, 1.5% KCl, pH 7.5; see Chapman and Powers, 1984) plus 100 μl of 10% SDS and 2 μl of 10 mg/ml Proteinase K. The tissue samples of larvae, finlets and muscle were incubated at 60 °C for 2–3 h with occasional shaking, while the eggs were kept in the digestive solution for 12–24 h longer. The standard phenol–chloroform method (Maniatis et al., 1982) was applied to the digested samples to extract DNA. The DNA from the larvae, eggs and broodstock fish was rehydrated with 10–30 μl TE buffer. The two primers CB3R-LT and 12SAR-H were used to amplify the *D-loop* region of mtDNA following Palumbi et al. (1991); their sequences are 5′-CACATTAAACCTGAATGATATTT-3′ and 3′-ATAGTGGGGTATCTAATCCCAGTT-5′, respectively. The PCR reaction was carried out in 25-μl total volume with an initial denaturation at 95 °C for 3 min 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 six restriction endonucleases (*Dde*I, *Hin*fI, *Mnl*I, *Msp*I, *Nla*III and *Taq*I). These enzymes were selected by preliminary screening of the RFLP in this DNA fragment using 10 Pacific and 2 Atlantic yellowfin individuals (data not shown). Digested PCR samples were electrophoresed through 2.5% agarose gel (Biogel, BIO101), followed by ethidium bromide staining that was photographed.

## 3. Results

### 3.1. Genotypes of brood stock individuals

The amplified fragment size of each mtDNA segment was estimated to be ca. 1900 bp, and no apparent size difference among individuals was observed. Among the 38 broodstock individuals examined, the numbers of observed genotypes in each restriction assay were three (*Dde*I and *Mnl*I), two (*Hin*fI and *Msp*I), eight (*Nla*III) and five (*Taq*I). Combining the genotypes obtained by the six restriction analyses (Table 2) yielded 27 composite genotypes, of which 18 established a single individual's identity, 8 were shared by two individuals and 1 type was shared by four individuals. All five individuals of group A and 28 of group B died by the end of October 2002. Gonad examination of these individuals revealed that 17 were females and 16 were males. The remaining five individuals are still alive and the sex is not known for four of these (Table 1).

Table 2
Composite genotypes of 38 broodstock individuals

| Composite genotype | Broodstock ID | | | | Genotypes | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Dde*I | *Hin*fI | *Mnl*I | *Msp*I | *Nla*III | *Taq*I |
| 1 | B-19* | | | | *A* | *A* | *A* | *A* | *B* | *B* |
| 2 | B-21 | | | | *A* | *A* | *A* | *B* | *B* | *B* |
| 3 | B-14 | B-18 | | | *A* | *A* | *B* | *A* | *B* | *B* |
| 4 | B-4* | | | | *A* | *A* | *A* | *A* | *B* | *C* |
| 5[a] | A-5* | B-5* | B-17 | B-29 | *A* | *A* | *A* | *A* | *B* | *D* |
| 6 | B-3 | | | | *B* | *A* | *A* | *A* | *B* | *D* |
| 7 | A-4* | B-20 | | | *A* | *A* | *A* | *B* | *B* | *D* |
| 8[a] | B-11* | | | | *C* | *A* | *A* | *B* | *B* | *D* |
| 9 | B-28 | B-33 | | | *A* | *A* | *B* | *A* | *B* | *D* |
| 10 | B-27 | | | | *A* | *A* | *B* | *B* | *B* | *D* |
| 11 | B-16* | B-24 | | | *A* | *A* | *A* | *B* | *B* | *E* |
| 12[a] | A-2* | B-30 | | | *A* | *A* | *B* | *A* | *B* | *E* |
| 13 | B-6 | | | | *A* | *A* | *B* | *B* | *B* | *E* |
| 14 | B-25 | | | | *C* | *A* | *B* | *B* | *B* | *E* |
| 15[a] | A-1* | B-32* | | | *A* | *A* | *A* | *A* | *B* | *F* |
| 16[a] | A-3 | B-12* | | | *A* | *A* | *A* | *A* | *C* | *D* |
| 17[a] | B-10* | | | | *A* | *B* | *A* | *A* | *C* | *D* |
| 18 | B-2 | | | | *A* | *A* | *B* | *A* | *C* | *E* |
| 19[a] | B-13 | | | | *A* | *A* | *B* | *A* | *D* | *D* |
| 20[a] | B-9* | | | | *A* | *A* | *B* | *A* | *E* | *C* |
| 21[a] | B-15* | | | | *B* | *A* | *A* | *A* | *E* | *D* |
| 22 | B-8 | B-23 | | | *A* | *A* | *B* | *A* | *E* | *D* |
| 23 | B-22 | | | | *A* | *B* | *B* | *A* | *E* | *D* |
| 24 | B-26* | | | | *A* | *A* | *B* | *A* | *F* | *D* |
| 25 | B-31* | | | | *A* | *A* | *C* | *A* | *H* | *D* |
| 26[a] | B-1* | | | | *A* | *A* | *A* | *A* | *I* | *D* |
| 27 | B-7 | | | | *A* | *A* | *B* | *A* | *J* | *D* |

Broodstock individuals with asterisk or underlined were revealed as female or male by gonad examination, respectively.

[a] Composite genotypes observed in the offsprings.

Table 3
Spawning profiles of nine identified yellowfin tuna dams

| mtDNA type | Dam ID | 1996 | 1999 | | | | | | | | | | | | | | | | | 2000 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | |
| | | | August | September | | | | October | | | November | | | | December | | | | | January | | | | | | |
| | | | 23 | 1 | 7 | 15 | 24 | 4 | 14 | 25 | 2 | 11 | 19 | 27 | 3 | 10 | 19 | 25 | 29 | 4 | 7 | 8 | 12 | 16 | 20 | |
| 15 | A1 | S | 22 | 10 | 3 | | 4 | 1 | | 8 | | | 1 | | 2 | | 6 | 7 | | 1 | 3 | 3 | | | | |
| 12 | A2 | S | | 3 | 2 | 14 | 1 | 4 | 4 | 9 | 10 | 5 | D | | | | | | | | | | | | | |
| 5 | A5 | S | | 4 | 4 | | 1 | 4 | 2 | 1 | | | (2) | (7) | (2) | | (1) | (4) | | (3) | | (1) | | | | |
| 26 | B1 | | S | | | | 1 | 1 | 3 | | | 1 | 2 | 1 | | 2 | 1 | 1 | 5 | 1 | 8 | | | 3 | 3 | |
| 5 | B5 | | S | | | | | | | | | | (2) | (7) | (2) | | (1) | (4) | | (3) | | (1) | | | | |
| 20 | B9 | | S | | | | | | | | | | 3 | 4 | 8 | | 2 | | | 3 | | | | 1 | | |
| 17 | B10 | | S | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | B11 | | S | | | | | | | | | | | 1 | | | | | 1 | | | | | | | |
| 16 | B12 | | S | | | | | | | | | 1 | 1 | 1 | 1 | | 2 | 2 | 5 | 1 | | 3 | | | 1 | |
| 19 | B13 | | S | | | | | | | 1 | | 1 | | 2 | 1 | 8 | | | 4 | 1 | 3 | 1 | 23 | 10 | 6 | |
| 21 | B15 | | | | | | | | | | | | | | | | | | | | | | | | | |
| mtDNA type | Dam ID | 2000 | | | | | | | | | | | | | | | | | | | | | | | | |
| | | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 |
| | | February | | March | | | | April | | | | | May | | | | | June | | | | July | | August | | |
| | | 6 | 15 | 11 | 18 | 27 | 28 | 4 | 5 | 16 | 18 | 26 | 3 | 9 | 17 | 24 | 31 | 9 | 13 | 20 | 28 | 3 | 19 | 8 | 18 | 26 |
| 15 | A1 | | | | | D | | | | | | | | | | | | | | | | | | | | |
| 12 | A2 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | A5 | | | | | | | | | | | (3) | (3) | (2) | | (2) | (2) | | (4) | (3) | (4) | (4) | (1) | (1) | (2) | (4) D |
| 26 | B1 | 2 | 1 | | | D | | | | | | | | | | | | | | | | | | | | |
| 5 | B5 | | | | | | | | | | | (3) | (3) | (2) | | (2) | (2) | | (4) | (3) | (4) | (4) | (1) | (1) | (2) | (4) D |
| 20 | B9 | 6 | | | | | | | 4 | 9 | 2 | 3 | 4 | 4 | 5 | 5 | 1 | 2 | 1 | 1 | | 4 | | 1 | 1 | 2 D |
| 17 | B10 | | | | | | | | | | | | | | | | | | | | 4 | 1 | 2 | 2 | 2 | 2 D |
| 8 | B11 | | | | | | | | | | | 4 | | | | | | | 1 | | | 1 | 1 | 2 | 1 | 2 D |
| 16 | B12 | 1 | 6 | | 4 | 10 | 3 | 7 | 1 | 1 | 2 | | | 3 | 2 | 1 | 3 | 2 | 2 | 4 | 2 | | 1 | 1 | | D |
| 19 | B13 | 1 | 1 | 7 | 11 | 2 | 7 | | 4 | | 5 | | | 3 | | 1 | | 2 | 1 | 2 | | | 2 | 2 | 4 | |
| 21 | B15 | S | | | | 1 | | | | | | 3 | 2 | | 2 | | 3 | 2 | 1 | | 1 | | | D | | |

Numbers of each cell indicate the number of individual eggs or larvae which had identical genotypes to each dam.

See Table 2 for the composite genotype. S: stocked, D: dead. Since dam B5 shared the same genotype with A5, number of their eggs or larvae was put in the parenthesis.

### 3.2. Genotypes of eggs and larvae

Ten composite genotypes were detected among 538 eggs and larvae analyzed, and these composite genotypes matched those of 16 broodstock fish (Table 2). One composite genotype (type 5) was shared by four broodstock fish, and types 12, 15 and 16 were each shared by two broodstock fish. By eliminating from the analysis, all males and individuals which were too small or not yet stocked on the sampling date (Table 1), 8 of 10 genotypes observed in the offspring established a single dam's identity. Broodstock fish A5 and B5 were both females and shared genotype 5.

### 3.3. Spawning profile

Water temperature in the tank reflected that of near-shore sea surface temperatures, as the water was continuously pumped into the tank via seawater intake lines located in waters just seaward of Achotines Bay. Spawning occurred daily during the 1-year sampling period except during late February to mid-March 2000 when the tank water temperature dropped from >24 to 22–23 °C.

Spawning profiles of the eight identified dams, and the two unidentified females that shared the same genotype, could be illustrated through analysis of the offspring's genotypes (Table 3). Based on the date when the genotype first occurred and the growth trajectories estimated for individuals (Wexler et al., in press), the size of a female at first spawning was estimated to be 12–28 kg and 75–112 cm. Including a genotype shared by two individuals (Type 5: A5 and B5) (Table 2), two to six genotypes were observed on 42 of 48 sampling days. This indicated that multiple females spawned on a given date. Although sampling was not conducted every day, the same genotypes such as types 12, 16, 19, 20 and 26 were observed on almost any sampling day until death. For example, offspring of dam A2 were present on nine consecutive sampling days during September and November 1999 until the fish died by striking the tank wall. Similarly, the offspring of dams B1, B9, B12 and B13 were observed on almost any sampling day for an extended period of time. In particular, dams B12 and B13 started spawning in October to November of 1999 and continued through July and August of 2000. On the other hand, no genotypes of four females (A4, B4, B16 and B19) were observed in the sampled offspring, although these females were in the same tank for a long period.

## 4. Discussion

The results from our study indicated that genetic monitoring of a captive spawning population of cultured fish is possible as long as we can sample their offspring routinely in a closed facility. For the first time in a large pelagic fish species, we have utilized mtDNA genotypes of captive broodstock, fertilized eggs and hatched larvae to identify individual spawning females and to monitor spawning behavior. We demonstrated that individual yellowfin tuna females are capable of spawning daily for extended periods of time as long as they remain in the appropriate range of water temperatures and have sufficient food. In addition, the genetic variation of the mtDNA *D-loop* region of yellowfin tuna proved to be

so high that it is probably useful not only for identification of individual fish but also for investigations of population structure in natural waters.

Histological analysis of the gonads of wild yellowfin tuna has indicated that females apparently can spawn at near-daily intervals, as fresh postovulatory follicles have been observed in the gonads of fully mature females that are presumed to have recently spawned (Schaefer, 1996, 1998). Schaefer (1998) estimated the mean interval between spawning events of reproductively active yellowfin females to be 1.27–1.52 days in the eastern Pacific, coinciding with our observations of captive yellowfin.

We cannot discount the possibility that these four females did not spawn at all during the study period. However, examination of their ovaries at the time of death indicated that all were sexually mature, two were reproductively active at the time of death and two were previously reproductively active but not at the time of death (K. Schaefer, personal communication). Furthermore, 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 (D. Margulies, personal communication). Therefore, it is likely that the number of spawning females was underestimated in our genetic analysis due to the conservative sampling frequency and sample sizes.

Our sampling frequency and sample sizes were chosen exclusively because of technical and cost issues, as the PCR-RFLP analysis adopted is conventional but very labor-intensive. Extraction of DNA, especially from eggs, is tedious and time-consuming. Time limitations and labor restrictions prevented us from analyzing large numbers on a daily basis. However, for future analyses of this type, alternative approaches such as single-strand conformation polymorphism (SSCP) analysis (Orita et al., 1989) may be better for the processing of large numbers of samples. Short DNA fragments (less than 350 bp) are necessary for better SSCP resolution, and the short but hypervariable region in the *D-loop* (see Alvarado Bremer et al., 1998, 1999; Reeb et al., 2000) may be the best candidate for such an analysis. If the SSCP assay were incorporated with much quicker DNA extraction and PCR amplification, nearly real-time monitoring of genetic variation of offspring and the determination of individual spawning profiles of yellowfin tuna would be possible.

The production of large numbers of tuna juveniles may soon become feasible for aquaculture and stock enhancement operations (S. Masuma, personal communication). The genetic analysis presented here may be useful in managing the genetic variation of offspring released into natural tuna populations in order to minimize the genetic impact to the wild stocks.

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