INDIAN OCEAN ALBACORE STOCK STRUCTURE STUDIES BY MORPHOMETRIC AND DNA SEQUENCE METHODS

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ABSTRACT

Both morphometric and DNA sequence analyses were applied in this study for throwing light on the stock structure of the albacore (Thunnus alalunga) resource in the Indian Ocean. A total of 144 albacore individuals, sampled from vessels fishing in six different localities of the Indian Ocean, were used for morphometric comparison. Two fisheries research surveys were carried out in 1990-1991 by the R/V Haikung, which belongs to the Taiwan Fisheries Research Institute, for collecting albacore specimens. Seven well-preserved (at -75℃) muscle tissue specimens, removed from seven albacores caught by the R/V Haikung from three different localities of the Indian Ocean, were used for DNA sequence comparison.

The results show that (1) the first two eigenvalues absorb about 99% of the total within-character variability, it is thus advisable to use the first two canonical variables for comparison of morphometric samples; (2) the resultant mutual relationships drawn from morphometric analysis agree surprisingly well with those from the DNA sequence comparison; (3) albacore samples from the Indian Ocean can be categorised into two major groups: those collected from the area west of 90℃E and those from the area east of that longitude; (4) within-group heterogeneity is much less than between-group heterogeneity, no matter which method is applied. Based on the results so far obtained, the authors believe that it is possible to have two albacore stocks delimited by the 90℃E longitude in the Indian Ocean.

INTRODUCTION

Taiwanese longliners began fishing in the Indian Ocean in the late 1960s, and albacore are the major target species. In the early 1980s, for instance, the annual longline catch of albacore ranged from 6,000 to 20,000 t. The annual total catch of albacore increased to about 40,000 t in the late 1980s when the driftnet fishery emerged mainly targeting albacore in the period from the mid-1980s to 1992. The Taiwanese fishing fleet has been historically one of the major fishing fleets in the Indian Ocean to utilise the albacore resource.

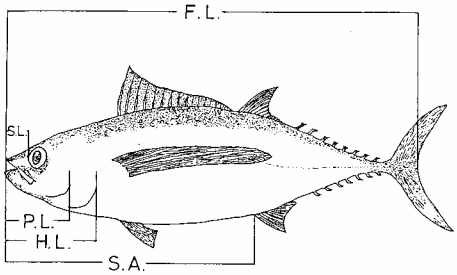
As one of the major fishing nations harvesting the Indian Ocean albacore resource, much research effort has been devoted by Taiwanese fisheries scientists to studying: (1) age and growth (Kuo, 1990; Lee, 1990); (2) stock assessment of the resource (Huang *et al.*, 1986; Lee and Liu, 1988) in addition to those previous studies of (1) larval distribution (Koto, 1969; Nishikawa, 1985); (2) movement and distribution (Suda, 1974); and (3) stock assessment (Shiohama, 1985). Although one stock hypothesis of Indian Ocean albacore resource has been adopted for many years, there is still no concrete evidence yet to fully support such postulation.

The main purposes of this study are to investigate between-sample comparison on (1) morphometric traits of Indian Ocean albacores landed at Kaohsiung fishing port in the period of 1988-1992, when Taiwanese driftnet fisheries were active; and (2) DNA sequences obtained by analysing albacore muscle tissue collected during the 1990-1992 Indian Ocean fisheries research cruises carried out by the R/V *Haikung*, which belongs to the Taiwan Fisheries Research Institute. The results thus obtained may throw light on the albacore stock structure in the Indian Ocean.

Table 1. **Information of Indian albacore samples collected for the present study**

Sample name	*Sample size*	*Range of fork length*	*Period of fishing*	*Name of Vessel*	*Type of gear*
G1	20	65-80	1989/11-1990/02	Yusing	Longline
G2	16	100-115	1990/11	R/Haikung	Longline
G3	10	78-111	1990/12	R/Haikung	Longline
G4	32	60-100	1987/12-1988/01	ChunYuFa	Driftnet
G5	36	50-100	1988/07-1988/09	TaoYuen	Longline
G6	30	50-90	1988/10-1988/12	HoChunMan	Driftnet

***Figure 1**. **Morphometric characters u**sed in the present study.*



MATERIALS AND METHODS

Morphometric Comparison

Measurements of five morphometric characters: (1) fork length; (2) snout length; (3) pre-operculum length (snout to pre-operculum); (4) head length (snout to operculum); and (5) length of snout to the insertion of the anal fin, as shown in Figure 1, were made on each individual specimen. A total of 144 albacore specimens were collected for morphometric comparison in the present study. Further information pertinent to the samples is shown in Table 1.

In the period of 1987-1992, six sets of morphometric measurement were collected from albacore specimens caught by vessels fishing in six different localities of the Indian Ocean, as shown in Figure 2. Four of these sets were taken from albacore landings at Kaohsiung fishing port, which is applicable only in years of 1987-1992, by Taiwanese commercial longliners or driftnetters fishing in the Indian Ocean. The other two sets were collected aboard the R/V *Haikung* during a fisheries research survey in the Indian Ocean in late 1990.

Canonical variate analysis, which is basically a principal component analysis supplemented by a normalisation of all within-variate variability into unity, is used in the present study. The schematic layout of the method can be expressed as follows:

Find a transformation: $\mathbf{Y} = \mathbf{C}\,\mathbf{X}$

To maximise: $\mathbf{C}\,\mathbf{Z_b}\,\mathbf{C'}$

subject to: $\mathbf{C}\,\mathbf{Z_w}\,\mathbf{C'} = \mathbf{I}$

where:

$\mathbf{X}$ =matrix of original groups of observations;

$\mathbf{Y}$ =matrix of transformed (orthogonal) variates;

$\mathbf{C}$ =transformation matrix;

$\mathbf{C'}$ =transpose of $\mathbf{C}$;

$\mathbf{Z_w}$ =matrix of within-group dispersion;

$\mathbf{Z_b}$ = matrix of between-group dispersion;

$\mathbf{I}$ = unity matrix.

***Figure 2.** Map of sampling areas or locations.*

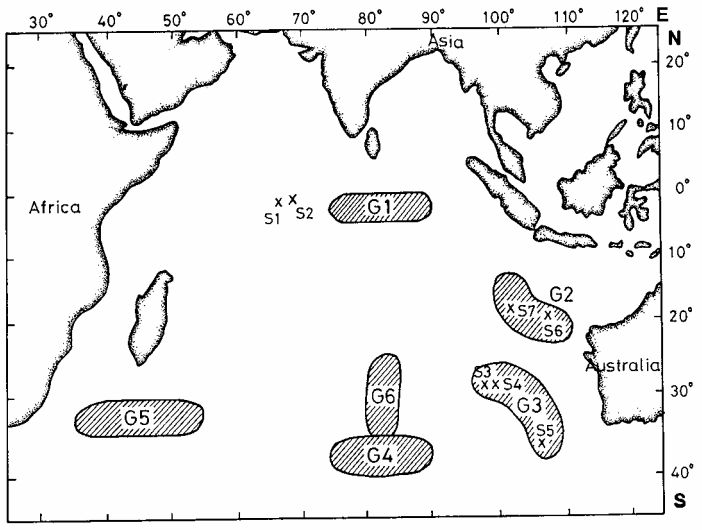


Table 2. **Localities, sampling dates, and designated codes of the seven albacore samples**

No.	*Sampling date*	*Locality*		*Code*
		S. latitude	E. longitude	
1	12-28-1991	1°00"	66°0"	s1
2	12-28-1991	1°00"	66°30"	s2
3	30-12-90	29°56"	99°30"	s3
4	30-12-90	29°56"	99°00"	s4
5	20-12-90	37°30"	106°29"	s5
6	30-11-90	20°09"	107°30"	s6
7	26-11-90	19°23"	102°23 "	s7

DNA Sequence Comparison

During the 1990 research survey, about 2 cc of muscle tissue from the central area between the dorsal fin base and the lateral line was removed from every albacore caught. As soon as an albacore was landed and morphometrically measured on the deck, about 9 cm^2 of skin was removed to expose the muscle tissue underneath and a cut of about 4 cc of muscle tissue was made. This piece of tissue was immediately brought to the laboratory, which is one floor underneath the deck, for further cleaning and trimming procedures. Then it was carefully packed into a small bottle for liquid nitrogen storage. These samples were then transferred to a -75°C refrigerator until the R/V *Haikung* completed the cruise.

Seven albacore specimens, which represented three area localities in the Indian Ocean, were intentionally chosen for DNA sequence analysis. The exact catch locations (marked as "X") of the seven specimens are shown in Table 2 and Figure 2.

DNA was isolated from seven individuals by the procedure of Hillis and Moritz (1990), with minor modifications. About 100mg of muscle was incubated in 1 ml of Digestion buffer (10mM EDTA, 100mM Tris-HCl pH 8, 100mM NaCl, 0.1% SDS, 50mM Dithiothreitol, 0.5mg/ml Proteinase K) for 2-4 hours at 50°C. The DNA was purified with standard phenol-chloroform extraction method. The DNA was precipitated with ethanol and washed with 70% ethanol. The DNA pellet was dried by vacuum and re-suspended in 50ul TE buffer (10mM Tris-HCl pH 8, 1mM EDTA pH 8) and stored at 4°C.

Double-stranded PCR amplifications were performed adopting the protocols of Innis *et al.* (1989). Amplifications were performed in 50ul of a solution containing 50mM KCl, 10mM Tris-HCl pH 8.3, 1.5mM MgCl2, 0.1% gelatin, 0.4mM dNTP, 10 to 25 ng of template DNA, 2 units of Super Taqpolymerase (HT Biotechnology Ltd.),and 20 pmole of each primer. A total of 35 cycles of 1 min. at 94°C for denaturing, 1 min. at 50°C for annealing, and 1.5 min. at 72°C for extension were carried out with a thermal cycler (PTC-100, MJR Inc.). The corresponding locations of the two primers in mtDNA (P3 and PB) used in PCR are shown in Figure 3. The nucleotide sequences of P3 and PB are as follows:

P3: 5'-AACTTCCATCCTCAACTCCCAAAGC-3'

PB: 5'-AGTGGGGTATCTAATCCCAG-3'

A 1.35 kb DNA fragment was amplified. The amplification product was separated by agarose gel electrophoresis, then visualized by staining with ethidium bromide, and the amplified fragment of DNA was excised from the gel. A JETsorb Gel Extraction Kit (GENOMED inc.) was used to extract DNA from excised gel. Finally, the purified DNA was resuspended in 25ul double-distilled H_2O and used as a template for DNA sequencing.

Nucleotide sequencing was performed by the dideoxy chain-termination method (Sanger *et al.*, 1977) using the Amplicycle sequencing kit. [o 35S-dATP] was used as a label. Taq DNA polymerase (Perkin Elmer Cetus), two units for each reaction, was added to the labelling mixture. Only one primer, P-phe (presented by Levy *et al.*, 1994, to amplify the D-loop), was adopted in the sequencing reaction. The corresponding location of P-phe is shown in Figure 3, its sequence is

5'-GCTTTAGTTAAGCTACG-3'.

A total of 29 cycles of 1 min. at 94°C, 1 min. at 50°C, and 1.5 min. at 72°C was carried out. After this sequencing reaction, 4ul Stop dye solution were added to each tube. The resulting single-strand DNA products were subject to electrophoresis in a 6% polyacrylamide gel. The gel was fixed, and then transferred to a 3-mm filter, dried and visualised by exposure to X-Omat film (Kodak) for 12 to72 hours (Sambrook *et al.*, 1989).

Figure 3. *Schematic diagram of the amplified region of the albacore mtDNA between P3 and PB. The locations and orientations of the three primers on the amplified region are indicated by arrows. Designated P3 and PB are PCR primers and P-phe is sequencing primer. Black area is the sequenced region.*

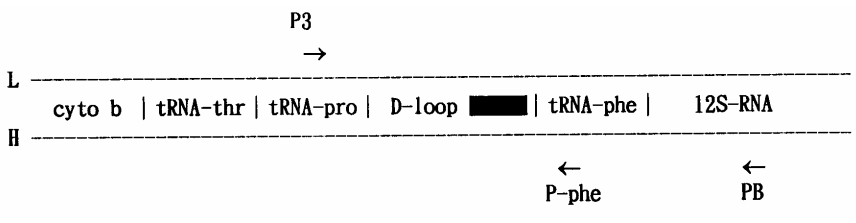


Table 3. **Mean vector, with its corresponding transformed first two canonical variates, of the morpho-metric characters for stock identification of albacore samples from six locations in the Indian Ocean**

Groups	*F. L*	*S. L*	*S. P*	*H. L*	*S. A*
			Mean Vector		
G1	71.7	7.5	16.4	21.0	45.6
G2	107.9	10.7	24.1	30.4	70.0
G3	101.3	10.2	22.7	29.1	66.7
G4	75.7	7.5	17.5	22.0	47.9
G5	77.8	8.5	18.0	23.0	49.5
G6	69.3	7.6	16.0	20.5	44.2
Canonical Axes					
1st	0.5884	-0.0761	0.1649	0.0868	0.0396
2nd	0.1684	12.2791	0.1609	0.0023	0.0086
Canonical variates			Groups		
	G1	G2	G3	G4	G5
1st	9.98	14.93	14.10	10.57	10.86
2nd	0.54	-0.09	0.14	0.04	1.01

F.L. = fork length; S. L. = snout length; H. L. = head length S.P. = snout to preoperculum; S. A = snout to anal fin

Nucleotide sequences of a portion of the mitochondrial D-loop region were obtained for seven albacore tunas. The corresponding nucleotide sequence from bluefin tuna (Pla *et.al*., 1994) was compared with this part of the D-loop region. Sequence alignments were accomplished with the Pileup program of the GCG software (Genetic Computer Group, version 8.01, University of Wisconsin). The Plot Similarity program in the GCG software was used to compare the similarity of each nucleotide between the seven nucleotide sequences. The inter- and intra-phylogenetic relationship was constructed by the unweighted pair-group method with arithmetic mean (UPGMA) by using the values of the distances matrix between nucleotide sequences (correction method: Kimura 2-parameter), which were estimated from the whole sequence of each pair by using the Distances program of the GCG software. The nucleotide sequence of bluefin tuna was used as an out group.

RESULTS OF MORPHOMETRIC COMPARISON

The sum of all eigenvalues thus obtained in the present study is 1,493. The first two eigenvalues: 1.151 (representing about 77% of the total) and 0.327 (about 22%), have already accounted for 99% of the total within-sample variability. It is thus advisable to use the first two canonical variates to represent the mutual relationships of albacore samples caught from different areas.

The mean vector, with its corresponding transformed first two transformed canonical variates, of the morphometric characters for stock identification of albacore samples caught from six localities of the Indian Ocean is shown in

Figure 4. *Plot of the first two canonical variates showing the mutual relations of the six samples. The circle represents its 90% confidence limits.*

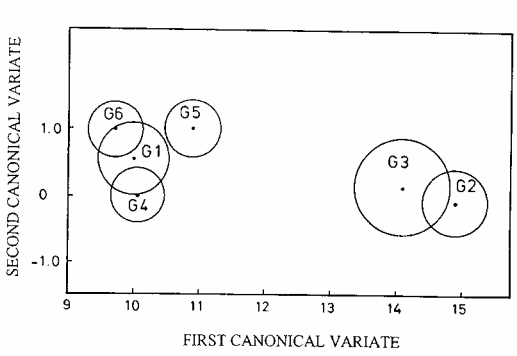


Table 3. The numerical figures, which are an indication of influential strength in the determination of its resultant canonical variate, of the first canonical axes indicate that the order is: fork length, pre-operculum length, head length, snout length, and length of snout to anal fin insertion. Those of second canonical axes are: snout length, fork length, preoperculum length, length of snout to anal fin insertion, and head length. It appears that the head portion of the fish plays an important role in the morphometric comparison.

The plot of six groups by the first two canonical variates is shown in Figure 4. Although the geographic distances among samples are significantly large, samples from the northern Indian Ocean (G1), central Indian Ocean (G4 and G6), and southwestern Indian Ocean (G5) show much closer relationships as compared to the two samples from the eastern Indian Ocean (G2 and G3). Furthermore, the period of fishing indicates that all the samples were collected in almost the same period of time (October to February) except G5, which was collected in July to September.

RESULTS OF DNA SEQUENCE COMPARISON

Figure 5 shows the aligned sequences of 300 bp of a part of the control region (D-loop) for the seven albacore tunas and one bluefin tuna. The 300 bp obtained represents approximately 30% of the complete D-loop region from the genus Thunnus (Pla *et al.*, 1994). Twenty variable nucleotide positions were identified among seven albacore sequences, but twenty-seven among the eight sequences. The most different region was between positions 83 to 148. The Plot Similarity program in the GCG package was also executed. The similarity score showed that the most variable position is located at position 99 (similarity is only 0.6) but the similarity of most locations is 1 (Figure 6).

Table 4. **Pairwise Distance between nucleotide sequences among the seven albacore samples and bluefin tuna by using the Distance program in the GCG software. The correction method is Kimura 2-parameter**.

Code	*Bluefin*	*s1*	*s2*	*s3*	*s4*	*s5*	*s6*	*s7*
Bluefin	0.00	22.90	23.35	23.35	23.82	23.36	22.45	23.82
s1	-	0.00	1.01	5.55	5.92	6.28	5.19	6.65
s2	-	-	0.00	6.28	6.65	7.02	5.92	6.65
s3	-	-	-	0.00	0.34	0.67	0.67	1.01
s4	-	-	-	-	0.00	1.01	1.01	1.36
s5	-	-	-	-	-	0.00	1.35	1.35
s6	-	-	-		-	-	0.00	1.70
s7	-	-	-	-	-	-	-	0.00

***Figure 5**. Sequences of a 300-bp region of the mitochondrial D-loop from seven albacore tunas and one bluefin tuna (Pla et. al., 1994). The positions of nucleotides for bluefin tuna is from 560 to 858. Dots represent matching sequences, and dashes represent gaps.*

```
                 1
   s3    ttatttttt ctctccttcc tctcatttgg catctcacag tgcaaataca
   s4    .......... .......... .......... .......... ..........
   s5    .......... .......... .......... .......... ..........
   s6    .......... .......... .......... .......... ..........
   s7    .......... .......... .......... .......... .......g..
   s1    .......... .......... .g........ .......... ..........
   s2    .......... .......... .g........ .......... .......g..
bluefin  .......... .......... .t.......t .g........ ...-...g..
         51                                                  100
   s3    acaatgatca gcaaggtaga acattttctt gcctgcaggg taatggttat
   s4    .......... .......... .......... .......... ..........
   s5    .......... .......... .......... .......... ........c.
   s6    .......... .......... .......... ..t....... ..........
   s7    .......... .......... .......... .......... ........g.
   s1    .......... .......... .......... ..t....... ...acagca.
   s2    .......... .......... .......... ..t....... ...atagca.
bluefin  .......... a......... .......... ..t....... ...atagcc.
         101                                                 150
   s3    tcatggttta aatccta-ta ttaaaataac cacatacttg gatatcatga
   s4    .......... .......-.. .t........ .......... ..........
   s5    .......... .......-.. .t........ .......... ..........
   s6    c......... .......-.. .t........ .......... ..........
   s7    .......... .......-.. .t..g..... .......... ..........
   s1    gt....c... .t.....-.. .c........ ......aag. .......c..
   s2    gt....c... .t.....-.. .c........ .....gaag. .......c..
bluefin  gc....c... .t.....t.. c.t....... ......aga. ..........
         151                                                 200
   s3    gcataatgat aatattaccc gtaaaatatc taagacaccc cctctcggct
   s4    .......... .......... .......... .......... ..........
   s5    .......... .......... .......... .......... ..........
   s6    .......... .......... .......... .......... ..........
   s7    .......... .......... .......... .......... ..........
   s1    .......... .......... .......... .......... ..........
   s2    .......... .......... .......... .......... ..........
bluefin  .......... .......... .......... .......... ..........
         201                                                 250
   s3    tttgcgcgtt aaaccccccc acccccctaa actcgtgata tcattaacac
   s4    .......... .......... .......... .......... ..........
   s5    .......... .......... .......... .......... ..........
   s6    .......... .......... .......... .......... ..........
   s7    .......... .......... .......... .......... ..........
   s1    .......... .......... .......... .......... ..........
   s2    .......... .......... .......... .......... ..........
bluefin  .......... .......... .......... .......... ..........
         251                                                 300
   s3    tcctgtaaac ccccgtaaa caggaaaatc tcgagtgggg tattttatgg
   s4    .......... .......... .......... .......... ..........
   s5    .......... .......... .......... .......... ..........
   s6    .......... .......... .......... .......... ..........
   s7    .......... .......... .......... .......... ..........
   s1    .......... .......... .......... .......... ..........
   s2    .......... .......... .......... .......... ..........
bluefin  .......... .......... .......... .......... ..........
```

Table 4 shows the distances among the eight nucleotide sequences, and this distance matrix was used in the analysis of the phylogenetic tree. The phylogenetic tree constructed by UPGMA is shown in Figure 7. From this molecular tree two pieces of information can be obtained: first, the molecular data can easily distinguish between bluefin tuna and albacore; second, two significant genealogical branches can be observed among the seven albacore tuna samples. The s1 and s2 cluster is one group, and the rest is another. This result indicates that the seven albacores from three different localities may have come from two reproductively isolated populations. Table 4 shows the pairwise similarity among the seven albacore samples. The intra-group similarity is about 0.99, but about 0.94 or 0.95 in inter-group.

CONCLUSION AND DISCUSSION

The results obtained in the study show that (1) the first two eigenvalues absorb about 99% of the total within-character variability, it is thus advisable to use the first two canonical variables for comparison of morphometric samples; (2) the resultant mutual relationships drawn from the morphometric analysis agree surprisingly well with those from the DNA sequence comparison; (3) albacore samples caught in the Indian Ocean can be categorised into two major groups: those collected from the area west of 90°E and those from the area east of that longitude; and (4) within-group heterogeneity is much less than between-group heterogeneity, no matter which method is applied. Based on the results so far obtained, the authors believe that it is possible to have two albacore stocks delimited by the 90°E longitude in the Indian Ocean.

***Figure 6**. Plot of similarity score versus sequence of base pair.*

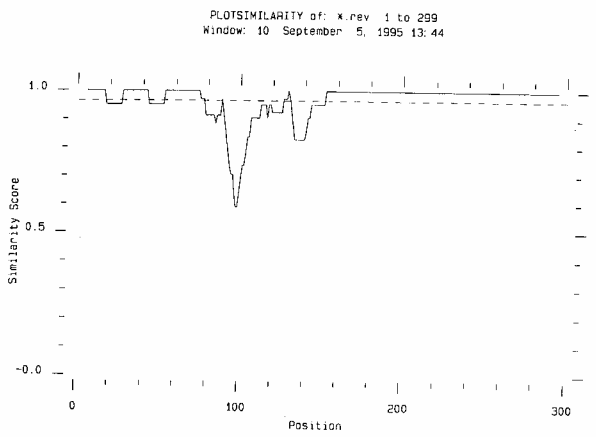
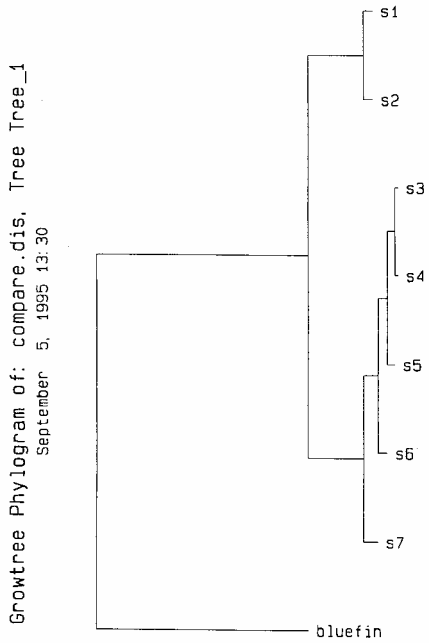


***Figure 7.** Plot of the phylogenetic tree constructed by UPGMA.*



Nishikawa(1985) indicated that dense larval distribution areas can be found both in the waters off northern Australia on the east side of the Indian Ocean and the areas near Madagascar, which is on the west side of the Ocean. It is very unfortunate, though, that there is virtually no information on larval distribution between the two extremes, thus it is very difficult to judge whether the spawning of Indian Ocean albacore is continuously distributed from east to west or whether there are two different spawning stocks at both ends of the east-west bounds of the Indian Ocean. The whole picture of larval distribution patterns certainly will provide very important information for the albacore stock structure in the Indian Ocean. Further information related to this matter is urgently needed.

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REFERENCES CITED

HILLIS, D.M. AND C. MORITZ. (1990) Molecular systematics. Sinauer Associates. Sunderland.

INNIS, M.A. AND D.H.GELFAND. (1989) Optimization of PCRs. Pp. 3-12 in M.A.Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, *eds.* PCR protocols. Academic Press Inc.

KOTO, T. 1969. Distribution and movement of the albacore in the Indian Ocean and the Atlantic Ocean based on the catch statistics of Japanese tuna longline fishery. Bull. Far Sea Fish. Res. Lab. 1:115-129.

LEE, Y.C. AND C.L. KUO. 1988. Age characters of albacore, *Thunnus alalunga*, in the Indian Ocean. IPTP.TWS/88/61:99-108.

PLA C., J. VINAS, J. M. PUOLAR AND J. A. LEVY. (1994) Biochemical genetics characterisation of large pelagic stocks (*Thunnus thunnus, Thunnus alalunga* and *Sarda sarda*) in the Mediterranean. ICCAT Working Document SCRS/94/171.

SANGER, F., S. NICHLEN AND A. R. COULSON. (1977) DNA Sequencing with Chain-terminating Inhibitors, Proc. Natl. Acad. Sci. USA. 74: 5463-5467.

SAMBROOK, J., E. F. FRITSCH AND T. MANITAIS. (1989) Molecular clonings, a laboratory manual, 2nd ed. Cold Spring Harbor Lab. 1200pp.