

PRELIMINARY AGE VALIDATION RESULTS FROM TAGGED-RECAPTURED
FLUOROCHROME LABEL ALBACORE IN NORTH EAST ATLANTIC.

V. Ortiz de Zárate¹, P. Megalofonou², G. De Metrio³ and C. Rodriguez-Cabello¹.

ABSTRACT

In 1991, during a tagging cruise, 1,494 immature albacore (Thunnus alalunga) were tagged and injected with oxytetracycline to produce a time mark in the skeleton structures (fin spine). A total of 29 whole fish were recovered after being at liberty for less than three months, one year and two years having all an oxytetracycline mark. The experiment was designed in an attempt to validate the age determination of immature albacore by using the first dorsal fin spine method.

Analysis of the translucent zones identified after the OTC mark shows that the albacore examined do not follow a single pattern in formatting the annuli. In most cases two annuli were formed in one year at liberty, but cases with one or three annuli were also present. Furthermore the first annuli identified after the OTC mark occurs sometime between July and October. Individual variability in growth is evident. Count of annulus formed after the OTC mark is not completely in agreement with previous studies based on the fin spine method. Number of annuli formed per year could suggest different migration pattern in the immature albacore, as well as a mixture of individuals from diverse populations.

RESUME

Au cours d'une campagne de marquage en 1991, 1.494 germons (Thunnus alalunga) immatures ont été marqués à l'oxytétracycline pour établir des marques chronologiques dans les structures osseuses (épines de nageoires). En tout, 29 poissons entiers ont été recapturés moins de trois mois après, au bout d'un an et au bout de deux ans, tous porteurs de marqueurs à l'oxytétracycline. Cette expérience visait à tenter de valider la détermination de l'âge du germon immature par la méthode utilisant les épines de la première dorsale.

L'analyse des zones translucides identifiées d'après les marqueurs OTC montre que les germons étudiés ne suivaient pas un mode unique de formation des anneaux. Dans la plupart des cas, deux anneaux s'étaient déposés en un an, mais il existait aussi des cas avec un ou trois anneaux. Par ailleurs, les premiers anneaux identifiés après le marquage à l'OTC se sont déposés entre juillet et octobre. La variabilité individuelle de la croissance est évidente. Le comptage des anneaux formés après le marquage à l'OTC ne concorde pas pleinement avec les études antérieures basées sur la méthode des épines de nageoires. Le nombre d'anneaux formés par an pourrait suggérer un mode distinct de migration chez le germon immature, ainsi qu'un mélange de poissons de différentes populations.

RESUMEN

En 1991, durante una campaña de marcado, se marcaron e inyectaron con oxitetraciclina (OTC) 1.494 atunes blancos inmaduros (Thunnus alalunga), con el fin de producir una marca de tiempo en las estructuras óseas (espinas de aletas). Se recuperaron 29 peces enteros tras un periodo de libertad inferior a tres meses, un año y dos años, todos con la marca de oxitetraciclina. El experimento tenía como objetivo intentar validar la determinación de la edad del atún blanco inmaduro aplicando el método de la espina de la primera aleta dorsal. El análisis de las zonas traslucidas, identificadas tras la colocación de la marca OTC,

¹. Instituto Español de Oceanografía. Apdo.240., Santander 39080, Spain.

². University of Athens, Department of Biology, Panepistimiopolis, Athens GR 157 84, Greece

³. Università degli Studi di Bari, Dipartimento di Produzione Animale, Via Giovanni Amendola, 165/A. 70126 Bari, Italia.

muestra que en los atunes blancos examinados, la formación de los anillos no sigue el mismo patrón. En la mayor parte de los casos, en el curso de un año en libertad se formaron dos anillos, si bien se observaron casos de uno y tres anillos.

Además, los primeros anillos identificados tras la colocación de la marca OTC se forman entre los meses de julio y octubre. Es evidente que hay una variabilidad individual en el crecimiento. Al contar los anillos formados tras la colocación de la marca OTC, se observa que la concordancia con anteriores estudios basados en el método de la espina de aleta no es total. El número de anillos que se forman por año, podría sugerir una pauta migratoria diferente en el atún blanco inmaduro, así como una mezcla entre peces procedentes de diversas poblaciones.

1. INTRODUCTION

Immature and pre-adult individuals of north Atlantic albacore (*Thunnus alalunga*) stock are exploited in the surface fisheries during their spring-summer migration to the Bay of Biscay and adjacent waters of the north east Atlantic.

Age determination of albacore using the first dorsal fin ray was addressed by Bard and Compean (1980). They identified, in the samples studied, two translucent zones (annuli) per year, the first one corresponding to autumnal migration and the second one to spring migration. The growth parameters estimated were fit to the von Bertalanffy growth curve model.

Later studies by González-Garcés and Fariña-Perez (1983), also using the first dorsal fin ray, agreed with the establishment of a pattern of two bands formed per year on immature albacore, although there was a discrepancy in the estimation of mean size at age one. Also, for interpretation purposes, the first annuli appears to be formed in fall-winter and the second in spring-summer. Nevertheless, this fin ray method involves a high degree of subjectivity depending on the interpreters.

Another study to introduce more objectivity was developed to analyze fin ray sections by means of automatic reading software based on image analysis (Troadeç and Antoine, 1993).

Alternatively, another approach to estimate age composition from size data of the north Atlantic albacore stock was applied by Santiago (1993a). This stochastic method contemplates the mean length and standard deviations in length for several age groups (Schnute and Fournier, 1980).

Comparisons of different methods applied to age albacore length distribution have been made in an attempt to verify certain techniques using mark-recapture data (Ortiz de Zárate *et al*, 1994), ageing from hard parts (Fernandez, 1992; Troadeç and Antoine, 1993) and length size analysis (Santiago, 1993a). However, no validation exists to prove that any of these techniques is accurate. The importance of validating an ageing technique has been widely mentioned (Beamish and McFarlane, 1983; Casselman, 1983).

Current assessment of the status of this stock requires accurate age composition catch data to apply structured evaluation models. Therefore, improvement in knowledge to determine age and growth rate is one of the priorities in the evaluation of this stock. Therefore, within the development of the Special Albacore Program (PSG) a direct study was designed by IEO (Instituto Español de Oceanografía) to validate the growth of immature albacore using hard parts structures (first dorsal fin ray) by means of tagging and injecting individuals with oxytetracycline (OTC) during the tagging program in the Bay of Biscay in the Summer of 1991 (Ortiz de Zárate *et al.*, 1993).

In this study we present these results in an attempt to validate the fin ray method of age determination, which includes only immature fish which have been at liberty for up to two years.

2. MATERIALS AND METHODS

During the tagging cruise carried out in August 1991 in the Bay of Biscay, in the Cantabrian sea, a total of 1,494 albacore were injected with oxytetracycline (OTC) and released, carrying a red spaghetti type FT-1 tag.

OTC was purchased under the commercial name "Oxipra-20" at a concentration of 200mg/ml. A dosage of 70mg/kg of fish weight (McFarlane and Beamish, 1987), roughly estimated, was administered, intramuscular, by means

of an Socorex automatic syringe into the body area of the dorsal fin. Fork length (FL) to the inferior cm was measured, and the date and location noted (Ortiz de Zárate *et al.*, 1994).

OTC was used to produce a time mark in the skeleton structures. The mark laid is detected by means of a yellow-gold fluorescence when exposed to ultraviolet (UV) illumination.

An informative campaign offering rewards for whole fish carrying a red tag took place in the fishing ports on the Cantabrian coast. As a result, 29 whole albacore were recovered during the fishing seasons in 1991, 1992 and 1993. The information recorded for each tagged and recaptured fish used in the study included date, location, length and weight. Size was recorded in fork length to the nearest one centimeter.

The first dorsal fin ray from those recaptured albacores were removed. From each fin ray two serial sections of 0.7 mm were cut using a low speed saw and mounted with a synthetic resin as described by González-Garcés and Fariña Perez (1983).

Fin ray sections were viewed in a darkened room using a Leitz Diaplan microscope equipped with an ultraviolet light system Leitz Ploemopak (3 lambda) with filter type D for tetracycline. A Quantimet 500 Plus image analyzer connected to the microscope by means of a Sony video camera was used to examine the spine sections.

The OTC mark was identified, as well as changing gradually to white light the number of annuli formed after the OTC mark were counted (Table 4). Measurements of OTC, annuli and spine section diameters (mm) were taken by image analyzer, employing the software Qwin. All measurements were taken according to the transversal position as described by González-Garcés and Fariña-Perez (1983).

Photographs were taken using a microscope equipped with a Wild Photomat MPs 45/51s system and a Fluotar 10/0.03 objective.

Back-calculated fork lengths were obtained for both the OTC mark diameter and spine section diameter in all samples, to examine length measurements, especially those taken during the tagging operations. The lineal regression fit was used, derived for a sample of 790 albacore from summer surface fishery over a size range of 45 cm to 117 cm ($b = 0.060091$; $a = -0.33407$; $r = 92.02$) which is described in Troadec and Antoine (1993). Chi-square tests were run to the observed and estimated lengths.

The growth rate of each fish in centimeters per day was estimated from the length increment and lapsed time observations, and thus the annual growth rate was obtained. In order to calculate annual mean growth, the released individuals were split into two size groups. The first group ranged from 57 to 63 cm length and the second one from 64 to 69 cm, according to two modes observed in the size distribution of released fish (Figure 1).

3. RESULTS

The total number of recoveries with OTC injected amounted to 23 in 1991, 47 in 1992 and 1 in 1993, which are summarized in Table 1. Length size distribution of recaptured fish is shown in Figure 2. From these, only 29 whole fish were recovered and spines were removed for the purposes of this study. All spine sections showed a narrow band of yellow-gold fluorescence, depicting the region of bone proliferation at the time of the OTC injection. The set of fish with spine removed is shown in Tables 2 and 3.

A decrease in the intensity of induced fluorescence was apparent when preparatives of fin ray sections were exposed to UV light for several minutes during examination. After several readings, some of the samples lost the fluorescence effect completely, reducing the number of observations to 22. The final data used are shown in Tables 2 and 3.

The number of annuli counted after OTC marking, the location of the OTC mark, as well as increment to the edge of the spine section are shown in Table 4.

Fish caught within a period of one to three months do not present annuli after the deposition of OTC, which was noticeable in the edge of the spine section. Only in one case was the presence of one annuli overlapping the OTC mark observed.

Examination of spine sections belonging to 17 fish, recaptured in the following fishing season, being at liberty between 11 and 13 months, showed a variability in the number of annuli formed after OTC marking. Number of annuli

counted after the OTC mark were: one in 12% out of total, two in 59% and three in 29% of the total cases. For these fish, it was also noticed that the formation of an annulus was strongly linked to the "time" mark. In fact, in all the cases an annulus was either coincident with the OTC mark or found just after the OTC mark, as has been represented in Table 4.

Only one albacore (NO-5946) was recaptured after two years (27 months) at liberty. The examination of its spine section shows that right after the OTC mark an annulus was formed and two more annuli were laid, making a total of three annuli at the time of recapture.

No differences were observed between the observed and estimated lengths for both OTC mark diameter and spine section from back-calculation, (X^2 , $p > 0.05$), H_0 was accepted at a level of significance of 95% for both cases.

Mean growth rates obtained for the two size groups are 12.2 cm/year and 10.7 cm/year respectively (Table 5). An F-test was applied to examine the null hypothesis for the variances ($H_0: S_1 = S_2$) gave a value of $F=7.2162$ and therefore the null hypothesis H_0 was rejected ($F(6,13)/0.05=2.92$). However, t-test showed that there is no significant difference in the mean growth observed between the two groups ($t=0,6266 < t_{19,0.05}=2.093$). Size increments are included in Figure 3.

4. DISCUSSION

The experiment was designed in an attempt to validate the age estimation of immature albacore by using the first dorsal fin ray method. The recovery percent (4.7) of fish tagged and injected with OTC gives a recovery rate slightly higher than un-injected fish (3%), indicating that there was no mortality introduced with the injection of OTC.

Likewise, all spines studied showed an OTC mark, indicating that the dosage of 70 mg/kg (McFarlene and Beamish, 1987) used is harmless for immature albacore and appropriate for these kind of growth studies.

It is known that the OTC attaches to the calcium of collagen fibers of bone matrix within a month (Manuel de Science Halieutique, FAO No 115). According to this knowledge, it was noticed that the development of a new annulus was associated with the OTC mark deposition in fish at liberty between 11 and 27 months (Table 4). It can be concluded that in 12 (60%) cases OTC was identified just before an annuli and in 6 (40%) cases OTC was placed on an annulus. Therefore, we concluded that sometime between July and October there is an annulus formed.

The above remark is also supported by the absence of an opaque growth zone to the edge of the spine in 7 fish. Actually, we detected the formation of an annulus right on the edge of the spine section in 41% of the total fish recaptured between 25 July and 20 September of the following year. However, we observed an increment on the border in all fish recaptured between 11 October and 26 November.

The count of the translucent and opaque zones identified after the OTC marking shows that albacore examined do not follow a single annual growth pattern. Mostly, two annuli were identified in one year after release, but also one and three annuli were developed in some fish.

Variability in the number of translucent and opaque zones is present in the studied specimen. As well, variance observed in the growth rate could support the great individual variability of albacore. The albacore recaptured after two years (NO-5946) seems to have followed a pattern of one annulus per year after the OTC mark and an opaque zone is found on the edge explaining the growth up to November, when it was taken. Three different recaptured fish being at liberty one year are shown in photographs (Figures 4, 5 and 6).

We could not achieve satisfactory explanations for the overall number of annuli formed in a year after OTC marking. Therefore, we considered that our findings could suggest different growth and migration patterns in the individual examined. The heterogeneity of north Atlantic stock exploited by surface fisheries has been previously documented (Hue, 1979, 1980; Aloncle et Delaporte, 1979).

ACKNOWLEDGEMENTS

We appreciate the great cooperation of fishermen from surface fleets who promptly reported the recaptured whole fish and relevant information. We should also like to extend our thanks to the staff of IFREMER who kindly collaborated by sending the recaptures obtained by French surface fleets.

Table 4. Estimated number of annuli formed after OTC mark was formed and growth zone to edge of spine. section from fish being at liberty for 1 to 27 months.

TAG CODE	REC. DATE	F.L. (cm)	Days at Liberty	Month	OTC * Mark	No. Annuli after OTC	Increment ** to Edge
NO-5482	10.11.91	66	58	2	ON	1	Y
NO-5506	11.07.91	67	84	3	A	0	Y
NO-5708	10.22.91	65	67	2	A	0	Y
NO-5723	09.15.91	66	30	1	A	0	Y
NO-6242	07.30.92	63	341	11	JB	2	Y
NO-6248	07.25.92	71	336	11	JB	2	Y
NO-6444	07.30.92	82	339	11	ON	1	N
NO-5816	07.27.92	74	345	11	ON	2	N
NO-7292	07.28.92	74	336	11	ON	1	Y
NO-5990	08.02.92	69	348	12	ON	2	N
NO-7378	08.07.92	65	345	12	JB	2	Y
NO-5785	08.18.92	75	368	12	JB	2	N
NO-5889	08.18.92	76	365	12	JB	2	Y
NO-5853	08.23.92	76	371	12	JB	3	Y
NO-6345	08.02.92	77	343	12	ON	2	Y
NO-7243	08.27.92	81	367	12	JB	2	N
NO-5713	09.07.92	72	389	13	JB	3	N
NO-5532	09.20.92	79	405	13	JB	3	Y
NO-6080	09.06.92	83	383	13	JB	3	Y
NO-5671	09.10.92	77	392	13	JB	3	N
NO-6347	09.13.92	80	385	13	ON	2	Y
NO-5946	11.26.93	91	829	27	JB	3	Y

* ON coincident with annulus
 JB just before annulus
 A after annulus

** Y YES
 N NO

Table 5. Comparison of mean growth observed in the two groups of fish analyzed being at liberty > 11 months.

Size group F.L. (cm)	Mean	SD	SE	No
57-63	12.2012	6.2294	2.3545	7
64-69	10.6761	2.3146	0.6186	14

Table 1. Recoveries of albacore released with OCT mark in summer of 1991.
August 1991

No. released	1,494
No. recaptured	71
Percents of recaptured	4.7
No. spines collected	29
Percents spines with OCT mark	100

Table 2. Albacore recaptured and spine samples collected at liberty less than 3 months.

RELEASES				RECOVERIES		
TAG CODE	DATE	F.L. (cm)	LOCATION	DATE	F.L. (cm)	LOCATION
* NO-6249	08.24.91	54	44 59°N/3 52°W	10.05.91	54	44 30°N/7 30°W
NO-5482	08.13.91	66	44 46°N/2 54°W	10.11.91	66	45 30°N/4 50°W
NO-5506	08.13.91	67	44 47°N/2 54°W	11.07.91	67	44 10°N/3 04°W
NO-5708	08.15.91	65	45 02°N/3 29°W	10.22.91	65	45 40°N/3 35°W
NO-5723	08.15.91	66	45 49°N/3 17°W	09.15.91	66	45 10°N/4 25°W
* NO-5915	08.20.91	57	44 50°N/3 46°W	10.06.91	57	44 03°N/3 20°W
* NO-5977	08.20.91	58	44 52°N/3 46°W	09.19.91	58	44 20°N/3 40°W
* NO-6386	08.25.91	67	45 18°N/3 45°W	09.08.91	67	45 10°N/4 25°W

Table 3. Albacore recaptured and spine samples collected at liberty over 3 months.

RELEASES				RECOVERIES		
TAG CODE	DATE	F.L. (cm)	LOCATION	DATE	F.L. (cm)	LOCATION
NO-6248	08.24.91	58	44 59°N/3 52°W	07.25.92	71	44 43°N/3 36°W
NO-7292	08.27.91	66	44 56°N/3 40°W	07.28.92	74	44 30°N/2 41°W
NO-5816	08.17.91	66	45 00°N/3 45°W	07.27.92	74	44 20°N/2 55°W
NO-6242	08.24.91	57	45 59°N/3 52°W	07.30.92	63	44 51°N/3 13°W
NO-6444	08.26.91	60	45 09°N/3 40°W	07.30.92	82	44 40°N/3 07°W
NO-5990	08.20.91	58	44 53°N/3 45°W	08.02.92	69	43 47°N/3 14°W
NO-6345	08.25.91	68	45 16°N/3 51°W	08.02.92	77	44 57°N/2 59°W
NO-7378	08.28.91	60	44 19°N/3 07°W	08.07.92	65	44 10°N/3 22°W
* NO-6260	08.25.91	67	45 09°N/3 52°W	08.14.92	73	43 50°N/2 50°W
NO-5889	08.19.91	65	44 36°N/3 22°W	08.18.92	76	44 53°N/4 25°W
* NO-7290	08.27.91	68	44 56°N/3 40°W	08.05.92	77	45 04°N/3 19°W
NO-5785	08.16.91	64	44 30°N/3 12°W	08.18.92	75	44 30°N/3 06°W
NO-5853	08.18.91	65	44 53°N/4 19°W	08.23.92	76	44 02°N/5 04°W
NO-5713	08.15.91	62	45 02°N/3 29°W	09.07.92	72	45 20°N/3 15°W
NO-5532	08.12.91	64	44 32°N/3 24°W	09.20.92	79	45 26°N/3 21°W
NO-5671	08.15.91	67	45 06°N/3 57°W	09.10.92	77	45 44°N/3 55°W
NO-6347	08.25.91	68	45 16°N/3 51°W	09.13.92	80	43 44°N/3 59°W
NO-6080	08.20.91	67	44 53°N/3 45°W	09.06.92	83	43 50°N/3 20°W
* NO-5834	08.18.91	66	44 59°N/3 46°W	08.20.92	79	43 50°N/3 00°W
NO-7243	08.26.91	69	45 01°N/3 41°W	08.27.92	81	43 42°N/4 32°W
NO-5946	08.20.91	57	44 52°N/3 46°W	11.26.93	91	35 31°N/9 20°W

* Spines which induced fluorescence vanished after being examined with UV several times.

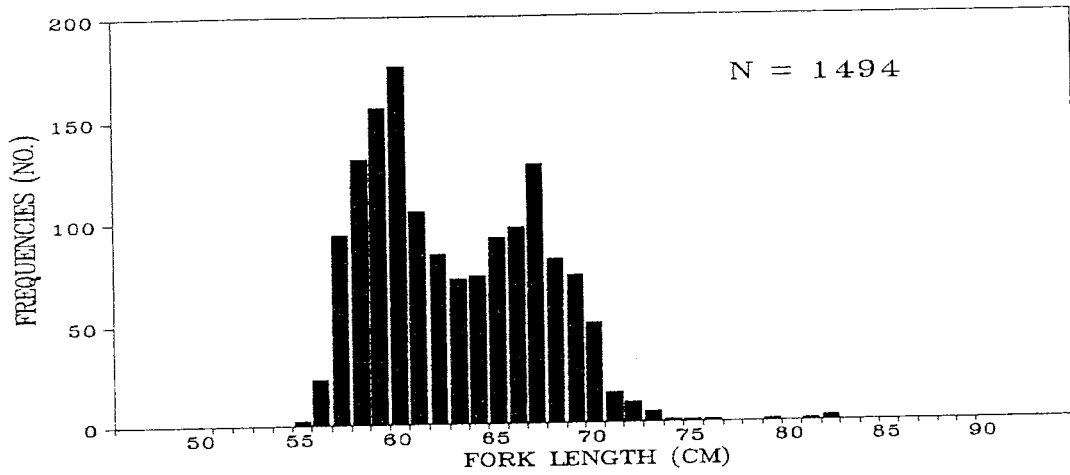


Fig. 1 Length distribution of tagged and OTC injected albacore released during 1991 cruise.

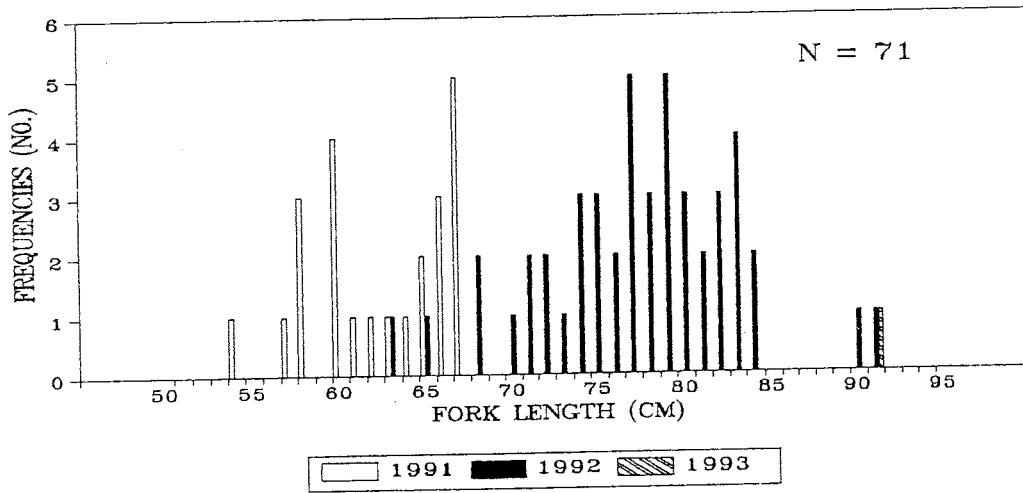


Fig. 2 Length distribution of OTC injected albacore recaptured during fishing season in 1991, 1992 and 1993.

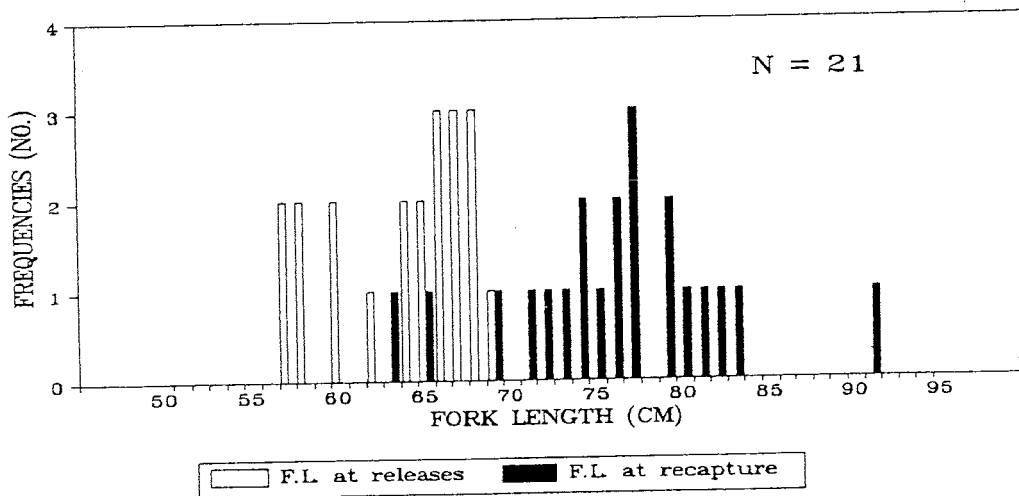


Fig. 3 Size increment of recaptured albacore injected with OTC and spine removed at liberty over 1 year.

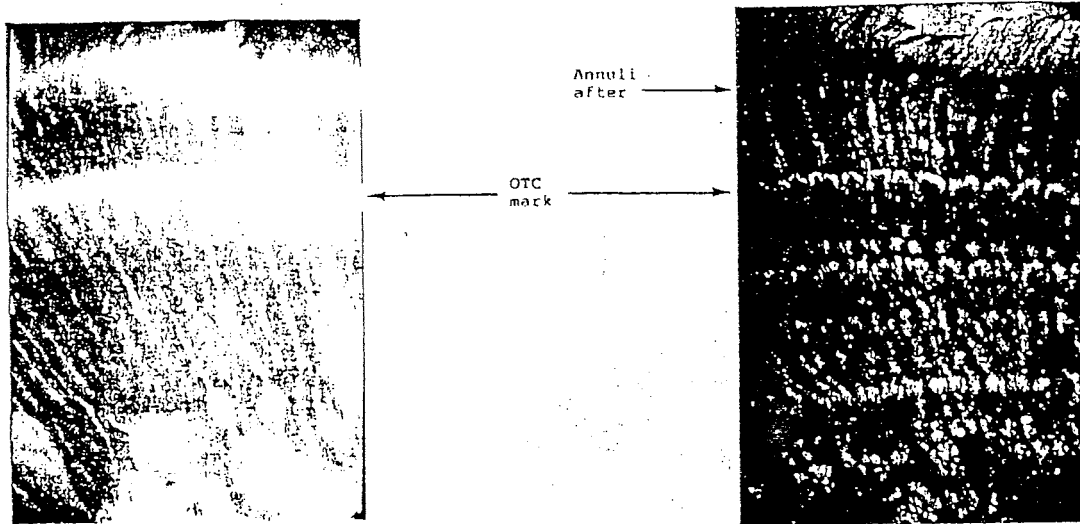


Fig. 4 Cross section of dorsal fin ray from albacore number NO-6444 photographed using reflected UV light and regular transmitted light. Recaptured in July 30, 1992 after 11 months at liberty. F.L. = 82 cm. OTC mark on an annulus and one annulus after OTC developed in the edge are indicated.

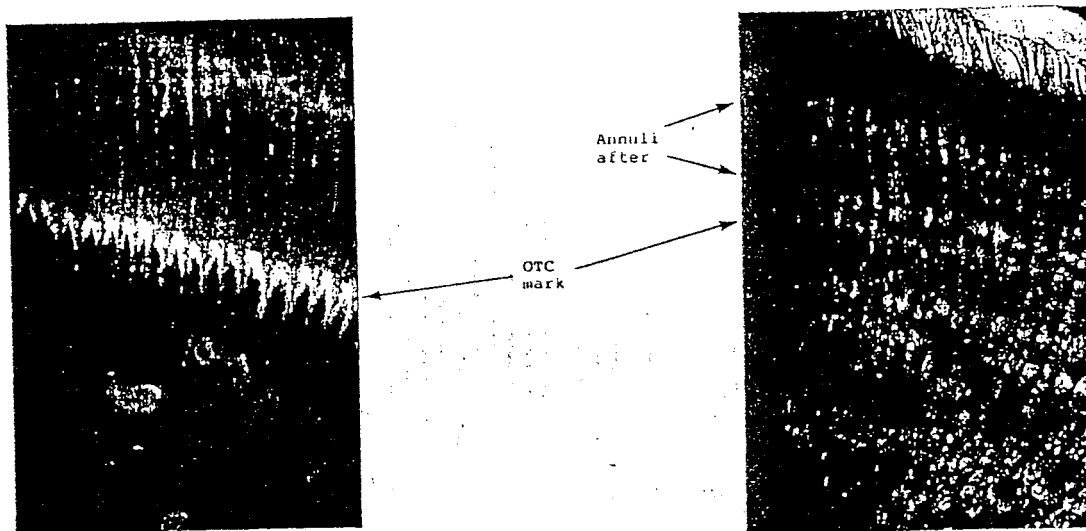


Fig. 5 Cross section of dorsal fin ray from albacore number NO-6347 photographed using reflected UV light and regular transmitted light. Recaptured in September 13, 1992 after 13 months at liberty. F.L. = 80 cm. OTC mark on an annulus and two annuli formed after OTC are indicated. Spine showing growth zone in the edge.

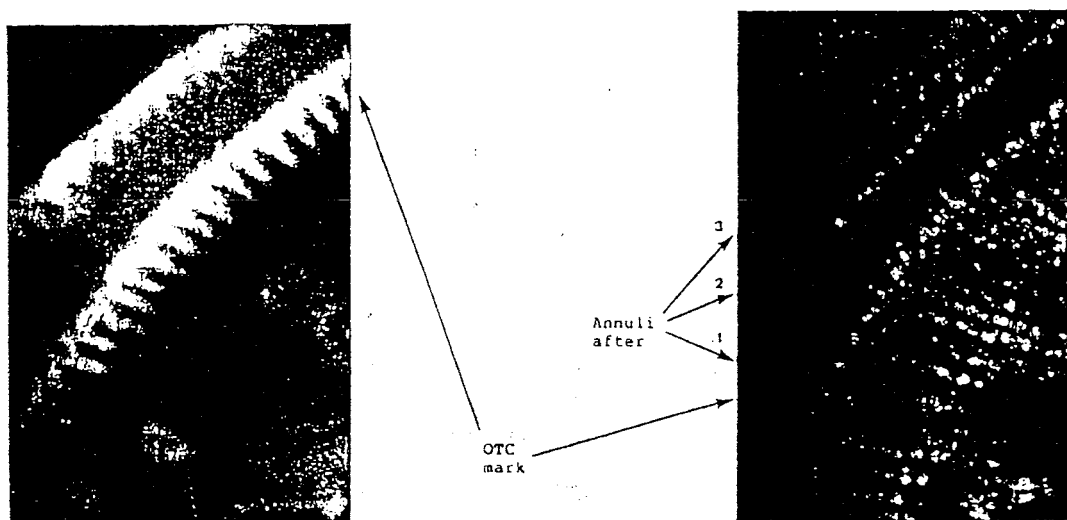


Fig. 6 Cross section of dorsal fin ray from albacore number NO-5853 photographed using reflected UV light and regular transmitted light. Recaptured in August 23, 1992 after 12 months at liberty. F.L. = 76 cm. OTC mark just before an annulus and three annuli formed after OTC are indicated. Spine showing growth zone in the edge.