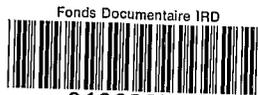


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SUMMARY PAPER

# Progress of Age and Growth Assessment of Atlantic Skipjack Tuna, *Euthynnus pelamis*, from Dorsal Fin Spines<sup>1</sup>

LOIC M. ANTOINE,<sup>2</sup> JEREMY J. MENDOZA,<sup>2</sup> and PATRICE M. CAYRÉ<sup>3</sup>



## ABSTRACT

The present study is a part of an ongoing international research program on Atlantic skipjack tuna, *Euthynnus pelamis*, coordinated by the International Commission for the Conservation of Atlantic Tunas (ICCAT). Methodology was developed for estimating age and growth rate based on counts of growth bands on sections of dorsal fin spines from 78 skipjack tuna.

The precision of counts of growth bands between eight different readers is assessed and the difficulties encountered in developing methodology and differences between readers were identified. A preliminary estimate of growth rate is presented based on samples from three origins. Estimates of age based on counts of growth bands on spines remain unvalidated, particularly the assumption of two bands per year we used for interpretation. However, ongoing studies using tetracycline as an internal tag to determine the periodicity of growth marks indicate this substance is deposited on spines, but longer times at liberty (> 1 yr) will be necessary for more definitive results.

## RÉSUMÉ

Cette étude fait partie d'un programme international de recherche sur le listao de l'Atlantique, *Euthynnus pelamis*, coordonné par la Commission Internationale pour la Conservation des Thonidés de l'Atlantique (CICTA). Une méthodologie est proposée pour estimer l'âge et le taux de croissance; elle est fondée sur l'étude des bandes de croissance lues sur des coupes de rayons de la nageoire dorsale chez 78 individus.

La précision relative de lecture a été étudiée chez huit expérimentateurs; les difficultés pour mettre au point cette méthodologie, ainsi que les différences entre expérimentateurs ont été abordées. Une estimation préliminaire du taux de croissance sur trois échantillons d'origines différentes est présentée. L'estimation de l'âge à partir des bandes de croissance sur les coupes d'épines nécessitent une validation, et particulièrement l'hypothèse faite sur la formation de deux bandes par an. Cependant les études en cours au moyen de tétracycline comme marqueur interne montrent que cette substance est déposée dans l'os des épines et peut aider à déterminer la périodicité des marques de croissance, mais il faudrait des temps de liberté plus longs (un an ou plus) que ceux observés à présent pour obtenir des résultats consistants.

## INTRODUCTION

Different approaches have been taken for estimating the age and growth rate of skipjack tuna, *Euthynnus pelamis*. A synopsis of past work is presented by Josse et al. (1979) and includes a review of length frequency analysis, modal progressions, mark and recapture studies, and counting growth bands on hardparts (i.e., vertebrae, otoliths, and dorsal fin spines). The rates of growth reported by different authors were quite variable and in some cases differences between studies were as much as two- or three-fold. These differences may be partially attributed to the diversity of methods and origins of samples.

The International Commission for the Conservation of Atlantic Tunas (ICCAT) is responsible for making management

recommendations for Atlantic scombrids and implemented the International Skipjack Year Program (ISYP) in 1981. Part of this research effort, with emphasis on skipjack tuna recommended by the ICCAT working group, included age and growth rate assessment of skipjack tuna in the eastern Atlantic Ocean. The objectives of this study were to develop a technique for estimating age and growth rate of skipjack tuna based on counts of growth bands on spine sections and to assess the precision of these counts by different readers. We chose the dorsal fin spine as a source of age and growth information because of the ease and utility of this structure reported by Shabotinet (1968), Batts (1972), and Cayré (1979) for estimating age and growth rate of skipjack tuna.

## METHODS AND MATERIALS

Our approach to age and growth assessment of skipjack tuna was developed during a series of meetings of the ICCAT skipjack working group (four scientists) held in Brest, France, and Dakar, Senegal, during 1980 and 1981. Specimens used for this

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<sup>1</sup>This research is part of the International Skipjack Year program coordinated by the International Commission for the Conservation of Atlantic Tunas.

<sup>2</sup>Centre Oceanologique de Bretagne, B.P. 337-29273, Brest Cedex, France.

<sup>3</sup>Centre de Recherche Oceanographique, B.P. 2241, Dakar, Senegal.

analysis were obtained by sampling purse seine and bait-boat landings during 1980 in Senegal, Ivory Coast, and Venezuela.

The first dorsal spine was extracted from each specimen and the fork length (cm FL), total weight (g), date of capture, and location were recorded. A series of three sections (500-700  $\mu$ m thick) were cut from the spines above the condyle base (3-5 mm according to length of individual fish), using an Isomet<sup>4</sup> low-speed saw.

Spine sections were mounted in a drop of 90% alcohol and viewed under a projector with transmitted light or with a binocular lens microscope using incidental light and a dark background. Sections were roughly cone-shaped and examinations were restricted to the distal surface of each section (side farthest to the condyle base). Sections of the second dorsal fin spine were also examined (when available) to aid interpretation. Translucent growth zones (see Glossary) appeared clear in transmitted light and dark in incidental light, whereas opaque growth zones were dark in transmitted light and light in incidental light. X-ray microradiographs done on several spine sections indicated that translucent bands represented zones of higher calcium concentration, which have been reported to represent areas of inhibited (slow) growth (Castanet et al. 1977; Compeán-Jimenez and Bard 1980). A series of photographs of spine sections were compiled and distributed to eight readers for counting, measuring, and interpreting growth bands.

We use the term "ring" to refer to translucent zones which were counted on each specimen. A code was defined to enable readers to standardize their interpretations. Previous reports indicate that rings on spines of Pacific and Atlantic tunas are often present in groups of two or more, which may represent annual cycles (Chi and Yang 1973; Compeán-Jimenez and Bard 1980; Cayré and Diouf 1981). Our observations also suggest this hypothesis for skipjack tuna and thus we have adapted this assumption for interpreting groups of rings to estimate age. Therefore, each group of rings we identified was assumed to represent 1 yr of growth. Owing to the sparse knowledge of the biology, life history, and behavior of skipjack tuna in different geographical areas, it was not possible to recognize rings as "accidental," "spawning checks," or attributable to other biological or environmental events.

Our code for rings, used by seven out of eight readers (reader 4 was unaware of the existence of this code), was as follows:

- A = ring
- AR = ring present in vascularized core
- AF = blurry ring; not well marked; limits slightly marked
- AE = narrow ring
- AL = large ring
- Ai = incomplete ring
- Ad = ring partially split along the longitudinal axis
- A<sup>t</sup> = ring particularly well marked.

The reader described each section by this code and then indicated the ring counts or groups that he used to assign an age to each sample. An example of our interpretation follows:

<sup>4</sup>Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

$$\begin{array}{l} \text{Code and ring number} \\ \text{Estimated age} \end{array} \quad \frac{AR}{1} + \frac{AE + AL}{2} + \frac{A^t + AF}{3} \\ + \frac{A + A}{4} + \frac{A}{+}$$

This example represents a total of eight rings with an estimated age of 4+ yr.

Measurements were taken with a profile projector fit with a stage coupled with a micrometer and a binocular lens microscope fit with an ocular micrometer. Measurements taken on spine cross sections included: 1) Spine diameter ( $d$ )—the distance between the outside margins of the spine above the notch in the posterior face through the approximate center of the spine (Fig. 1), 2) radius of growth band ( $r$ )—the distance from the estimated center of the spine to the outside margin of each growth increment, and 3) diameter of growth band ( $d^1$ )—the distance from the outside spine margin through the spine center to the outside margin of each growth band (Fig. 1).

When using a profile projector, a line was drawn through the center axis ( $a^1$  to  $a^2$ ), bisecting the spine in the mid-sagittal plane (Fig. 1). The location where this line ( $a^1$  to  $a^2$ ) intersects the spine diameter ( $d$ ) was the estimated center of the spine.

Depending on the measurement, the radius of each growth band was given by the value of  $r$  or ( $d^1 - d/2$ ). A  $t$ -test of the mean values of the difference between  $r$  and ( $d^1 - d/2$ ) for 30

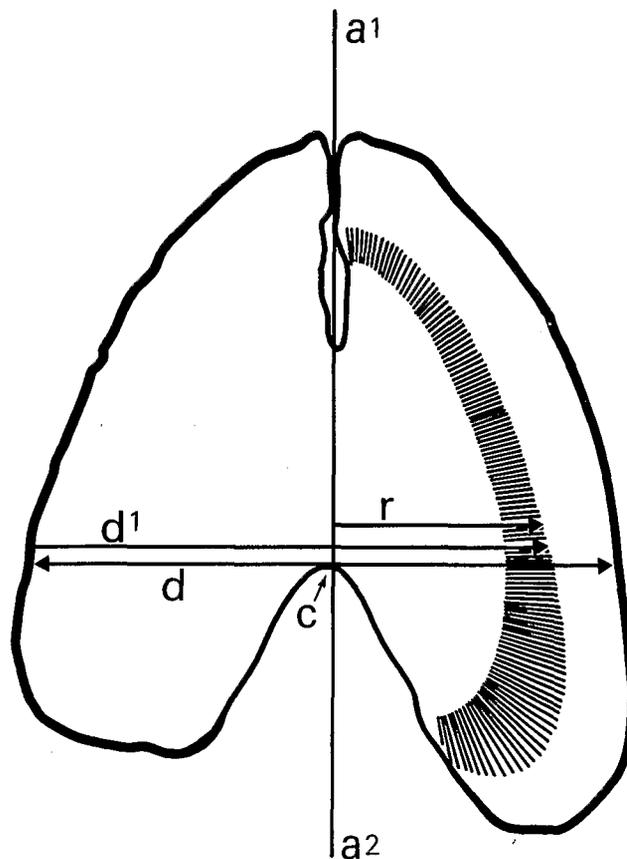


Figure 1.—Cross section of dorsal fin spine of skipjack tuna.  $a^1$ - $a^2$  = sagittal plane;  $c$  = estimated center of the spine;  $d$  = spine diameter;  $d^1$  = growth diameter;  $r$  = growth ring radius.

different section readings did not show a significant difference ( $\alpha = 0.05$ ) between the two methods; therefore, observations from both were pooled for ageing analysis.

In skipjack tuna  $\geq 50$  cm FL, the first several rings were often obscured (masked) due to enlargement of the vascular core. We attempted to resolve the problem following the general methods outlined by Cayré and Diouf (1983), Berkeley and Houde (1983) and Gonzales-Garces and Fariña-Perez (1983). This approach entails calculating the average number and location of the first several bands observed in very young fish to correct for obscured bands in larger (older) individuals.

In order to compare interpretations of different readers, photographs of 78 dorsal spine sections were sent to eight readers. The readers did not have the characteristics of the fish (length, origin), in order to avoid biasing the readings. The photographic magnification of all prints was the same. Readers 1, 2, 3, 5, and 7 participated in developing the reading code and applied it, readers 6 and 8 applied the code without having participated in its development, and reader 4 did not apply the method code for age estimation but rather counted his interpretation of annual bands to assign an age. The 78 samples were deliberately chosen from fishes coming from different origins (Caribbean, central Atlantic, Gulf of Guinea), and for this reason we will not try to interpret results from the point of view of skipjack tuna growth since the major objective of this experiment was to determine the level of agreement between readers.

A mean age was initially calculated for the spine sections read by each reader. Variances between readers were tested for homogeneity and were found to be significantly different ( $F$  max test;  $\alpha \leq 0.05$ ). Therefore, statistical comparisons between readers was accomplished by establishing an age-length relationship for each reader's data set. We chose to represent length as a function of age by a least squares linear model and this yielded predictive regression lines for each reader (an example is given in Fig. 2). Because residual variances of the different regressions were not homogenous, variance analysis was not used to compare the regression lines. An alternative approach using the joint confidence region for a given probability level for both slope and elevation of the regression lines was adopted (Draper and Smith 1966). This region takes the shape of an elongated ellipse. Differences between paired estimates

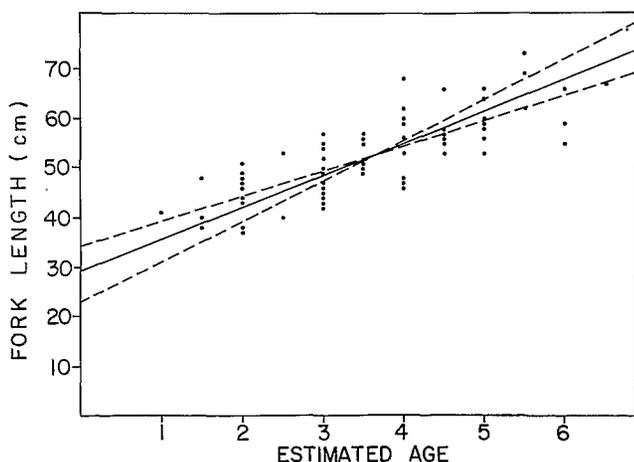


Figure 2.—Example of fork length vs. estimated age regression obtained for reader 5. Solid line is the functional regression, dashed lines are the predictive regressions.

for elevation and slope between readers were declared significant when the ellipses did not intersect. Details of the method are given by Conan (1978). All statistical inferences were made with a significance level of  $\alpha = 0.05$ .

Two different methods (back calculation and age-length relationships) were used to study growth. The estimated length at different ring formation based on spine measurements was determined by back calculation. This method increases the number of observations but may be biased from the dependence of the different age-length estimates and from the correction of obscured rings in larger fish.

For growth estimated by back calculation, the predictive regressions obtained for each sample were used in calculations. The formula used in back calculations follows Lee (1920):

$$FL_i = a + (FL - a) \frac{A_i}{A} \quad (1)$$

where  $FL_i$  = fork length at time  $i$   
 $FL$  = observed fork length  
 $a$  = bias adjustment parameter  
 $A_i$  = radius of ring  
 $A$  = radius of section.

We also examined growth by observing estimated age-length relationships; this method tends to lend itself better for adjustment to mathematical models.

## RESULTS AND DISCUSSION

From a total of 78 photographs of spine sections, 17 (21.8%) were considered unreadable by at least one person. Only one specimen (1.3%) produced total agreement among all readers, and two others produced agreement when interpreted to within  $\pm 0.5$  yr (assuming two rings per year, 0.5 yr is represented by one isolated ring). This represents a total agreement within  $\pm 0.5$  yr of 3.8%. It is noteworthy to mention that a similar comparison on cod otolith readings showed 39% agreement between 10 readers (Lopez-Veiga et al. 1977). Berkeley and Houde (1983) found that only 13% of swordfish, *Xiphias gladius*, spines were unreadable. Therefore, it appears that agreement between readers was unusually low in our study, and unreadable spines are relatively numerous compared with what we had expected and as indicated in other reports.

Comparisons between different pairs of readers (Table 1) indicated  $< 40\%$  agreement, except for readers 2 and 4 (56%) and readers 7 and 8 (73%). Lowest values were between read-

Table 1.—Percent agreement between pairs of readers for counts of rings on cross sections of 78 skipjack tuna spines captured off Venezuela, Senegal, and Ivory Coast, 1980-81.

Reader	Agreement between pairs of readers (%)							
	1	2	3	4	5	6	7	8
1		31	38	31	30	23	13	14
2	31		25	56	31	9	13	10
3	38	25		20	39	24	38	30
4	31	56	20		24	8	16	13
5	30	31	39	24		14	23	26
6	23	9	24	8	14		21	21
7	13	13	38	16	23	21		73
8	14	10	30	13	26	21	73	

ers 4 and 6 (8%) and 2 and 6 (9%). Close agreement between readers 7 and 8 could be related to their close geographical proximity, which gave them an opportunity to work together longer during the development of the methodology in their laboratory. In addition, these readers did not attempt to age within  $\pm 0.5$  yr. However, readers 2 and 4 also achieved a comparatively high level of agreement even though they used different methods and did not work together.

When comparisons between pairs of readers were tabulated for counts to within  $\pm 0.5$  yr, a much higher rate of agreement was observed (Table 2). Sixteen pairs of readers had an agreement rate that exceeded 50% and 13 pairs of readers exceeded 60% agreement. The low rates of agreement may be related to reading closely spaced rings near the outer margin of the sections. This has also been shown to be a problem in reading bluefin tuna, *Thunnus thynnus*, vertebrae (Lee et al. 1983).

The mean bias shown in Tables 3 and 4 is a comparative index defined as the sum of overestimated and underestimated

age and illustrates the tendency of a reader to count rings in relation to the entire set of readings. Thus, readers 2 and 4 clearly tend to underestimate age compared with the other readers (indicated by a minus sign), while readers 7 and 8 clearly overestimate age. Readers 3, 5, and 6 slightly overestimated age and reader 1 slightly underestimated age.

The coded interpretation from each reader indicated that, except in several particularly easy cases with well-marked rings, there was considerable variation in the counts and measurements of rings by individual readers. We felt these discrepancies were due, in part, to differences in the individual reader's ability to recognize groups of rings.

Figure 3 shows that two groups of readers may be clearly distinguished by non-overlapping ellipses (i.e., these groups were significantly different from each other): 1) readers 3, 5, 6, 7, and 8, 2) readers 2 and 4. Reader 1 occupies an intermediate position between these groups. Readers 3, 5, and 6, and readers 7 and 8 may also be grouped (quasi-concentric ellipses). Parameters of the functional and predictive regressions of these analyses are given in Table 5.

The determination of age in skipjack tuna by the use of dorsal fin spines remains difficult. Even when a common methodology is used, interpretations show important divergences.

**Table 2.**—Percent agreement between pairs of readers within  $\pm 1$  ring for counts on cross sections of 78 skipjack tuna spines from Venezuela, Senegal, and Ivory Coast, 1980-81.

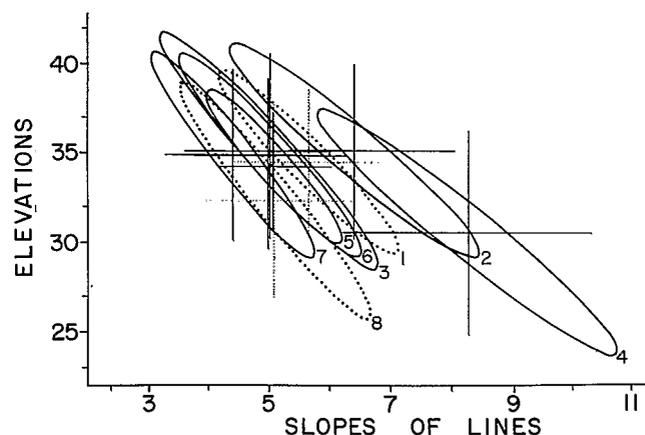
Reader	Agreement between pairs of readers (%)							
	1	2	3	4	5	6	7	8
1		67	66	62	61	50	40	46
2	67		48	72	41	54	25	22
3	66	48		46	63	65	67	61
4	62	72	46		35	50	26	24
5	61	41	63	35		60	45	48
6	50	54	65	50	60		66	61
7	40	25	67	26	45	66		73
8	46	22	61	24	48	61	73	

**Table 3.**—Bias between percent pairs of readers within  $\pm 0.5$  yr. Bias is measured as % overestimated average age, - % underestimated average age. These values are only relative in the comparative sense since absolute age is not known.

Reader	Agreement between pairs of readers (%)								Mean bias
	1	2	3	4	5	6	7	8	
1		49	-26	47	-24	-23	-42	-64	-12
2	-49		-68	5	-61	-63	-64	-90	-56
3	26	68		70	-2	2	-42	-46	11
4	-47	-5	-70		-57	-41	-42	-87	-50
5	24	61	2	57		5	-29	-27	14
6	23	63	-2	41	-5		-45	-42	5
7	42	64	42	42	29	45		1	38
8	64	90	46	87	27	42	-1		50

**Table 4.**—Bias between percent pairs of readers within  $\pm 1$  yr. Bias is measured as % overestimated average age, - % underestimated average age. These values are only relative in the comparative sense since absolute age is not known.

Reader	Agreement between pairs of readers (%)								Mean bias
	1	2	3	4	5	6	7	8	
1		31	-20	36	-23	-16	-54	-44	-13
2	-31		-51	9	-57	-44	-76	-79	-47
3	20	51		54	0	-13	-27	-29	8
4	-36	-9	-54		-58	-50	-74	-76	-51
5	23	57	0	58		-3	-31	-29	11
6	16	44	13	50	3		-22	-28	11
7	54	76	27	74	31	22		1	41
8	44	79	29	76	29	28	-1		41



**Figure 3.**—Ellipses of joint confidence limits for slope and evaluation for the relationship between fork length and estimated age (see details in text) for 8 readers. Readers grouped together are: 3, 5, 6, 7 and 8; 2 and 4. Reader 1 is transitional between the groups. Solid and dotted vertical and horizontal axis for each ellipse denote the elevation ( $y$  intercept) and slope, respectively.

**Table 5.**—Parameters of functional and predictive regressions  $FL = a + b$  (age) for each reader where  $a$  = intercept,  $b$  = slope,  $r$  = coefficient of correlation,  $N$  = number of individuals.

Reader	Functional regression		Predictive regression		$r$	$N$
	$a$	$b$	$a$	$b$		
1	27.57	7.77	34.51	5.65	0.727	78
2	25.72	9.78	35.14	6.40	0.654	77
3	24.06	8.12	35.12	5.01	0.616	75
4	21.98	1.47	30.55	8.27	0.721	61
5	29.23	6.47	34.25	5.09	0.787	76
6	26.34	7.39	34.86	5.03	0.680	78
7	26.14	6.54	34.90	4.41	0.674	78
8	22.19	7.60	32.32	5.10	0.671	78

Differences arise from the number of rings seen and coded and from the way in which these are grouped. The absence and/or the blurry nature of rings in the altered central zone most likely increases the bias in readings, especially when the fish are more than 50 cm FL. Finally, the nature of the edge of the sections is difficult to interpret. Nevertheless, the use of a common methodology allows comparisons of precision between readers. When possible, samples should be read by several investigators before drawing any conclusion on skipjack tuna age and growth. Although we considered the precision of our age estimates, accuracy of these estimates (see Glossary) was not addressed.

Our results show that there was a comparatively high level of agreement between readers 1, 3, and 5 (Tables 1-5). Each of these readers examined samples from landings at Cumana, Venezuela ( $N = 150$ ), from Dakar, Senegal ( $N = 49$ ), and from Abidjan, Ivory Coast ( $N = 99$ ), and regression lines were adjusted to estimates of age at length (Fig. 4). The comparison between regression lines from the three areas was done by means of ellipses of joint confidence limits because the residual variances between areas were not homogenous ( $F$  max test significant;  $\alpha = 0.05$ ). Figure 4 indicates that samples from these three areas could not be statistically distinguished from each other.

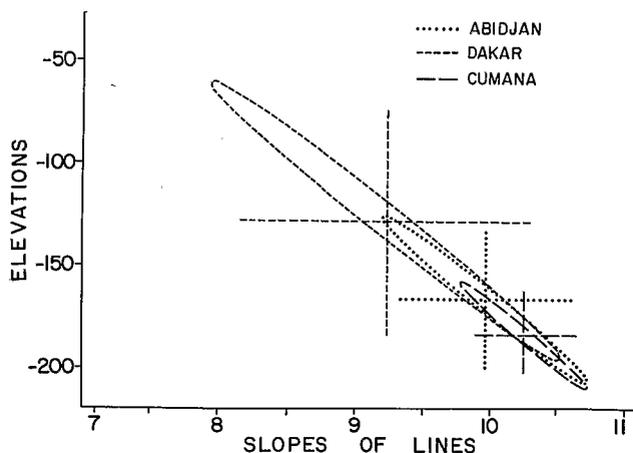


Figure 4.—Ellipses of joint confidence limits for slope and elevation of ring radius vs. fork length regressions for three samples from Ivory Coast (dotted line), Senegal (small dashed line), and Venezuela (large dashed line). Vertical and horizontal axes for each ellipse denote the elevation ( $y$  intercept) and slope, respectively.

We found that rings within the central altered zone of sections, especially for fish with fork lengths  $> 50$  cm, were often obscured. The measurements of growth rings (Fig. 5) from each fish identifies (on the average) the location of the first three rings (800, 1,000, and 1,300  $\mu\text{m}$ , respectively). These data were used to estimate rings obscured in fish larger than 50 cm FL due to enlargement of the core.

The significant relationship between the diameter of the dorsal fin spine section and fork length (Table 6) provides strong rationale for back calculation of length at ring formation. The fork lengths at estimated age based on back calculation and from observed data (Table 7) indicate about 4 to 5 cm FL between cohorts. There was a significant relationship between estimated age and fork length for each of the three areas (Fig.

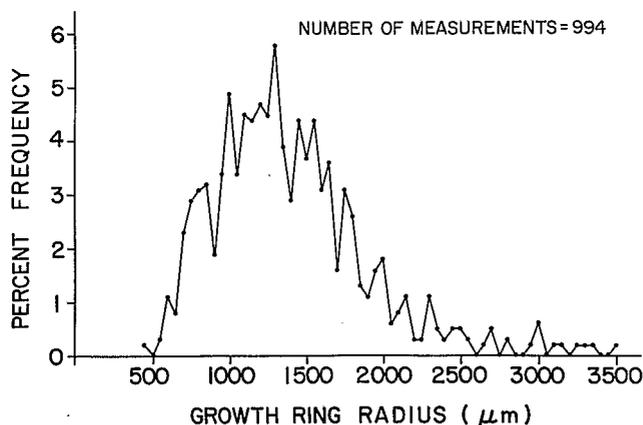


Figure 5.—Frequency of growth ring radius for 994 measurements in all skipjack tuna spine samples combined.

Table 6.—Parameters for regression analysis of the relationship between fork length and spine diameter by location.  $FL = a + bd$  where:  $a$  = elevation;  $b$  = slope;  $r$  = coefficient of correlation;  $N$  = sample size.

Location	$a$	$b$	$r$	$N$
Cumana (Venezuela)	19.6722	0.09275	0.88	150
Abidjan (Ivory Coast)	19.8645	0.09133	0.84	99
Dakar (Senegal)	19.4613	0.09216	0.88	49

Figure 7.—Fork length (cm) at estimated age obtained by back calculation and average fork length at estimated age based on spine analysis from three geographical areas. FL = fork length; SD = standard deviation.

Estimated age	Cumana (Venezuela)				Abidjan (Ivory Coast)				Dakar (Senegal)			
	Back calculation		Average fork length at estimated age		Back calculation		Average fork length at estimated age		Back calculation		Average fork length at estimated age	
	FL	SD	FL	SD	FL	SD	FL	SD	FL	SD	FL	SD
1	34.10	1.81	34.68	4.60	34.50	2.21	35.75	4.89	34.20	12.00	35.24	3.69
2	39.00	2.70	39.09	4.57	38.80	2.72	39.92	4.84	39.50	2.55	40.27	3.47
3	44.10	2.90	43.50	4.54	43.20	3.08	44.09	4.80	45.10	2.87	45.30	3.49
4	47.90	2.95	47.91	4.53	47.50	3.52	48.26	4.78	49.80	2.08	50.33	3.46
5	51.60	3.69	53.32	4.52	52.40	4.68	52.43	4.77	54.00	3.16	55.36	3.47
6	53.60	5.13	56.73	4.52	55.60	5.54	56.60	4.78	57.70	3.69	60.39	3.53
7	62.80	6.18	61.14	4.53	58.70	3.78	60.77	4.81				

6), but more detailed analyses were not justified, because the first few rings obscured by the vascularized core were all corrected from the same pooled data base (Fig. 5). Although the observed and back-calculated fork length and estimated ages were very close (Table 7), we did find slightly higher values from Dakar. Statistical comparisons of these data were not made because of the heterogeneity of sample variances. Overall, these data tend to verify that skipjack tuna from the three geographical areas were generally reacting to the same environmental stimuli.

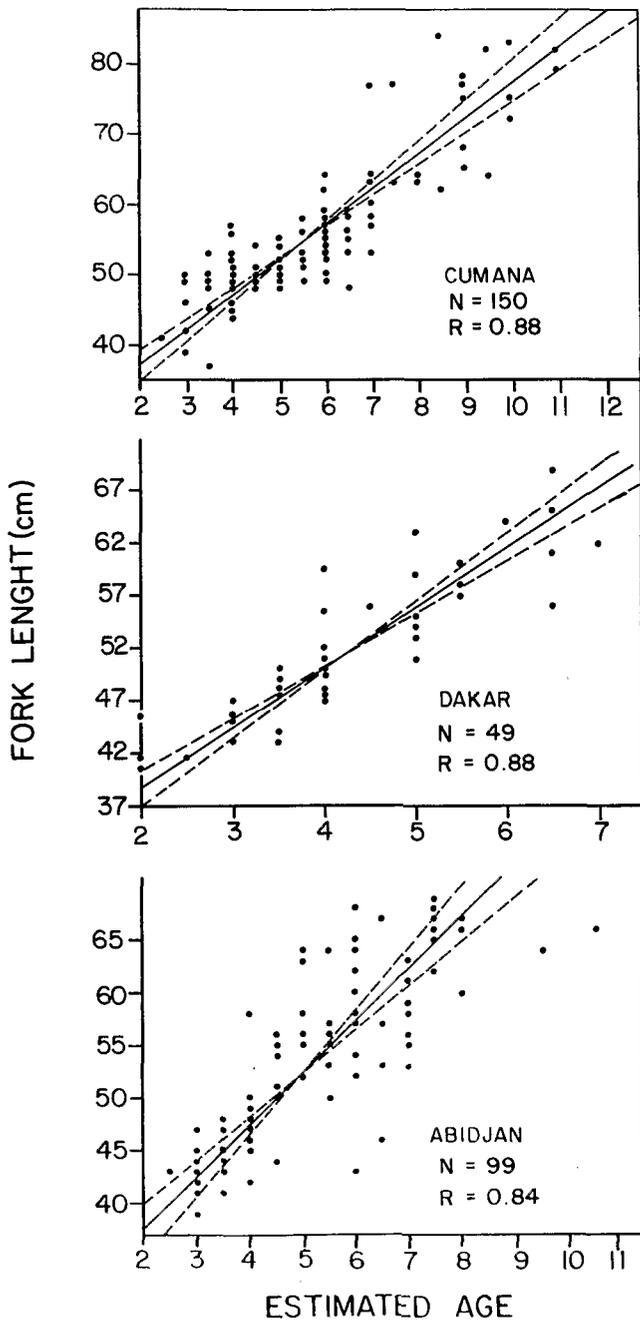


Figure 6.—The relationship between fork length (cm) and estimated age for skipjack tuna sampled at (from top to bottom) Cumana (Venezuela), Dakar (Senegal), and Abidjan (Ivory Coast). Solid lines are functional regressions and dashed lines are the predictive regressions.

We have mentioned that the hypothesis of two rings per year assumed for several other species of tuna was also assumed in this study. We attempted to substantiate this assumption by observing the nature (translucent or opaque) of the edge of skipjack tuna dorsal spine sections from fish landed at Dakar during 1980. The proportion of translucent edges was calculated per month. Figure 7 suggests that from January to June there was a long period of inhibited growth (translucent edge). From July to September, growth appeared to resume (opaque edge), and later in October a new translucent edge appeared. Finally, growth resumed in November and December. This pattern seems to suggest the formation of two rings a year. Nevertheless, several reservations include: 1) Monthly samples were small and did not take into account possible interschool differences or differential growth between sexes (Cayré 1981). 2) The interpretation of the edge of a section is difficult and is highly variable from one reader to another. 3) A period of inhibited growth from January to July seems too long to discount the possibility that several rings may form during this period.

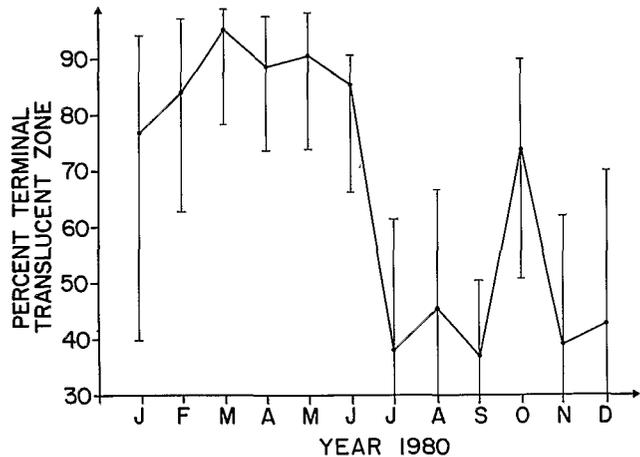


Figure 7.—Percent terminal translucent zone ( $\pm$  range) by month during 1980 for skipjack tuna caught off Dakar, Senegal.

On the basis of annual periodicity, the increments examined in this study (averaging 4 to 5 cm FL between cohorts) are generally two times less than other estimates for Atlantic skipjack tuna based on hardparts (Batts 1972; Carles Martin 1975; Cayré 1979). It seems obvious that our results must be regarded as provisional. The continued research during the ISYP skipjack tuna program should provide additional data on this topic. In particular, tetracycline marking may clarify doubts concerning the time of ring formation and related interpretation of bands on spine sections. Following methodology described by Wild and Foreman (1980), skipjack tuna have been injected with tetracycline during ISYP tagging cruises. The first returns from injected skipjack tuna show that the antibiotic is visible on dorsal spine sections under fluorescence microscopy. The present number of tetracycline marked and recaptured fish (52) and their time at liberty (maximum time: 5 mo for one individual) are not sufficiently large to permit a study of growth at this time. Only fish with at least 1 yr at liberty could validate ring periodicity for the annual cycles and

only for growth during the time each returned fish was at liberty.

In summary, readers of this study have been led to note that: 1) Inhibited growth bands are numerous and may be large, indicating frequent and/or long periods of inhibited growth. 2) Growth bands may also be narrow, indicating short periods of rapid growth. 3) Bands are frequently different from one fish to another (from the same area), which indicates a great variability of individual growth.

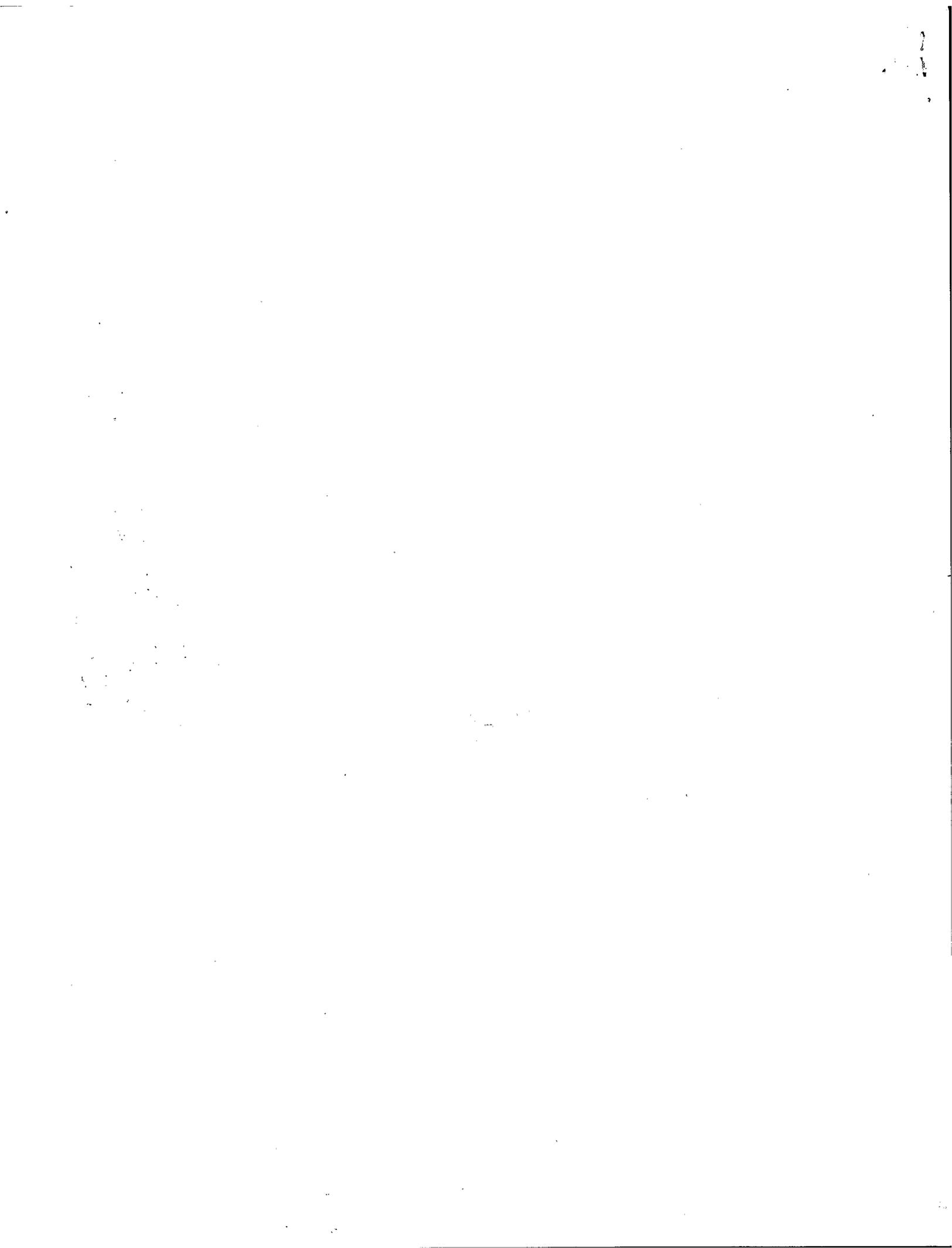
These remarks lead us to propose a relatively high growth rate for skipjack tuna which may be related to favorable local environmental conditions. This hypothesis has already been advanced based on gonad maturation studies (Cayré 1981). Although reading skipjack tuna spines to assign ages is a simple and easy method to employ for age estimation, the major difficulties we identified need to be addressed before this method is widely used.

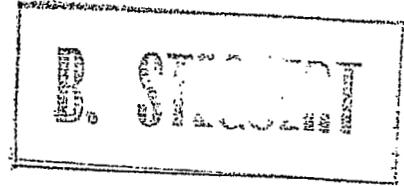
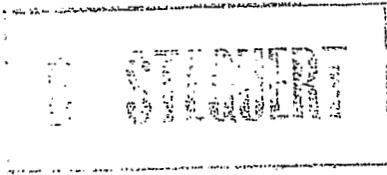
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