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Age estimation and annulus-formation in dorsal spines of juvenile bluefin tuna, *Thunnus thynnus*, from the Mediterranean Sea

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The first dorsal fin spines were used to estimate the age of juvenile bluefin tuna, *Thunnus thynnus*, from the Mediterranean Sea. Observations on the type of growth zones occurring on the outside margin of each spine section by month and marginal increment analysis indicated that only one translucent zone is formed per year between February and April, when lower sea surface temperatures are observed. Age estimations were based on counts of translucent zones on the spine sections and mean size at estimated age was calculated for the first three years of life.

There has been little work estimating the age of juvenile bluefin tuna, *Thunnus thynnus* L., from the Mediterranean using hard parts (Radtke & Morales, 1989) and size–frequency distributions (Liorzou & Bigot, 1994; Orsi-Relini et al., 1997). In this study, fin were chosen spines as source of age information since they are easy to sample and well-defined growth marks are evident on them. The goals were: (a) to validate the use of spines to age juvenile bluefin tuna by marginal increment analysis; and (b) to estimate the age of juvenile bluefin tuna sampled from the Mediterranean Sea.

Bluefin tuna specimens were obtained during 1992–1998 from catches by Greek and Italian fishing fleets operating in the Aegean, Adriatic and Ionian Seas in troll line, albacore long line and clupeoids purse seine fisheries. Samples were collected over most of the year (except February and March). The first spine of the first dorsal fin was removed from each specimen and fork length (FL), round weight (RW), place and date of capture were recorded. Length measurements were taken to the nearest millimetre (mm) and weight to the nearest gram (g).

Three serial cross-sections of ~0.7 mm thick were obtained from each spine at the point near the condyle spine base using a low-speed saw. Spine sections were observed with a binocular lens microscope under transmitted light. Interpretation of growth bands was based on the recognition of narrow translucent zones and wider opaque zones, assumed to represent slow and fast growth respectively.

The first approach to validate the ageing method was based on marginal increment analysis. Observations on the type and frequency of growth zones occurring on the outside margin of each spine section per month were made (Cayré & Diouf, 1981) and mean marginal increments were calculated as a proportion of the analogous complete growth increment (Berkeley & Houde, 1983). A PC connected to the microscope by means of a video camera and equipped with image analysis software was used to perform measurements. The diameter of the spine (D), the distances from the outside edge of each translucent zone to the opposite edge of the cross-section (D_i) and the marginal increment (MI) were measured (Figure 1A). Spine radius (R) and translucent zone radii (R_i) were calculated using Cayré & Diouf (1981) method.

The number of translucent zones was counted in order to assign an estimated age to every bluefin tuna and build a sizeage key. Length–frequency distribution was used to verify the ageing results. The relationship between the spine radius

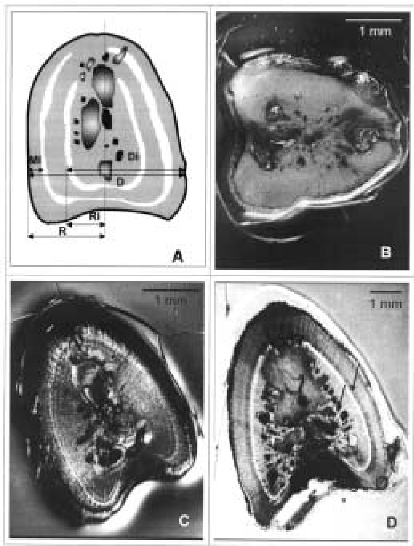

Figure 1. Images of bluefin tuna spine sections. (A) Schematic diagram showing the measurements: D, diameter of the spine; D_I, distance from the outside edge of translucent zone i to the opposite edge of the cross-section; MI, marginal increment; R, radius of the spine; and R_I, radius of the i translucent zone. (B) Spine section of a 49 cm bluefin tuna caught on the 25 January; estimated age 0^+. (C) Spine section of a 59 cm bluefin tuna caught on the 24 May; estimated age 1^+. (D) Spine section of a 87 cm bluefin tuna caught on the 12 September; estimated age 2^+. Arrows indicate the first and second translucent zone.

and the fork length was determined using regression analysis. Back-calculations of lengths at the time of translucent zones formation were obtained using the formula of Ricker (1975).

Spine sections of 303 specimens ranging in fork length from 22 to 118 cm were examined (January, N=18; April, N=10; May, N=14; June, N=1; July, N=15; August, N=4; September, N=117; October, N=64; November, N=54; December, N=6). Opaque zones appeared at the spine margins of all fish (N=271) sampled from June to January whereas translucent zones appeared at the spine margins of fish sampled during April and May in a percentage of 60 and 29% respectively. The marginal increment analysis indicated that the mean percentage marginal increments increased steadily beginning from April to January

Table 1. *Length–age key of 303 bluefin tuna sampled from the Mediterranean Sea, 1992–1997. Fork length (FL) is regrouped in classes of 5 cm.*

	Age estimations							
FL (cm)	0+	1	1+	2	2+	3	3+	Total
20	2	0	0	0	0	0	0	2
25	0	0	0	0	0	0	0	0
30	3	0	0	0	0	0	0	3
35	9	0	0	0	0	0	0	9
40	35	0	0	0	0	0	0	35
45	69	1	0	0	0	0	0	70
50	17	16	0	0	0	0	0	33
55	0	6	2	0	0	0	0	8
60	0	1	8	0	0	0	0	9
65	0	0	17	0	0	0	0	17
70	0	0	50	0	2	0	0	52
75	0	0	17	0	1	0	0	18
80	0	0	3	0	1	0	0	4
85	0	0	0	0	1	0	0	1
90	0	0	0	0	21	0	2	23
95	0	0	0	0	16	0	0	16
100	0	0	0	0	0	0	1	1
105	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0
115	0	0	0	0	0	0	2	2
120	0	0	0	0	0	0	0	0
SUM	135	24	97	0	42	0	5	303
%	44.2	8.0	32.2	0.0	14.0	0.0	1.7	100.0
Mean FL	45.3	53.6	70.8	—	92.6	—	104.4	
±SD	4.0	2.7	4.6		6.0		12.8	

(April 3.9%, May 4.3%, June 8.3%, July 28.0%, August 43.2%, September 58.1%, October 81.9%, November 87.6%, December 94.6%, January 97.8%). According to these findings it could be suggested that only one translucent zone (annulus) is formed per year in the spines of juvenile bluefin tuna. This consideration should be valid for the first year of life because the young-of-the-year specimens were sufficiently present in all monthly samples. Besides, it could be suggested that the formation of the annulus takes place between February and April and its completion occurs during April and May. In the eastern Mediterranean, the lower water temperatures (SATMER, 1994, 1995, 1996) usually occur in February, March and April (12–15°C). By May, temperatures increase and summer temperature usually peaks in August (25–26°C). Since the translucent zones are identified as slow growth zones, the incidence of their formation may be related to environmental conditions and especially to low water temperature. However, in a study for the Atlantic bluefin tuna, Cort (1991) suggested that translucent zones are formed between autumn and winter and can be single or double.

Based on the counts of the translucent zones, we estimated ages between 0+ years to 3+ years (Figure 1B–D). The correlation of numbers of translucent zones and modes from length–frequency distributions also suggests that translucent zones observed in spines are formed once a year and could be a valid indicator of age for juvenile fish. As with many rapidly growing species, distinct modes were observed in size–frequencies of juvenile bluefin tuna. Three length groups with modes at 47.5, 72.5 and 92.5 cm were well defined and the estimated age groups 0, 1 and 2 coincided satisfactorily with them (Table 1).

A significant linear relationship was found between spine radius and fork length ($FL=4.455+0.0403R$, $r^2=0.93$). Mean back-calculated lengths at estimated ages one, two and three were 54.5, 81.2 and 100.1 cm respectively. These results appear to be realistic and seem consistent with what is known about the growth of the species in the Mediterranean (Liorzou & Bigot, 1994; Orsi-Relini et al., 1997).

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