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Comparison of otolith growth and morphology with somatic growth and age in young-of-the-year bluefin tuna

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Otolith morphological characteristics were studied using image analysis techniques and the relationships between otolith growth and somatic growth and age, as estimated from counting daily otolith increments, were examined in young-of-the-year (YOY) bluefin tuna *Thunnus thynnus* ranging in fork length (L_F) from 8·5 to 55·5 cm. Whole otolith length, width, area and perimeter, and three shape indexes, circularity, *E* value and rectangularity, were extracted for each pair of sagittae. Since no statistical significant differences between left and right otolith morphometrics were found, only one otolith from each fish was used for correlations. Statistically significant relationships were observed between otoliths measurements and fish somatic growth when a linear regression was applied after logarithmic transformation of all variables tested. Among the variables, otolith length was the one that showed the highest correlation with L_F, followed by otolith area and perimeter, whereas otolith rectangularity exhibited the lowest correlation. Statistically significant relationships were also observed between the otolith variables tested and the age of the fish, which ranged from 20 to 129 days. The ages estimated using otolith mass were very close to those assessed using daily increment counts (bias ranged from 1 to 24 days). Therefore, otolith mass could represent a valuable criterion for age estimation in YOY bluefin tuna that is objective, economic and easy to perform compared to daily increment counting method.

Key words: age; bluefin tuna; otolith morphology; somatic growth.

INTRODUCTION

The estimation of age structure of a fish population is very important in fisheries management and gives information not only on the biology of the species but also on its population dynamics. Different techniques have been applied to age fishes mainly by counting growth increments from hard parts such as otoliths, fin spines, scales and vertebrae. In specimens of the 0+ year group, age estimation by counting the daily increments from whole or sectioned otoliths is the only method that gives accurate estimation of the age of individual fish in a population. Many different laboratories and authors have used this

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technique in order to provide precise and accurate estimation of fish age (Itoh & Tsuji, 1996; Szedlmayer, 1998; Itoh *et al.*, 2000; Neuman *et al.*, 2001) but as it has been pointed out by Cardinale *et al.* (2000), many disadvantages occur when dealing with age estimation from fish otoliths. The main disadvantages are that the method is time consuming and expensive in manpower and a consistent age reading depends on the reader's experience.

The use of simpler technique than counting sectioned otolith increments is important to provide an easier way for fisheries biologists to age fishes. Now, with image analysis technology available, morphological characteristics such as otolith length, width or shape in relation to fish age have demonstrated a clear relationship between the variables (Fossen *et al.*, 2003; Tuset *et al.*, 2003). Otolith mass has been extensively used because it has been shown that a strong relationship exists between fish age and otolith mass for different species (Cardinale *et al.*, 2000; Araya *et al.*, 2001). Therefore, the otolith mass and other otolith morphological variables could constitute a valid alternative to the traditional methods for the estimation of young-of-the-year (YOY) age.

Otoliths of bluefin tuna *Thunnus thynnus* (L.,) have been used rarely for age estimations because it is extremely time consuming to collect, prepare and read the otoliths (Brothers *et al.*, 1983; Foreman, 1996; Itoh *et al.*, 2000; Megalofonou *et al.*, 2003). During the past decade, the expansion of the fishing industry and the resulting heavy fishing pressure, especially on juvenile bluefin tuna in the east Atlantic and the Mediterranean Sea, resulted in the urgent need to improve stock assessment and management of this valuable species, in particular to improve the ageing procedures and the catch at age data. Although the daily increment method is probably an excellent technique for ageing juvenile bluefin tuna (Brothers *et al.*, 1983; Foreman, 1996; Itoh *et al.*, 2000; Megalofonou *et al.*, 2003), it is argued that it would not be practical to use this method for routine determination of the age composition of the catch. In the present study, otolith morphological characteristics of juvenile bluefin tuna were examined using image analysis techniques and the relationships between otolith variables and somatic growth were analysed. The hypothesis that otolith size or shape is directly related to fish age, as estimated from otolith increment counting, is also considered, with the aim of providing strictly objective measurements that could be used to determine age.

MATERIALS AND METHODS

SAMPLE COLLECTION AND MEASUREMENTS

All bluefin tuna samples were obtained from Greek and Italian purse seine boats fishing for small pelagic fishes in the Mediterranean Sea during the period 1997–2002 (Fig. 1). Place and date of capture were recorded for each sample. All fish were frozen for later dissection and measurements in the laboratory. Fork length (L_F) to the nearest mm and total mass (M_T) to the nearest g were measured for each fish. Otoliths were removed from the thawed fish with the aid of minute insect needles mounted on wooden rods. Despite the minute size and fragility of the otoliths, the efficiency of the otolith extraction was particularly high: at least one or two sagittae were obtained from each fish. The dissected otoliths were separated from extraneous organic material, cleaned with distilled water and dried. All broken otoliths were systematically detected

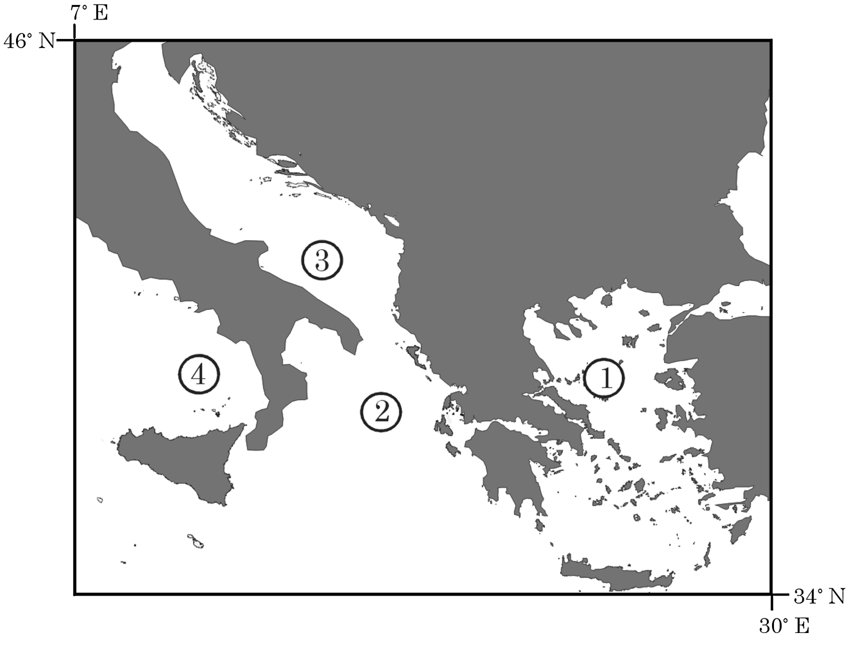

FIG. 1. Map indicating the areas in the Mediterranean Sea where bluefin tuna samples were collected during 1997–2002: Aegean Sea (1), Ionian Sea (2), Adriatic Sea (3) and Tyrrhenian Sea (4).

under a Nikon stereoscope and discarded from the analysis. The rate of otolith breakage was estimated at 30·7%. Broken or visibly damaged otoliths were not measured. Sagittal otoliths were weighed (M_O) to the nearest 0·001 mg. Whole otolith length (L_O), width (W_O), area (A_O) and perimeter (P_O) were measured for each sagitta using a binocular stereoscope equipped with image analysis system (Software Image Pro-Plus 4·1). The L_O was recorded as the greatest distance measured from the anterior rostrum to the posterior edge, parallel to the sulcus. The W_O was recorded as the greatest distance measured from the right to the left edge (Fig. 2). The L_O, W_O and P_O were measured to the nearest 0·001 mm and A_O to the nearest 0·001 mm^2. Three shape indexes, circularity (C_O), rectangularity (R_O) and E value (E_O), were obtained by combining size variables in various ways (Russ, 1990; Volpedo & Echeverría, 2003). Rectangularity is a measure of the otolith area divided by the area of its minimum-enclosing rectangle [$R_O = A_O\ (L_O\ W_O)^{-1}$] and circularity is the perimeter of the otolith squared divided by its area ($C_O = P_O^2\ A_O^{-1}$). Circularity gives information on the similarity of various features to a perfect circle, taking a minimum value of 4π (12·57). The E value is the per cent ratio between otolith width and length ($E_O = W_O\ L_O^{-1}$) and expresses the tendency in the shape of the otolith, circular or elongate.

OTOLITH MICROSTRUCTURE AND AGE ESTIMATION

The surface morphology of the otolith was examined with a scanning electron microscope (SEM). The sagittae were mounted on observation stubs, gold coated and scrutinized with the SEM. For internal structural examination and increment counting, otoliths were placed on microscope slides for preliminary viewing after immersion in methylbenzoat reinst ($C_8H_8O_2$, Merck, Darmstadt, Germany). Each otolith was kept with a small quantity of methylbenzoat reinst in a glass vial for 3 days (Megalofonou *et al.*, 2003) to increase the otolith transparency. Continuous daily micro-increments were visible with high magnification and counts could be made from the core to the

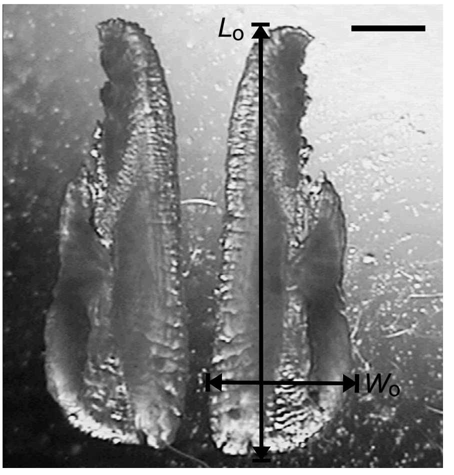

FIG. 2. Digitalized image of bluefin tuna (L_F = 377 mm) sagittal otoliths (left and right) observed with a stereoscope and the length (L_O) and width (W_O) measurements obtained using the image analysis software, Image Pro-Plus 4.1. Scale bar = 1 mm.

margin of the antirostrum especially for the smaller sagittae. When focusing on critical regions and micro-increment counts were prevented by otolith thickness and projecting surfaces, further preparation was necessary. The sagitta was embedded in epoxy resin (Spurr, 1969) sectioned in the transverse plane on a Buehler Isomet saw, and polished to 0·5 mm thickness with 600 grit sandpaper and 0·3 μm alumina polish. Increment counts were performed under transmitted light with a binocular lens microscope (Zeiss Axiolab) adapted for video viewing. Counts of successive increments from the core to the ventral edge were made from the video monitor and confirmed with direct observation through the eyepieces (Megalofonou *et al.*, 1995). For each otolith, two independent counts were performed without knowledge of L_F, M_T or M_O and the mean values were used in age estimations. The precision, or the reproducibility of repeated measurements on a given otolith, was estimated using the coefficient of variation (CV), expressed as the ratio of the S.D. to the mean (Chang, 1982). Counting on the same otolith with both the SEM and the optical microscope and obtaining similar counts for the micro-increments corroborated the accuracy, or the closeness of the age estimate to the true value. Sagittae-estimated ages were obtained by adding 4 days to total counts (Itoh *et al.*, 2000). This correction assumes that the first counted increment was formed 4 days after spawning or fertilization.

DATA ANALYSIS

The relationship between L_F and M_T was determined using a multiplicative regression model ($y = ax^b$). A linear regression model ($y = a + bx$) was used to examine the relationship between fish age and L_F and to calculate the mean growth rate within the size interval analysed. Length-at-age data were also fitted with non-linear models. The von Bertalanffy growth model was excluded because the model parameter L_∞ was deemed biologically unrealistic. The linear regression model was used to determine the relationships between otolith morphometric measurements, L_F, or otolith morphometric measurements, age. Because the variables were not normally distributed, logarithmic transformation was applied to all variables tested before any statistical procedure was applied. The null hypothesis that there was no significant difference in the morphometrics from left and right sagittae was tested using paired *t*-test as the variables of the differences were normally distributed. When otoliths did not differ statistically, one of each pair was selected randomly for the statistical analyses. All statistical differences were based on a significant level of $\alpha = 0{\cdot}05$. The relationships between L_F and M_O, and age and M_O were determined to evaluate if M_O could be used as an indicator of somatic growth and age in juvenile bluefin tuna.

ESTIMATION OF AGE USING OTOLITH MASS

The relationships between M_O and fish age as well as between L_O and fish age were estimated using a linear regression considering age as the independent variable (*x*) and the other variable as dependent (*y*). Then the ages of 10 individual fish (not used in the previous analysis) were assessed using M_O. To obtain a discrete variable of the age in days, ages were rounded to the nearest integer. The bias of the estimations was calculated as the estimated age from increment counts minus the assessed age from M_O. The CV was used to evaluate the degree of differences between age estimated using counting of micro-increments and age assessed using M_O (Chang, 1982). The CV was calculated as the S.D. of estimated ages in each specimen divided by the corresponding mean estimated age for that specimen. This value is 0 when the age is assessed by counting daily increments, and the age assessed using M_O matches for all the individuals sampled, and increases when frequency and magnitude of age estimation errors increase (Chang, 1982).

RESULTS

The otoliths of 67 YOY bluefin tuna were used in this study. Bluefin tuna ranged from 8·5 to 55·5 cm L_F and from 8 to 3000 g [Fig. 3(a)]. The L_F and M_T relationship ($M_T = 0{\cdot}0000085544\ L_F^{3{\cdot}20}$) [Fig. 3(b)] was relatively strong and the model explained 99·7% (r^2) of the variability.

Sagittae of YOY bluefin tuna were small, quite complex calcified structures with an elongated form in larger fish and rounded and squared in smaller fish.

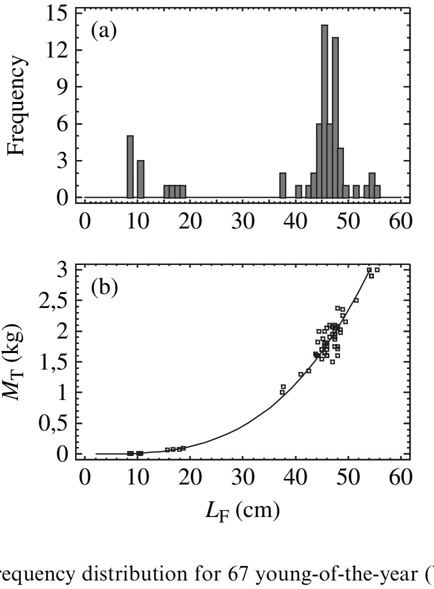

FIG. 3. (a) Fork length (L_F) frequency distribution for 67 young-of-the-year (YOY) bluefin tuna sampled in the Mediterranean Sea. (b) The L_F and total mass (M_T) relationship for 67 YOY bluefin tuna sampled in the Mediterranean Sea. The curve was fitted by $y = 0{\cdot}0000085544\ L_F\ 3{\cdot}20$ ($r^2 = 0{\cdot}997$).

They had a well-demarcated sulcus, which appeared to increase in depth with increase in fish size. The rostrum was rounded, exaggerated and well separated from the antirostrum. The concave surface of the rostrum was rugose and the micro-ridges were visible with the scanning microscope. The micro-ridges began from the core region where they were concentric in this area (Fig. 4).

In order to assess the similarity of left and right otoliths a paired sample comparison, *t*-test, was computed for all morphometric measurement for 22 pairs of otoliths and no significant difference was observed ($P > 0{\cdot}05$). Further, only one otolith was used for statistical analysis for all morphometric measurements and shape indexes (Table I). A statistically significant relationship was observed between $\log_{10}$ otolith variables (except $\log_{10} R_O$) and $\log_{10} L_F$ when a linear regression was applied. Among the otolith variables, L_O showed the highest positive correlation with L_F, followed by A_O and R_O and only E_O exhibited a negative correlation with L_F.

Daily increment counts ranged from 16 to 125 in fish ranging from 8·5 cm to 55·5 cm L_F. The mean CV of the two series of repeated counts was equal to 5·26%. A linear regression model of estimated age and L_F ($a = 5{\cdot}2$; $b = 0{\cdot}47$; $r^2 = 79{\cdot}2$; *t*-test for slope $b = 0$, $P < 0{\cdot}001$) yielded a moderately strong relationship between the variables. The slope of 0·47 indicates a growth rate of 4·7 mm day^{-1} within the size interval analysed (Fig. 5). Non-linear models yielded stronger relationships between the estimated age and L_F. Among them the double reciprocal model $y = [(a + b)\,x^{-1}]^{-1}$ showed the highest correlation (regression analysis, $a = -0{\cdot}008$; $b = 2{\cdot}552$; $r^2 = 96{\cdot}3$).

A linear regression model applied to determine the relationships that might exist between $\log_{10}$ otolith measurements and $\log_{10}$ age of the fish showed high correlations. A strong statistically significant relationship ($P < 000{\cdot}1$) was observed for the morphometric variables (L_O, W_O, A_O and P_O) and the age of the fish, with the L_O having the highest correlation. A moderately strong

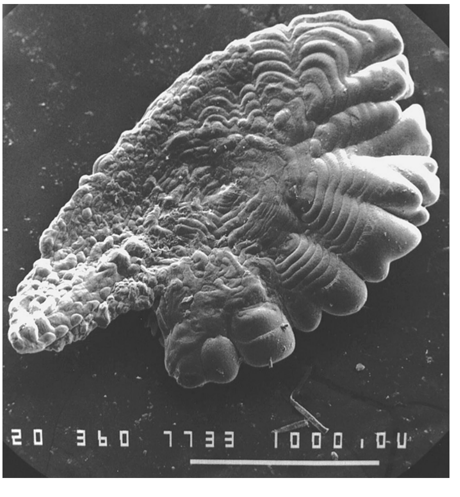

FIG. 4. Scanning electron micrograph of the sagitta otolith from a 85 mm L_F bluefin tuna caught in the Tyrrhenian Sea.

TABLE I. Summary statistics of otolith measurements (sagittae) and shape indexes for 57 young-of-the-year bluefin tuna from the Mediterranean Sea

	n	Mean ± S.D.	Range
Otolith measurements			
Mass (M_O) (mg)	57	7·45 ± 3·14	0·11–13·70
Length (L_O) (mm)	57	6·22 ± 1·69	1·74–8·07
Width (W_O) (mm)	57	2·01 ± 0·33	1·11–2·42
Area (A_O) (mm^2)	57	9·73 ± 3·29	1·43–14·06
Perimeter (P_O) (mm)	57	16·34 ± 4·19	5·09–20·33
Shape indexes			
Circularity (C_O)	57	28·18 ± 4·04	18·05–36·83
E ratio (E_O)	57	0·35 ± 0·01	0·25–0·65
Rectangularity (R_O)	57	0·74 ± 0·04	0·67–0·84

relationship was indicated between the C_O and the age, while there was not a statistically significant relationship ($P > 0{\cdot}05$) between R_O and age.

The relatively strong relationships between L_F and M_O, age and M_O, L_F and L_O and age and L_O, showed that both otolith size variables could be used as indicators of bluefin tuna somatic growth and age (Fig. 6). Using the appropriate age (y) and M_O relationship ($y = 27{\cdot}39 + 6840\ M_O$; $r^2 = 76{\cdot}1$; t-test for slope $b = 0$, $P < 0{\cdot}001$), ages were assessed from the masses of a sample of 10 fish (Table II). These were compared to ages estimated directly from otolith increment counts and the absolute values of bias ranged from 1 to 24 days, with a mean value 11·9. The mean CV was 13·5%.

DISCUSSION

This study provides new data useful for the prediction of age in YOY bluefin tuna through morphological measurements of their otoliths. The linear regression models applied show that there are statistically significant relationships between otoliths measurement and age of the fish over the very small range of L_F and ages that were examined and therefore, it is possible, that otoliths morphometric measurements (size and shape variables) could provide a good alternative way to age juvenile bluefin tuna.

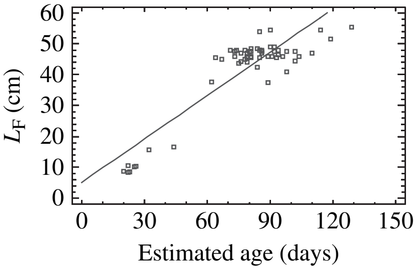

FIG. 5. Relationship between fork length (L_F) and estimated age in days for 57 young-of-the-year bluefin tuna sampled in the Mediterranean Sea. The curve was fitted by $y = 5{\cdot}2 + 0{\cdot}47x$ ($r^2 = 0{\cdot}792$).

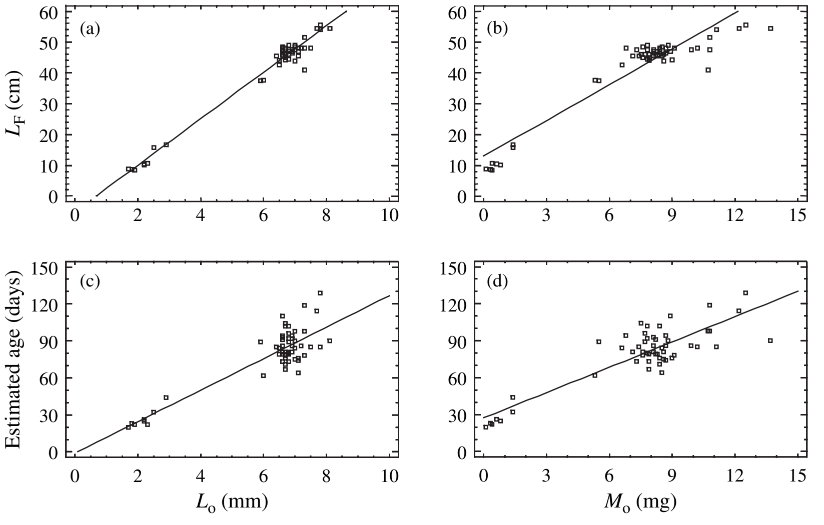

FIG. 6. The relationships between (a), (c) otolith length and (b), (d) otolith mass and (a), (b) fork length and (c), (d) estimated age for 57 young-of-the-year bluefin tuna sampled in the Mediterranean Sea. The curves were fitted by: (a) $y = -5{\cdot}06 + 7{\cdot}54x$ ($r^2 = 0{\cdot}975$), (b) $y = 12{\cdot}95 + 3{\cdot}87x$ ($r^2 = 0{\cdot}884$), (c) $y = -1{\cdot}03 + 12{\cdot}77x$ ($r^2 = 0{\cdot}771$) and (d) $y = 27{\cdot}39 + 6{\cdot}84x$ ($r^2 = 0{\cdot}761$).

There are very few studies on age and growth of bluefin tuna and especially on juveniles (Brothers *et al.*, 1983; Foreman, 1996; Itoh *et al.*, 2000; Megalofonou & De Metrio, 2000; Megalofonou *et al.*, 2003) because of the difficulties in sampling and processing hard parts. The constraints and difficulties of sampling YOY specimens from the wild populations had an effect on the number of samples used in this study, which was relatively limited and did not cover satisfactorily the size range studied (85–555 mm). In the Mediterranean Sea the catch of bluefin tuna <6·4 kg is prohibited and there are no special fishing gears to target the 0+ year age class.

TABLE II. Comparison of age estimations from counts of increments (estimated age) and otolith mass (assessed age) for 10 young-of-the-year bluefin tuna sampled in the Mediterranean Sea

Estimated age (days)	Assessed age (days)	Mean age ± S.D. (days)	CV	Bias (days)
20	28	24·0 ± 5·7	0·236	−8
22	30	26·0 ± 5·7	0·218	−8
49	37	43·0 ± 8·5	0·197	12
89	65	77·0 ± 16·9	0·220	24
103	79	91·0 ± 16·9	0·186	24
87	86	86·5 ± 0·7	0·008	1
92	104	98·0 ± 8·5	0·097	−12
92	103	97·5 ± 7·8	0·080	−11
114	111	112·5 ± 2·1	0·019	3
129	113	121·0 ± 11·3	0·094	16

The daily formation of otolith increment has already been validated in previous studies for larvae of 5 days after fertilization to fish of 68 cm L_F by the otolith marking method with oxytetracycline (Foreman, 1996) or by examination of reared larvae and juveniles in the laboratory (Itoh *et al.*, 2000). In this study, the micro-increments observed were assumed to be daily and increment counts were used to age YOY bluefin tuna from the Mediterranean Sea. The mean growth rate estimated, although higher, was consistent with that found in other studies for larval and juvenile specimens reared in laboratory and enclosures (Table III). The precision of the readings was quantitatively assessed using the mean CV, which is clearly favoured for otolith microstructure studies (Campana, 2001). Considering the difficulties in ageing YOY bluefin tuna from the micro-incremental structure of their otoliths, the mean value of the CV found was relatively low. The CV is usually *c.* 40% higher than the average per cent error for any given set of ageing data (Campana, 2001).

The relatively strong relationships found between age and otolith mass or age and otolith length indicate that otoliths grew at a predictable rate over the early life span of the fish and the use of otolith size for age determination of YOY bluefin tuna could represent a valuable method which is objective, economic and easy to perform, compared to increment counting method.

The need for a reliable and quick predictor of age for fish populations has resulted in many attempts at relating the size of otolith to age and several authors have shown that an alternative approach to ageing fishes is to develop a relationship between age and mass of otoliths. It has been suggested that the otolith mass and age relationship could provide more reliable estimate of age than those based on variables such as length (Cardinale *et al.*, 2000; Pilling *et al.*, 2003), as they pointed out that otolith mass is a much stronger predictor of age than fish length.

The present attempt to predict the age of 10 bluefin tuna using their otolith mass revealed that the estimated ages are very close to those assessed using daily increments counts. Nevertheless, it was evident that otolith mass did not precisely discriminate between ages as the estimated bias ranged between 1 and 24 days. Probably the obvious variation in otolith mass within ages reduced the precision of ages predicted using the otolith mass and age relationship. The relatively reduced capacity of the method to estimate precisely the individual ages in days may still lead fisheries scientists to continue using

TABLE III. Average growth rates for young-of-the-year bluefin tuna

Author	Area	Method	Fork length range (L_F in mm)	Growth rate (mm day^{-1})
Brothers *et al.* (1983)	Atlantic Ocean	Otoliths	306–413	1·4
Itoh *et al.* (2000)	Pacific Ocean	Otoliths of reared fish	238	3·4†
Present study	Mediterranean Sea	Otoliths	85–555	4·7*

*Derived from the slope of the regression (see Fig. 5). †The oldest fish sampled was 71 days and 238 mm L_F.

otolith daily increments to age individual fish. Otolith mass and age relationships, however, could be used to generate age structure data that require less precision and probably this could prove to be a valuable method for age estimations of bluefin tuna using a year scaling. Therefore, it will be very interesting to test this hypothesis in large juveniles and adult specimens. Length and age keys already exist for bluefin tuna and it is far easy to measure the length of a fish than its otolith mass, for common stock assessment purposes. The use of ageing through otolith mass (if the method is validated), however, could be useful to assess the accuracy of the length and age keys over time since fish length and age relationships can be very variable from one year to another. It has long been known that otolith growth is more conservative (steady) than body growth with the effect of slow-growing fishes having larger otoliths than faster growing fishes of the same size (Templeman & Squires, 1956). Another advantage is that otoliths grow continuously through out the life span of the fish.

Classically, the number of growth increment and age relationship is preferably validated over the whole size range of the species (Campana, 2001). This preliminary work on YOY bluefin tuna is based on a restricted number and size range of fish. Further experiments using older juveniles and adult fish should be conducted to validate the method for all ages. Since the shape of the otolith of older fish is far more complex, the within-age class variability of otolith mass or morphology could be far greater than in the case of juvenile fish. This point should be investigated thoroughly to determine if it is possible to age older bluefin tuna with otolith mass.

Of particular interest is that otolith length and mass could provide a direct estimate of somatic growth. Several studies have shown a linear relationship of otolith length and fish length (Hunt, 1993; Harvey *et al.*, 2000; Lychakov & Rebane, 2000; Fossen *et al.*, 2003). Huang & Chiu (1997) have suggested that linear growth of otoliths might be a common otolith and body length relationship in the juvenile stage. In the present study, non-linear models were not applied although they might, in some cases, have shown stronger correlation between the variables.

Otolith area, perimeter and shape indexes have been used to separate stocks and they have been suggested as an easier way to discriminate stocks from other more sophisticated methods such as Fourier series analysis (Bolles & Begg, 2000; Tuset *et al.*, 2003). Using image analysis techniques, otolith shape measurements were taken easily and very strong correlations were found for A_O and P_O with L_F and age. Moreover, the relationships between shape indexes and age revealed that bluefin tuna otolith morphology varies from a round shape in younger specimens (20 days old) to an elongate one in older fish (129 days old). The results were similar to those obtained for the otolith shape in larval and juvenile bluefin tuna reared in laboratories. According to Itoh *et al.* (2000), bluefin tuna otolith shape is spherical at first, then hemispherical with a flat distal side up to the fifth increment. It seems that during the period of life examined, bluefin tuna otolith morphology does undergo significant bidimensional changes and as it has been mentioned that for other species (Lagardère *et al.*, 1995), otolith morphology varies from a roundness of shape in larval individuals to the specific shape in adults. Consequently, the shape indexes, which represent in a bidimensional plane the pattern of otolith growth probably, could provide additional information for a better estimation of age during the early life of bluefin tuna.

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