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Age and growth rate of juvenile bluefin tuna *Thunnus thynnus* from the Mediterranean Sea (Sicily, Italy)*

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SUMMARY: The microstructural analysis of the sagittal otoliths of juvenile Atlantic bluefin tuna, *Thunnus thynnus*, can be used to estimate the age and growth rate of young-of-the-year individuals born in the Mediterranean. Samples of juvenile bluefin tuna, obtained as by-catch of the local small-scale pelagic fishery for Atlantic bonito and dolphinfish, were collected off the northern coast of Sicily between late August and early November 2002. Otolith age readings were carried out on 56 specimens ranging between 195 and 400 mm fork length and between 112 and 1266 g total weight. Based on microincrement analysis along a counting path from the otolith core to the antirostrum, juvenile fishes were found to be between 77 days and 153 days old. The estimated growth rate was practically linear over the whole size range, accounting for about 2.0-2.37 mm/day. Derived from the length-weight relationship and the estimated length-at-age data, the mean weight-at-age was 168, 429 and 813 g for 2, 3 and 4 month-old juveniles respectively. Furthermore, the hatching date distribution of bluefin tuna, obtained by means of the back-calculation of ageing data, indicated a spawning period of at least two months, namely from mid-May to mid-July, with a peak in mid-June. Our data indicate that juvenile bluefin tuna have a very high growth rate in the first part of their life, reaching a weight of more than 1 kg in four months.

Keywords: age and growth, bluefin tuna, otoliths, Mediterranean Sea.

RESUMEN: EDAD Y TASA DE CRECIMIENTO DE LOS JUVENILES DE ATÚN *THUNNUS THYNNUS* DEL MAR MEDITERRÁNEO (SICILIA, ITALIA). – El análisis microestructural de los otolitos sagitta de juveniles del atún *Thunnus thynnus*, ha permitido estimar la edad y tasa de crecimiento de individuos del primer año de vida, nacidos en el Mediterráneo. Los juveniles de atún, obtenidos como especie acompañante de la pesquería artesanal pelágica del bonito y lampuga, se recolectaron en las costas de Sicilia desde finales de agosto a principios de noviembre de 2002. Las lecturas de edad de los otolitos se realizaron en 56 ejemplares entre 195 mm y 400 mm de longitud de furca y entre 112 g y 1266 g de peso total. En base a los análisis de microincrementos a lo largo del eje de lectura, desde el núcleo del otolito hasta el antirostrum, se determinó que los juveniles tenían entre 77 y 153 días de edad. La tasa de crecimiento estimada era prácticamente lineal a lo largo de todo el rango de tallas, representando alrededor de 2.0-2.37 mm/dia. A partir de las relaciones talla-peso y talla estimada-edad se determinó el peso medio-edad en 168 g, 429 g y 813 g para juveniles de 2, 3 y 4 meses de edad, respectivamente. Además, la fecha de eclosión del atún, estimada a partir del retrocálculo de la edad, indica que el período reproductor se extiende al menos durante dos meses, desde mediados de mayo a mediados de julio con un pico a mediados de junio. Nuestros datos indican que los juveniles de atún presentan una elevada tasa de crecimiento en las fases tempranas de vida, alcanzando un peso de mas de 1 kg en cuatro meses.

Palabras clave: edad y crecimiento, atún, otolitos, Mar Mediterráneo.

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INTRODUCTION

The northern bluefin tuna, *Thunnus thynnus*, is a highly migratory species occurring in temperate and subtropical waters of the Atlantic and Pacific Oceans (Collette and Nauen, 1983). Two subspecies have been recognised: *T. thynnus thynnus* (Linnaeus) in the north Atlantic and *T. thynnus orientalis* (Temminck and Schlegel) in the north Pacific. Atlantic bluefin tuna are distributed throughout the Atlantic Ocean, as well as in the Mediterranean Sea and the Gulf of Mexico (Gibbs and Collette, 1967), which represent the most important spawning areas of this subspecies (Mather *et al.*, 1995; Magnuson *et al.*, 1994). In May-June, adult bluefin tuna migrate through the Strait of Gibraltar into the Mediterranean for feeding and spawning activities. Soon after spawning, which occurs from the beginning of June to the end of August (Arena, 1979), most individuals leave the Mediterranean to overwinter in the Atlantic off Morocco (Sella, 1929; Rodriguez-Roda, 1964; Sara, 1973; Compeán-Jimenez and Bard, 1983).

Besides its importance as a spawning area for Atlantic bluefin tuna, the Mediterranean is one of the most important fishing grounds, accounting for 53% of total catch of this species (ICCAT, 2000). In addition, being a large pelagic species, it is considered to be the most economically valuable fish in both the Atlantic and the Mediterranean (CEC, 1995).

As for the western Atlantic and Gulf of Mexico population of bluefin tuna (see in Brothers *et al.*, 1983), most studies concerning the spawning period and the age and growth of juveniles of *T. thynnus* in Mediterranean waters were undertaken using indirect methods. Indeed, the spawning time was frequently estimated according to the seasonal occurrence of eggs and larvae or younger juveniles (Scaccini *et al.*, 1975; Richards, 1976; Piccinetti and Piccinetti-Manfrin, 1979; Piccinetti and Manfrin, 1993), as well as through the study of gonadal maturation in adult fish (Serna and Alot, 1992; Susca *et al.*, 2000; Medina *et al.*, 2002; Hattour and Macías, 2002).

Available data on growth rate of juvenile Atlantic bluefin tuna are mainly based on length frequency analysis (Furnestin and Dardignac, 1962; Le Gall and Bard, 1979; Cort, 1990; Liorzou and Bigot, 1995; Orsi Relini *et al.*, 1997), as size distributions are well-defined and correspond to early age classes, as a consequence of fast growth and a single and relatively short spawning period (Compeán-Jimenez and Bard, 1983). Additional data on the growth rate of juveniles come from tag-and-recapture studies (Cort and Rey, 1985; Cort, 1990), and rearing experiments (Le Gall and Bard, 1979; Katavic *et al.*, 2002). Very few data on age estimation of juvenile Atlantic bluefin tuna have been reported to date. Larvae and young-of-the-year bluefin tuna caught in the western Atlantic and the Gulf of Mexico were aged by means of microincrement counts in sagittal otoliths (Brothers *et al.*, 1983). In the Mediterranean Sea, age estimation of juvenile bluefin tuna was determined by otolith microstructure analysis (Radtke and Morales-Nin 1989; Santamaria *et al.,* 2003) and by annulus-formation in dorsal spines (Megalofonou and De Metrio, 2000).

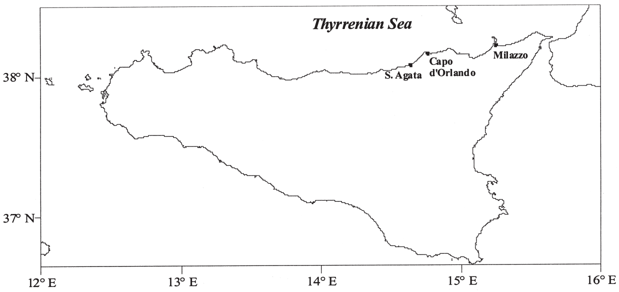

FIG. 1. – Sampling area off the northern coast of Sicily, showing the three main landing locations.

In order to provide new data on age and growth of young-of-the-year bluefin tuna, samples were collected off the northern coasts of Sicily on a monthly basis, from just after the spawning season (spring-summer) until the beginning of winter. The main aim was to determine the growth rate of the early life of juveniles until their first winter of life, probably a period of fundamental importance for the subsequent year class strength and survival. Sagittal otoliths were thus collected and aged by counting the daily growth increments. In addition, the hatch date distribution obtained from the back-calculation of ageing data allowed us to gain new insight into the time and duration of the spawning season of the Atlantic bluefin tuna in this area

MATERIAL AND METHODS

The study area was the northern coast of Sicily between Milazzo and S. Agata di Militello (Fig. 1). Samples were collected from commercial landings of the local small scale fishery, in the framework of the ICRAM Project "Eolide". Juvenile bluefin tuna were caught offshore (about 10 miles) as by-catch, close to floating fish aggregating devices (FADs)

TABLE 1. – Sampling data of juvenile bluefin tuna collected off the northern coast of Sicily in 2002.

Date	Area	Gear	Type
18-08	S. Agata	palamitara	drift net
29-08	Milazzo	cianciolo	purse-seine
04-09	Milazzo	cianciolo	purse-seine
05-09	S. Agata	cianciolo	purse-seine
10-09	S. Agata	cianciolo	purse-seine
12-09	S. Agata	palamitara	drift net
15-10	S. Agata	ferrettara	drift net
16-10	S. Agata	ferrettara	drift net
17-10	S. Agata	ferrettara	drift net
24-10	C. d'Orlando	ferrettara	drift net
28-10	S. Agata	palamitara	drift net
13-11	C. d'Orlando	palamitara	drift net

and by means of drift and purse seine nets. Sampling details are summarised in Table 1.

Each specimen was measured as total length (TL) and fork length (FL) to the lowest mm and weighed as total weight and eviscerated weight (g). Fish were dissected and the heads removed and frozen. To study age and growth of juvenile bluefin tuna, we decided to use otoliths, as they are not susceptible to resorption or alterations once formed (Campana and Neilson 1985, Campana *et al.*, 1995). In addition, otoliths are particularly useful for age determination of species with minute scales, such as bluefin tuna. Otolith removal was carried out following the "open the hatch method" (Secor *et al.*, 1992). A cranio-caudal frontal cut was made through the head to expose the brain, which was removed allowing the localisation of the sagittae. The sagittae were extracted by means of forceps, carefully cleaned from adhering vestibular tissue and stored dry in plastic vials. We preferred this method to the more common procedure (Nichy and Berry, 1976), as we were unable to properly locate the right position of the vertical cut through the head without breaking or loosing the sagittae into the cranial cavity.

Following Brothers *et al.* (1983) and Foreman (1996), sagittal otoliths were placed in a drop of immersion oil on a depression slide to improve resolution and examined under a light microscope at 100-400x magnification. The light microscope was equipped with a television camera connected to a video-analysis image program (OPTIMAS 6.5). The software was also used to measure the maximum diameter (OD), i.e. the maximum distance between the rostrum and the postrostrum (see Secor *et al.*, 1992), of each otolith to an accuracy of 0.01 mm. In addition, the sagittae were weighed to mg.

Increment counts were made on the distal side, as the overburden deposition is very thin on this side and increments are almost exposed on the otolith surface. According to Brothers *et al.* (1983), the increment counts were made from the core to the margin of the antirostrum (Secor *et al.*, 1992), an area with a relatively unambiguous and continuous pattern of increments. Each increment was a bipartite concentric ring comprised of a discontinuous and an incremental zone (defined D-zone and L-zone respectively, Secor *et al.*, 1995). The number of D-zones were counted as the number of increments, and assumed to be the age of the fish (see below). Two counts were made from the core to the margin and vice-versa, and the mean value was considered. When the counts differed by more than ten increments they were discarded. Out of 72 otoliths examined, about 22% were discarded as they were difficult to read. The index of average percent error (APE) (Beamish and Fournier, 1981), as well as the mean coefficient of variation (CV) (Chang, 1982), were calculated to estimate the relative precision between the counts.

Owing to the thickness of the antirostrum, there was a considerable individual variation in otolith readability. However, the particular three-dimensional morphology of the otolith, namely the projecting surfaces and the deep *sulcus acusticus*, did not allow the otolith surface to be ground without loss of increments in the core or on the margin of the antirostrum.

By means of ordinary least squares linear regression analysis, a straight line was fitted to the age-length data pairs obtained by the increment counts of otoliths. Furthermore, from the ageing data and the date of capture, the monthly distribution of hatching dates of our specimens was back-calculated.

The relationship between fish length (FL) and maximum otolith diameter was investigated by means of linear regression analysis. Finally, the length-weight relationship was calculated according to the exponential equation in the commonly used form:

$$W = a\ FL^{b}$$

where W is the total wet weight (g), FL the fork length of fish (mm) and a and b are regression parameters. By linearisation of the above equation, the $\log_{10}$-transformed length-weight data were computed to determine the regression parameters. All statistical inferences were based on a significance level of $P = 0.05$.

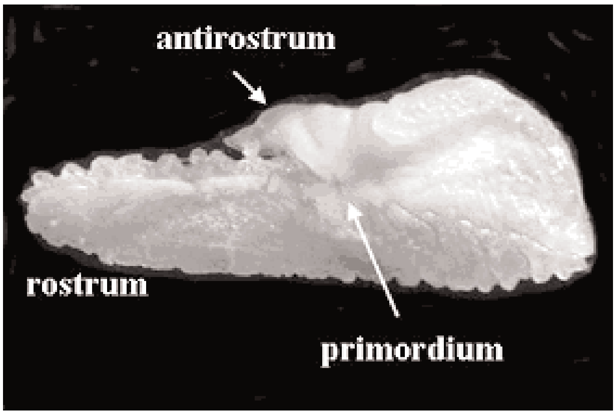

FIG. 2. – Sagittal otolith of juvenile bluefin tuna, showing the counting path of micro increments from the core to the margin of the antirostrum.

RESULTS

Otolith morphology

The sagittae of juvenile bluefin tuna are elongated along the anterior-posterior axis, flattened and with a pronounced thin rostrum. The external (distal) side of the otoliths is slightly concave and has an evident protrusion in the dorsal-posterior area. The internal (medial) side has a very deep *sulcus acusticus*. The antirostrum, i.e. an otolith protrusion opposite the rostrum, is well developed and its thickness is highly variable (Fig. 2). The primordium is an optically dense and irregular round area in the centre of the otolith. It is surrounded by two diffuse layers, or non-incremental rings, forming together the core (Fig. 3). Following some authors (Brothers *et al*., 1983; Itoh *et al*., 2000), these rings were not included in increment counts due to their faintness and anomalous width.

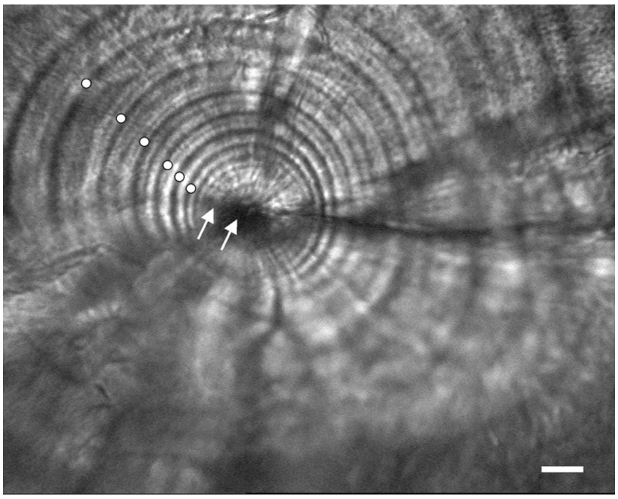

FIG. 3. – Sagittal otolith of juvenile bluefin tuna, showing the core region. Arrows indicate the two diffuse layers surrounding the primordium; spots indicate the first six daily rings. Scale bar = 10 μm.

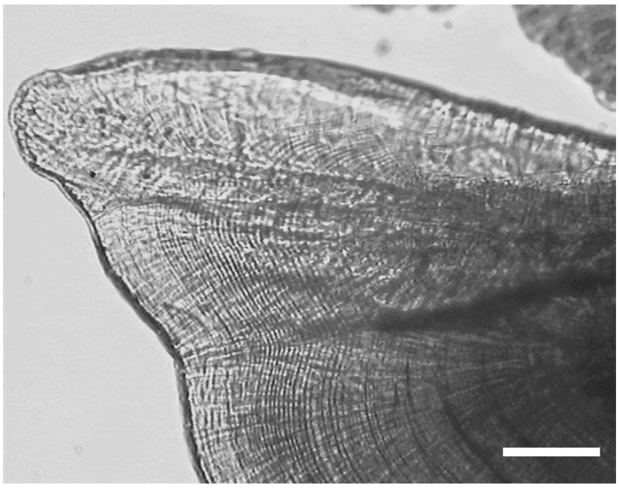

FIG. 4. – Sagittal otolith of juvenile bluefin tuna, showing the margin of antirostrum with more regular and distinct increments. Scale bar = 100 μm.

Off the core area, the first four-five increments are quite regular, spherical and of similar thickness (1-2 μm). Increments then become progressively wider and elongated along the posterior-anterior axis. Similarly, within such bipartite rings, the L-zone (or light subunit) becomes progressively wider than the D-zone (dark subunit). In correspondence of the thicker zone of antirostrum, approximately after the fifteenth-twentieth increment, growth increments are optically very dense and their thickness increases up to 20 μm. Close to the margin of the antirostrum, the increment thickness decreases and they tend to progressively become more regular and distinct (Fig. 4). As observed in previous studies (Brothers *et al*., 1983), subdaily growth increments are quite common along the counting path, but a proper reading method consisting in the use of a high focal point of the light microscope largely prevented their enumeration.

The maximum otolith diameter, recorded in fishes between 195 mm and 400 mm FL, ranged from 3.3 mm to 6.26 mm. The relationship between maximum otolith diameter (OD) and fork length of fish (FL) was linear (Fig. 5) and is summarised in the following equation:

$$OD = 0.898 + 0.013\ FL\ (n = 35,\ r^2 = 0.94)$$

Age and growth

Diel periodicity of growth increment formation has been largely documented in several scombrids, such as skipjack tuna, *Katsuwonus pelamis*, black skipjack tuna, *Euthynnus lineatus,* yellowfin tuna, *Thunnus albacares*, and albacore, *Thunnus alalunga*

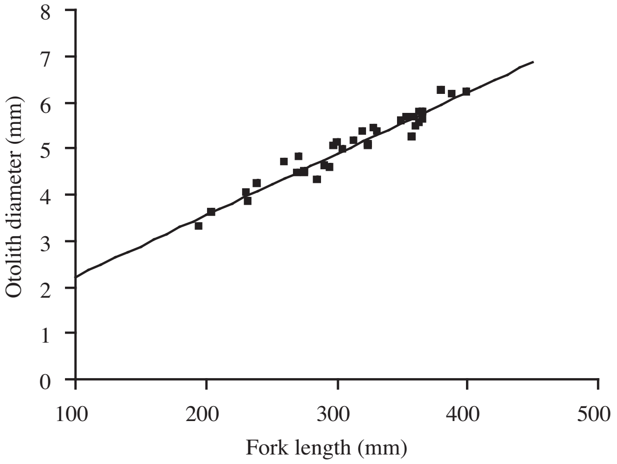

FIG. 5. – Relationship between maximum otolith diameter (OD) and fork length (FL) of juvenile bluefin tuna.

(Wild and Foreman, 1980; Uchiyama and Struhsaker, 1981; Laurs *et al.*, 1985; Wexler, 1993). Similarly, age estimation by means of otolith increment counts has been validated in larval and juvenile bluefin tuna, *Thunnus thynnus*, from fertilisation to fish of 68 cm in fork length (Foreman, 1996; Itoh *et al.,* 2000), as well as in adult specimens (Radtke, 1984). Following these authors, we assume that the growth increments we describe for young-of-the-year bluefin tuna are laid down daily, providing the true age (in days) of aged specimens. Furthermore, as the first increment is formed on approximately the fourth day after hatching, i.e. at the onset of feeding (Brothers *et al.*, 1983; Itoh *et al.*, 2000), age estimates were corrected by adding four days to total increment counts.

Overall, 56 specimens between 195 and 400 mm FL and between 112 and 1266 g total weight were aged. Age estimates ranged from 77 days to 153 days and they are summarised in an age-length key (Table 2). As far as the precision of readings is concerned, the low value of imprecision indices observed (0.020 and 0.029 for APE and CV, respectively) indicates a successful consistency between subsequent readings, supporting the suitability of the preparation techniques adopted. Based on the mean value of fork length at age (see Table 2), the growth rate was about 70 mm between the second and third month of life, then it decreased to 60 mm between the third and fourth month of life. Unfortunately we had only one fish older than four months, so we were unable to give the growth rate between the fourth and fifth month of life.

TABLE 2. – Age length key of juvenile bluefin tuna from the northern coast of Sicily.

FL (mm)	Age classes (months) II	III	IV	V
190	2	1		
200	2			
210	1			
220	1			
230		1		
240				
250		2		
260	1	3		
270	1	3		
280				
290		3		
300		2		
310		3	2	
320		5	2	
330		4	1	
340			2	
350			2	
360			6	1
370			1	
380			3	
390				
400			1	
N	8	27	20	1
Mean	220.2	290.4	350.6	360
Std	3.0	3.5	2.5	

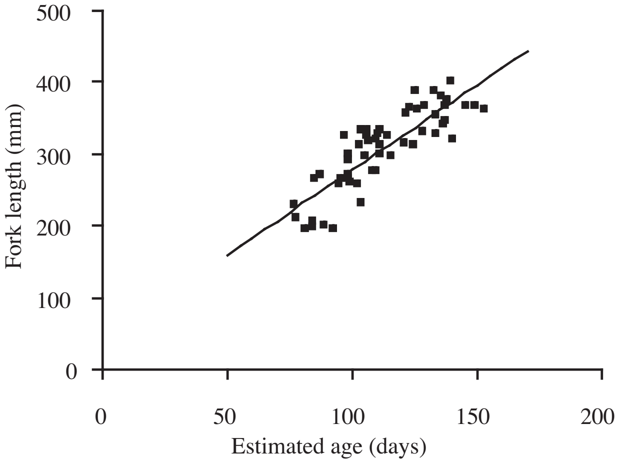

FIG. 6. – Simple linear regression fitted to age-length data of juvenile bluefin tuna from the northern coast of Sicily.

The growth model which best fits age estimates-length data was a simple linear regression (Fig. 6), providing the following relationship:

$$FL = 41.20 + 2.37 \text{ age (days) } (n = 56, r^2 = 0.71)$$

The slope of the linear fit thus indicates a growth rate of 2.37 mm/day. Fork lengths of fish were plotted against catch dates to provide an estimate of growth rate and to compare such results with those obtained from the age-length data. A least square regression was then fitted to the data, giving a growth rate of 2.02 mm/day. This value was not significantly different from that obtained from the age-length relationship (F = 1.61, P> 0.1).

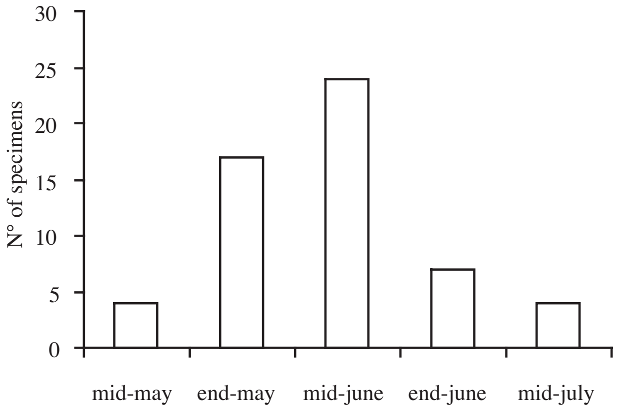

FIG. 7. – Monthly pattern of hatching dates of juvenile bluefin tuna, back-calculated from the ageing data and date of catch.

Starting from the age estimates and date of capture, hatching date distribution was back-calculated (Fig. 7). Estimated hatching period was quite long, lasting from mid-May to mid-July with a peak on mid-June.

Length-weight relationship

The relationship between fish length (FL) and weight was determined from 72 specimens (Fig. 8), ranging from 195 mm to 400 mm FL and from 110 g to 1266 g. The values of parameters a and b of the allometric power function, derived from linear regression of the $\log_{10}$-transformed length-weight data pairs, were the following:

$$W = 1.92*10^{-6}\ FL^{\ 3.39}\ (n = 72, r^2 = 0.98)$$

A positive allometric growth (i.e. b>3) was thus observed in the early life of bluefin tuna. Coupling of the length-weight relationship and the estimated length-at-age data revealed the mean weight-at-age of two, three and four month old juveniles to be 168, 429 and 813 g respectively.

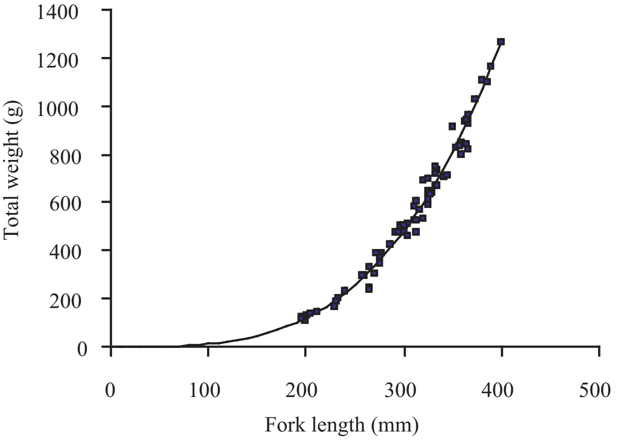

FIG. 8. – Fork length-weight relationship of juvenile bluefin tuna from the northern coast of Sicily.

DISCUSSION

The bluefin tuna fishery can be considered one of the most traditional and ancient activities in the Mediterranean Sea, with evidence dating back to 7000 BC (Doumenge, 1998). As a consequence of the considerable exploitation of this marine resource, studies concerning the bluefin tuna biology and its fisheries began in the early 20th century, especially in the Mediterranean Sea (Sanzo, 1910, Roule, 1924; Buen, 1925; Heldt, 1926, 1932; Sella, 1929, 1930; Scordia, 1933).

Similarly to other Atlantic tunas, the bluefin tuna fishery is currently managed by the International Commission for the Conservation of the Atlantic Tuna (ICCAT), through a complete stock assessment performed every two years since 1970 and based on an age-structured population model. However, the stock management of this species recently encountered some difficulties, because of increasing uncertainties concerning catch and effort data and lack of information on some biological and ecological features, such as age, growth rate and natural mortality (Fromentin, 2003).

A further problem is the stock delimitation and the degree of intermixing between different stocks, as the bluefin tuna is a highly migratory species that is widely distributed throughout the north Atlantic and the Mediterranean Sea (ICCAT, 2002). On the basis of elemental analysis of otoliths, used as natural tags which reflect differences in the chemical and physical environment of a fish, some studies have recently attempted to identify Atlantic bluefin tuna stocks from putative nurseries, i.e. the Gulf of Mexico and the Mediterranean Sea (Secor *et al.*, 2002; Rooker *et al.*, 2003). Similarly, chemical characteristics of otolith and mercury body burden have been used to discriminate the two postulated populations found in the Mediterranean, one that spends longer periods of time in the Mediterranean and one that migrates from the Atlantic to the Mediterranean just for spawning, returning to the Atlantic after breeding (Renzoni *et al.*, 1978; Morales-Nin and Fortuño, 1990).

In the present paper we analysed the inner structure of the otoliths, using the microincrement patterns to estimate the age and growth of juvenile bluefin tuna. In this species, otoliths (sagittae and probably lapilli) are known to be present at hatching (Sanzo, 1932). Bluefin tuna eggs hatch in just two days, and the early larvae resorb their yolk two or three days after hatching when the onset of exogenous feeding probably occurs. At this stage, the first otolith increment is laid down (Sanzo, 1932; Brothers *et al.*, 1983). We thus added four days to increment counts to obtain the age of fish from fertilisation to catch.

Although we were unable to validate our age estimates, the daily formation of otolith increment has been recently validated both in larvae and juvenile bluefin tuna, i.e. from larvae 5 days after fertilisation to fish 68 cm in fork length ((Foreman, 1996; Itoh *et al.,* 2000). However, we attempted to indirectly evaluate the accuracy of age estimates by comparing the results obtained from increment counts with other independent measures of age and growth. The growth rates, i.e. the slopes of the least squares regression lines fitted to age-length and date-of-catch-length pair data, were very similar to each other. The estimated growth rate of our samples was between 2.0 mm/day and 2.4 mm/day, which is similar to that reported for juvenile bluefin tuna of similar size sampled in the Ionian Sea and obtained by means of otolith increment counts (2.9 mm/day, Santamaria *et al.,* 2003). On the other hand, such values are slightly higher than those reported for juveniles bluefin tuna caught between October-November in the Ligurian Sea and obtained by means of length-frequency analysis and mark-recapture (0.71-2.0 mm/day). It should be mentioned, however, that such discrepancies are probably due to the different sampling period and techniques used. Similarly, the growth rates for juvenile bluefin tuna reported from the Atlantic stocks are very different among each other, again as a consequence of different approaches (see Rivas, 1954; Mather and Schuck, 1960; Furnestin and Dardignac, 1962; Brothers *et al*., 1983).

A further evidence to test the reliability of age estimates is the comparison of the back-calculated hatching dates with the spawning period of adults and the seasonal appearance of early larvae. Our data show a hatching period of bluefin tuna extending from mid-May to mid-July, with a peak in mid-June. The present results are fully consistent with previous studies on reproduction of bluefin tuna in Mediterranean, which report a spawning period between May and July indicated by the presence of hydrated oocytes (Rodriguez-Roda, 1967; Susca *et al*., 2001; Medina *et al*., 2002; Corriero *et al*., 2003). Similarly, the backcalculated hatching dates are in agreement with data on the occurrence of eggs and larvae of bluefin tuna in the Mediterranean from mid-June to early August (Piccinetti and Manfrin, 1993; Piccinetti *et al*., 1996; Cavallaro *et al*., 1998).

In conclusion, the analysis of otolith microstructure and chemical composition in juvenile bluefin tuna can be considered of great importance in stock management, providing information on age and growth rate and on putative nursery areas. Furthermore, as previously tested in other studies, the microincrements or growth units found in the otolith microstructure of juvenile bluefin tuna are laid down daily, allowing individuals to be aged with a high degree of accuracy.

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