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# Size and age at sexual maturity of female bluefin tuna (*Thunnus thynnus* L. 1758) from the Mediterranean Sea

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## Summary

The ovaries of 501 female eastern Atlantic bluefin tuna (*Thunnus thynnus* Linnaeus, 1758) captured in the Mediterranean Sea from May to September between 1998 and 2004 were analysed histologically. Body size at median sexual maturity ($L_{50}$) was 103.6 cm fork length (FL), while 100% maturity was reached above 135 cm FL. The age analysis, based on the count of the translucent zones of the first spiniform ray of the first dorsal fin, showed that most of the specimens with FL = $L_{50}$ were 3 years old while 100% maturity was reached between 4 to 5 years. The reported evidence indicates that for the eastern Atlantic bluefin tuna stock, the size and age of first sexual maturity of females was lower than in the western Atlantic stock.

## Introduction

The Atlantic bluefin tuna (*Thunnus thynnus* Linnaeus, 1758) is an important fishing resource in the Atlantic Ocean and the Mediterranean Sea. This species has been considered to be overexploited since the 1980s (Sissenwine et al., 1998). The International Commission for the Conservation of Atlantic Tunas (ICCAT) regulates this fishery and currently recognizes two stocks, the west and the east Atlantic stock (separated by 45°W meridian), the latter including the Mediterranean Sea. The western Atlantic population spawns in the Gulf of Mexico and in the Florida straits in April–July (Richards, 1976; Montolio and Juarez, 1977; Rivas, 1978; Baglin, 1982), whereas the eastern Atlantic population spawns in the Mediterranean during May–July (Rodríguez-Roda, 1967; Susca et al., 2001; Medina et al., 2002; Corriero et al., 2003; Karakulak et al., 2004). Western Atlantic bluefin tuna mature at the age of 6 and are considered fully mature by the age of 8, at a weight of 135 kg (Baglin, 1982; National Research Council (NRC), 1994). On the other hand, according to Rodríguez-Roda (1967), eastern Atlantic bluefin tuna mature at the age of 3, at a weight of 15 kg and are fully mature by the age of 5.

Knowledge of first sexual maturity has important implications for stock management and regulation of the fishery. The aim of this paper is to accurately indicate the size and age of first sexual maturity for female eastern Atlantic bluefin tuna.

## Materials and methods

### Sampling

Ovary and spine samples were collected from 501 bluefin tuna from May to September between 1998 and 2004 in the waters around the Balearic and Malta islands, in the South Adriatic, North Ionian, South Tyrrhenian and Northern Levantine seas and in the Sardinia Channel. The fish were caught by commercial vessels using long-lines, drift nets and purse seines and also traditional traps (*tonnare*) operating in Sardinia, Italy. For each fish caught the fork length (FL) was measured to the nearest centimetres and the date and place of capture recorded.

Ovary samples were fixed in Bouin's solution or 10% buffered formalin prior to histological analysis.

For age determination, the first spiniform ray of the first dorsal fin was taken and stored at −20°C.

### Ovary histology and reproductive state

Ovary samples were dehydrated in increasing ethanol concentrations, clarified in Histolemon and embedded in paraffin wax. Sections were cut (5 $\mu$m thickness) and stained with haematoxylin–eosin. The oocyte developmental stages were classified according to Corriero et al. (2003) and the reproductive state was assessed following Schaefer (1998). Oocyte atretic stages were classified according to Hunter and Macewicz (1985). On the basis of the classification scheme used, the distinction between immature and mature inactive fish was based on the presence of atresia of vitellogenic follicles, a sign of past reproductive activity. As previously reported (Corriero et al., 2003), no signs of atresia were observed in bluefin tuna captured some months after the reproductive season, due to their complete re-absorption. For the present study, samples collected during a temporal window from May to September only were used. This periodic sampling allowed a clear distinction between mature and immature specimens.

### Size at first sexual maturity

The body length at median sexual maturity ($L_{50}$) was estimated by fitting a logistic function to the fraction of mature fish per 5 cm FL intervals by nonlinear regression using the FISHPARM program (Saila et al., 1988). $L_{50}$ was defined as the smallest length interval in which 50% of the specimens were mature.

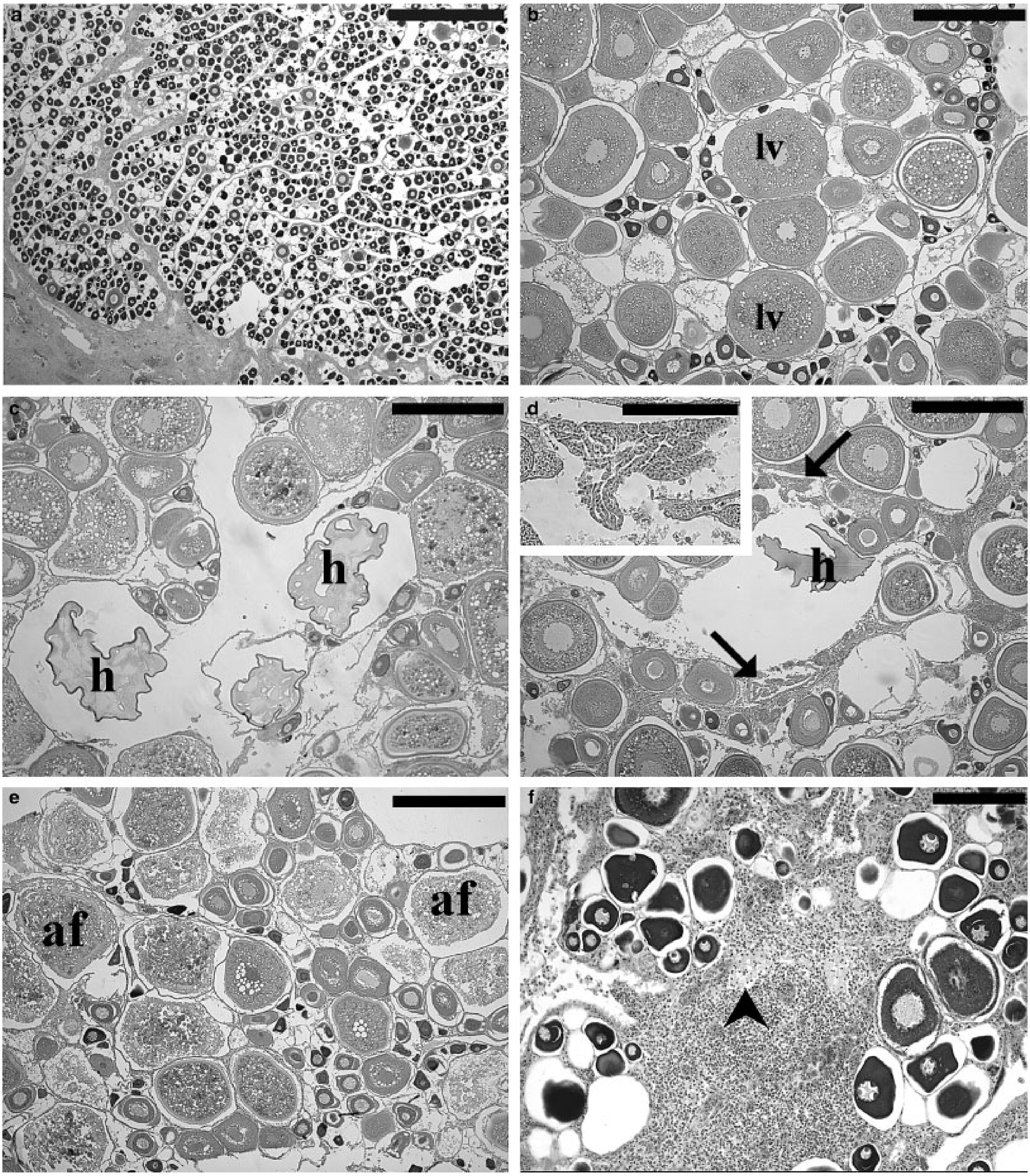


Fig. 1. Micrographs of the ovaries from bluefin tuna specimens captured in the Mediterranean Sea. Haematoxylin–eosin staining: (a) Ovary from an immature fish showing only perinucleolar stage oocyets (bar = 1000 $\mu$m); (b) Ovary from an active non-spawning specimen with late vitellogenic oocytes (bar = 500 $\mu$m); (c) Ovary from a spawning bluefin tuna with hydrated oocytes (bar = 500 $\mu$m); (d) Ovary from a spawning individual with both hydrated oocytes and post-ovulatory follicles (bar = 500 $\mu$m). Inset: higher magnification of a post-ovulatory follicles (bar = 300 $\mu$m); (e) Ovary from an inactive mature specimen characterized by major $\alpha$ atresia of vitellogenic follicles. (bar = 500 $\mu$m); (f) Ovary from an inactive mature fish showing perinucleolar stage oocytes and $\delta$ atretic follicles (bar = 100 $\mu$m). Arrow, post-ovulatory follicle; arrowhead, $\delta$ atretic follicle; af, $\alpha$ atretic follicles; h, hydrated oocyte; lv, late vitellogenic oocyte

**Age determination**

The age was determined for all fish belonging to size classes corresponding to $L_{50}$ (n = 20) and $L_{100}$ (the size for which all fish were mature; n = 40) using the technique described by Cort (1991) and Megalofonou (2000). Briefly, three serial cross-sections about 0.7 mm thick were obtained from each spine at the point near the condyle base using a low speed saw and diamond wafering blades. Spine sections were observed with a binocular lens microscope under transmitted light connected to the image analyser Quantimet 500 W (Leica, Cambridge, UK). Interpretation of growth bands was based on the recognition of the narrow translucent and wider opaque zones that are assumed to represent slow and fast growth, respectively. The number of translucent zones or rings, interpreted as annual events, was counted in order to assign an estimated age to the fish. As the nucleus of the spine is reabsorbed and the first rings begin to disappear at age 3, the mean diameter of the first rings of younger specimens was used to date the first visible ring of older specimens (Rodríguez-Marín et al., 2004). Two readings of each spine were made independently by one reader. When there was disagreement between counts of translucent bands, spines were read again for a third time.

## Results

### Ovary histology and reproductive state

On the basis of the ovary histological pattern (Fig. 1), 57 individuals or 11.3% of the specimens analysed were immature whereas 444 or 88.7% were mature.

### Body lengths at sexual maturity

Frequencies of immature and mature ovaries in different FL groups are shown in Fig. 2. No mature fish were found below 100 cm FL. The estimated body length at median sexual maturity ($L_{50}$) was 103.6 cm FL (SE = 0.99). Fittings of the logistic model resulted in:

$$Y = \frac{1}{1 + \exp[-0.1739(X - 103.6)]}$$

where $Y$ = % mature; and $X$ = body size (FL).

All fish above 135 cm FL were found to be mature.

### Age at sexual maturity

Twenty specimens constituted the size class that contained $L_{50}$ (100–104 cm FL), while 40 fish were included in the size class beyond which 100% maturity was reached (135–139 cm FL). All spines analysed showed the complete formation of the ring corresponding to their last year of life. Among the 20 fish included in the 100–104 cm size class, 16 belonged to age group 3 (Fig. 3a) and 4 to age group 4 (Fig. 3b). The 40 fish contained in the 135–139 cm size class belonged to age group 4 (4 specimens) and 5 (36 specimens; Fig. 3c).

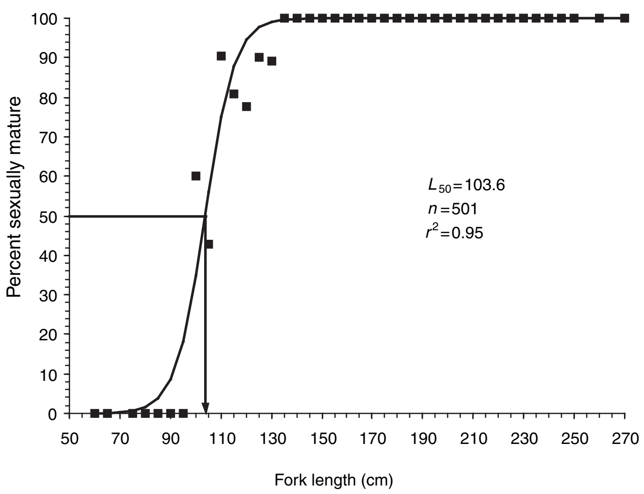


Fig. 2. Percent mature female bluefin tuna by 5-cm fork length interval, fitted to a logistic function. Arrow indicates body length at median sexual maturity ($L_{50}$). n = sample size


Fig. 3. Images of bluefin tuna spine sections. (a) Age 3 specimen with 103 cm FL captured on the 7 July; two rings are visible and one ring was reabsorbed. (b) Age 4 fish with 104 cm FL caught on 16 May; three rings are visible and one was reabsorbed. (c) Age 5 bluefin tuna with 138 cm FL sampled on 19 May; four rings are visible and one was reabsorbed. Arrows indicate visible rings. Magnification bars = 2 mm

## Discussion

The present paper represents the first attempt to determine size at sexual maturity for eastern Atlantic female bluefin tuna using a method based on the statistic elaboration of data coming from histological analysis (Lowerre-Barbieri et al., 1996; DeMartini et al., 2000).

On the basis of the macroscopic evaluation of the ovary maturity stage, Rodríguez-Roda (1967) estimated that 50% of the female bluefin tuna of the eastern stock are reproductively active at a size of 97.5 cm FL, while 100% maturity is reached between 115 and 120 cm FL. Tawil et al. (2002), in a preliminary approach to the study of sexual maturity based on the histological analysis of the ovaries of 21 bluefin tuna, found mature specimens above 115 cm FL. Further approximate information on the first sexual maturity of the eastern Atlantic bluefin tuna comes from investigations carried out for different aims. In a stereological study on bluefin tuna fecundity, Medina et al. (2002) reported that the smallest mature female sampled in Balearic waters was 116 cm FL. During a histological description of the ovarian cycle, mature females over 110 cm FL were found (Corriero et al., 2003).

Spine analyses of fish captured between May and September indicated that all specimens had completed the formation of the ring corresponding to their last year of life. This finding is in agreement with Cort (1991) and Megalofonou and De Metrio (2000), who reported that ring completion occurs during April and May for bluefin tuna caught in the Mediterranean. Our data indicates that the estimated age of most of the specimens with $FL = L_{50}$ is 3 years while 100% maturity is reached at 4–5 years. Age estimates reported in the present work are consistent with previous studies regarding age and growth of eastern Atlantic bluefin tuna carried out by the count of translucent zones in the dorsal spines. Cort (1991) and Megalofonou and De Metrio (2000) found bluefin tuna with $100 \leq FL < 105$ cm belonged to age group 3; Cort (1991) reported that most of the analysed fish of $130 \leq FL < 135$ cm were 5 years old.

Although to our knowledge there is no study reporting the size at 50% sexual maturity for the Western Atlantic bluefin tuna, the available data indicate that in this population, maturation starts at age 6 and 100% maturity is reached by age 8 at a size of 190 cm FL (Baglin, 1982; National Research Council (NRC), 1994).

The evidence reported in this paper confirms that the eastern Atlantic female bluefin tuna have a size and age of first sexual maturity that is markedly lower than the western Atlantic stock. This represents further evidence that leads to the scientific correctness of the separated management of the two stocks.

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