

First age and growth estimates in the deep water shark, *Etmopterus Spinax* (Linnaeus, 1758), by deep coned vertebral analysis

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Abstract The velvet belly *Etmopterus spinax* (Linnaeus, 1758) is a deep water bottom-dwelling species very common in the western Mediterranean sea. This species is a portion of the by-catch of the red shrimps and Norway lobsters otter trawl fisheries on the meso and ipo-bathyal grounds. A new, simple, rapid, and inexpensive vertebral preparation method was used on a total of 241 specimens, sampled throughout 2000. Post-cranial portions of vertebral column were removed and vertebrae were prepared for ageing readings. Band pair counts ranged from 0 to 9 in females, and from 0 to 7 in males. Von Bertalanffy growth equations estimated for both sexes suggested a higher longevity for females (males: $L_{\infty} = 394.3$ mm $k = 0.19$ $t_0 = -1.41$ $L_0 = 92.7$ mm $A_{99} = 18.24$ years; females: $L_{\infty} = 450$ mm $k = 0.16$ $t_0 = -1.09$ $L_0 = 72$ mm $A_{99} = 21.66$ years). Age estimations are discussed in the context of deep water shark species. This is the first successful attempt at delineating faint growth bands in the poorly calcified deep coned vertebrae of *E. spinax*. This technique may be used in other difficult poorly calcified species.

Introduction

In cartilaginous fish, hard and calcified structures, such as spines and vertebral centra, exhibit growth zones, identifiable

by an alternation of translucent and opaque areas (Ride-wood 1921; Urist 1961; Cailliet et al. 1983). Vertebral dimensions, as well as their degree of calcification, vary considerably within the elasmobranch group (La Marca 1966; Applegate 1967; Moss 1977). For example, vertebrae of coastal and pelagic species are more calcified than those of bottom dwelling deep-water sharks (Cailliet et al. 1986; Cailliet 1990). These differences are also reflected in variations of shape and in growth zone appearance, such as the presence and quality of bands and/or rings. Due to these differences, a general protocol for the elasmobranch group is not really available because of the high variability of calcification degree among species (Applegate 1967; Cailliet et al. 1983). Based on identification and count of band pairs, many techniques have been developed to assess age in cartilaginous species, assuming an annual periodicity (Cailliet et al. 1990). Even if deposition time should be properly validated within each species (Beamish and McFarlane 1983; Cailliet 1990; Campana 2001), age estimates have not always been validated (Campana 2001).

Etmopterus spinax is a bottom dwelling shark, typical of the bathyal stratum (200–1,000 m depth) of the Mediterranean sea (Tortonese 1956; Fischer et al. 1987; Notarbartolo Di Sciara and Bianchi 1998). It is caught as by-catch by commercial otter trawlers (Bertrand et al. 2000; Relini et al. 2000). *E. spinax* is characterized, as well as other deep bottom dwelling sharks (*Etmopterus baxteri*, *Galeus melastomus*, *Galeorhinus galeus*), by typical amphicoelous “deep coned” vertebrae; additionally its vertebrae are very small (Fig. 1), have little calcification and outer margins tend to refold on themselves as fish size increases (Fig. 11). Therefore, these features, together with the faint markings and fragile condition of the vertebrae, render them unsuitable to both most traditional ageing and validating increment periodicity techniques. The simple, inexpensive, and rapid

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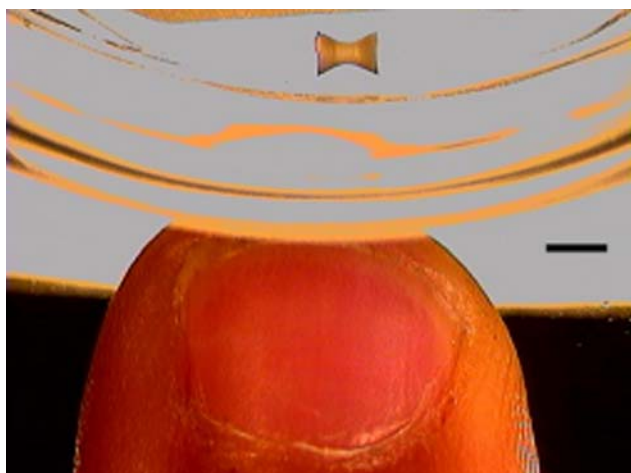


Fig. 1 Typical vertebral size compared to an observer's finger. Scale bar 1 mm

method proposed in this work, modified from Hoenig and Brown (1988), produced band elucidation for *E. spinax* vertebrae, leading to the first, but unvalidated, age results for this species.

Materials and methods

Samples were obtained from commercial otter trawl operations, conducted by Fiumicino marine, off the Latium coast (central Tyrrhenian sea) (Fig. 2). A combined effort of 16 hauls distributed in 3 bathymetrical strata (300–490 m, 491–580 m, and 581–900 m) was made seasonally during 2000. A total of 241 specimens of *Etmopterus spinax* (Linnaeus 1758) were collected and dissected. Each specimen was sexed (152 females and 89 males), measured (over the body total length and pre-caudal length, TL and PL respectively) to the nearest millimetre, and weighed (eviscerated weight, WE) to the nearest centigram. According to the estimates of velvet belly size at first maturity in the Mediterranean sea (Vacchi and Relini Orsi 1979; Fischer et al. 1987), specimens were divided into 3 size classes: “juveniles” (I: 100–200 mm TL, $n = 56$), “subadult” (II: 201–301 mm TL, $n = 139$), and “adult” (III: 301–435 mm TL, $n = 46$). Sex ratio was calculated for size class as follow: Females/(Females + Males) 100. A small post-cranial section of vertebral column (3–4 vertebrae) was removed from each specimen.

Vertebrae were prepared according to the method proposed by Hoenig and Brown (1988), but modified for this study as follow:

1. After removal, vertebral column section was stored at -20°C .
2. Section was dipped in a 10% in volume NaClO–water solution for 15 min. After being rinsed using distilled

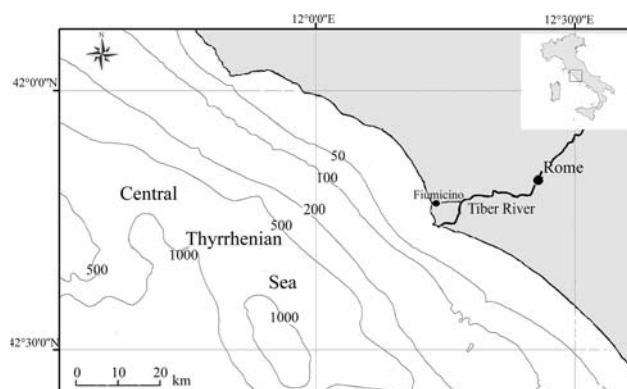


Fig. 2 Trawl grounds off the Latium coast where *Etmopterus spinax* specimens were sampled

water, it was dipped again in a new but same concentrated NaClO–water solution, for the same amount of time, and so on until complete removal of connective and muscular tissues, and separation of each single vertebra.

3. To measure the vertebral length (VL), each vertebra was put under transmitted light on a stereo-microscope provided with digital still camera, and millimeter paper for calibration. A digital image was acquired, and measurements were taken using Image-Pro[®]Plus. Due to the small sizes of these vertebrae, VL, rather than vertebral diameter, was chosen to analyze vertebral growth.
4. Each centrum was dipped into a 5% in volume $\text{Co}(\text{NO}_3)_2\text{-H}_2\text{O}$ solution in order to stain calcified areas. Time of immersion varied between 1 and 5 min, depending on vertebral dimension and hence on its degree of calcification. Each test tube had to be stirred gently in order to allow $\text{Co}(\text{NO}_3)_2$ penetration into the pronounced cavities of the deep-coned vertebra.
5. Vertebral centrum was rinsed with distilled water for few seconds in order to remove excess $\text{Co}(\text{NO}_3)_2$.
6. To enhance the bands, each centrum was dipped into an alcohol acid–water solution (obtained adding hydrochloric acid into a 70% in volume ethanol–water solution, with a ratio of 1:20) between few seconds to one minute, depending on vertebral dimension. Time of immersion was the most critical step of the entire preparation: a shorter time could not allow band enhancement, whereas a prolonged dipping could destroy the centrum.
7. After being rinsed using distilled water, the stained centrum was then viewed under the transmitted light using a stereo-microscope, and a digital image was obtained and read independently by two readers.

The criterion chosen for band readings is based on the identification of a dark layer followed by a lighter one defined

as a band pair (Cailliet et al. 2006). Each band was considered a temporal growth zone (Fig. 3).

Each reader counted, twice for each centrum, dark bands on the vertebral outer surface, from the core toward the distal margin (EA = estimated age). Counting was performed by each reader without knowledge either of the specimen length or the other reader's count.

Statistical analysis

Differences in sex ratio among sizes classes were tested by a chi-square Test on a 2×3 contingency table.

Linear regression analysis was used to calculate length-weight relationships (total length on transformed natural logarithm of eviscerated weight), as well as the VL-TL, and EA-TL correlations. A natural logarithmic transformation of (EA + 1) was also needed due to the presence of "0" values. ANCOVA analysis was then used to investigate sex differences for all the aforementioned relationships.

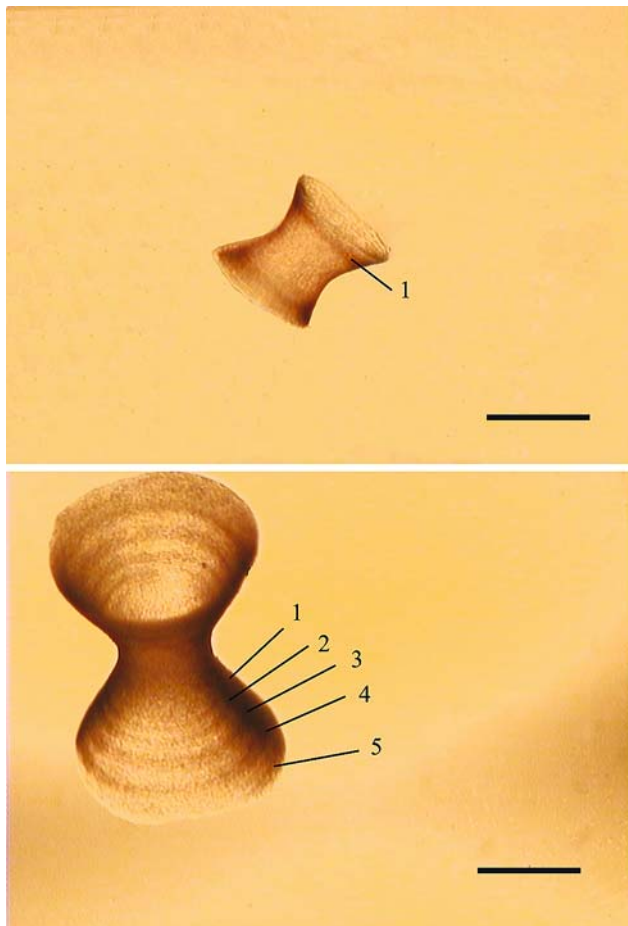


Fig. 3 Magnified digital images of deep coned vertebrae of *Etmopterus spinax* immersed in rinsed water and observed through stereomicroscope transmitted light after band enhancing treatment. *Upper* one band (age group 1), male, 150 mm TL; *Lower* five bands (age group 5), female, 275 mm TL. Scale bar 1 mm

Difference in percent of occurrence between type errors (± 1 and ± 2) was tested by a Chi-square test.

Estimates of precision were evaluated following the methodology suggested by Goldman (2004): total percentage agreement (PA), percentage agreement plus-minus one band ($PA \pm 1$) and percentage agreement by size classes PA (I, II, III) were calculated both between and within readers. Chi-square test on contingency tables was utilized to check for bias in all these cases. The age-bias curve (Campana et al. 1995) was utilized to test for bias between readers within age groups. In addition the non-parametric Wilcoxon Test (Conover 1971), index of average percent error (IAPE), and mean coefficient of variation (CV) (Beamish and Fournier 1981) were calculated in order to provide further estimates of precision in band count estimation between readers. The statistical package Statistica (6.0) 2006 was used for the aforementioned analyses.

The von Bertalanffy growth parameters were determined for each sex using the fishery program FISHPARM (Prager et al. 1987), using the equation $L(t) = L_{\infty}(1 - e^{-k(t-t_0)})$ (Bertalanffy 1960). Longevity was estimated through the algorithm $A_{99} = 5 \cdot \ln(2)/k$ (Fabens 1965), where A_{99} is the time (in years) passed before reaching 99% of L_{∞} and k is the growth constant derived from the von Bertalanffy growth equation. Extrapolated longevity, based upon A_{99} , is believed to give a more realistic estimate of the maximum age rather than A_{97} and A_{95} (Skomal and Natanson 2003).

Results

Systematic error did not occur in estimates of PA, $PA \pm 1$, and PA by length classes both between readers and within readers 1 and 2 (Table 1). PA by size classes decreased significantly with increasing fish length both between and within readers, resulting the lowest in the largest size class. The confidence intervals of all age groups (Fig. 4) enclosed the reference diagonal (line that bisects graph area passing through the origin with an angle of 45°) demonstrating no significant bias occurred in band counting within all age groups between readers. Error type ± 1 band occurred at frequency (14.11%) significantly ($\chi^2 = 14.22$, $df = 1$, $P < 0.001$) much higher than type ± 2 bands (0.83%) between readers whose counts did not show significant difference (Wilcoxon Test, $T = 297.50$, $n = 241$, $P < 0.6$; IAPE = 1.86%, mean CV = 1.02%).

Ten (0+ to 9) and eight (0+ to 7) age groups were found in females and males, respectively (Fig. 5). Band deposition periodicity was assumed annual due to the impossibility of validating it.

Females significantly ($\chi^2 = 16.71$, $df = 2$, $P < 0.001$) outnumbered males in all sizes classes with the most dramatic difference in the adult class (I = 58.93%, II = 56.12%,

Table 1 Precision in estimate of total percent agreement (PA), percent agreement plus/minus one band ($PA \pm 1$) and percent agreement by size classes (PA I, PA II, PA III) between and within readers in *Etmopterus spinax* from central Tyrrhenian Sea

	Between readers	Within reader 1	Within reader 2
PA	85.06%	89.21%	85.48%
df	1	1	1
χ^2	59.25	74.11	60.66
P	<0.001	<0.001	<0.001
$PA \pm 1$	99.17%	98.34%	97.51%
df	1	1	1
χ^2	116.53	112.63	108.80
P	<0.001	<0.001	<0.001
PA (I)	87.50%	92.86%	87.50%
PA (II)	88.49%	91.37%	89.93%
PA (III)	71.74%	78.26%	69.56%
df	2	2	2
χ^2	7.97	7.17	9.40
P	<0.05	<0.05	<0.01

III = 89.13%). Since no differences were found in the slopes of TL-EW and of TL-VL relationships between sexes, data were pooled resulting in single regressions (Figs. 6, 7, respectively). Mean eviscerated weight and vertebral length resulted both higher in females compared to males (ANCOVA, $F_{1,238} = 5.02$, $P < 0.05$ and $F_{1,238} = 6.45$, $P < 0.05$, respectively). Conversely, VL-EA relationships (Fig. 8) differed between sexes, revealing a significantly

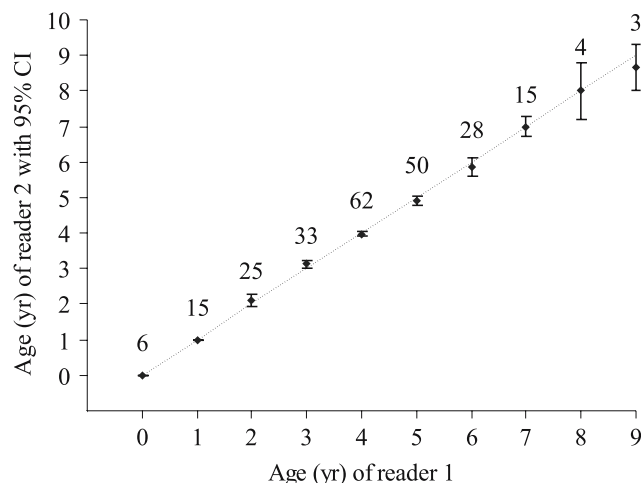


Fig. 4 Age bias graph for age groups of *Etmopterus spinax*. Dotted line, vertical bars and numbers above refer respectively to main diagonal, mean estimated age confidence intervals and sample size for each age groups

higher slope for the female regression therefore indicating that estimated age varied with vertebral length depending on sex. For this reason the Von Bertalanffy growth equation was calculated separately for females (Fig. 9) and males (Fig. 10). Males attained a smaller L_{∞} with a higher k value than females (Table 2). Estimated size at birth (L_0) and longevity (A_{99}) were 72.0 mm and 21.66 years, respectively, for females, and 92.7 mm and 18.24 years, respectively, for males.

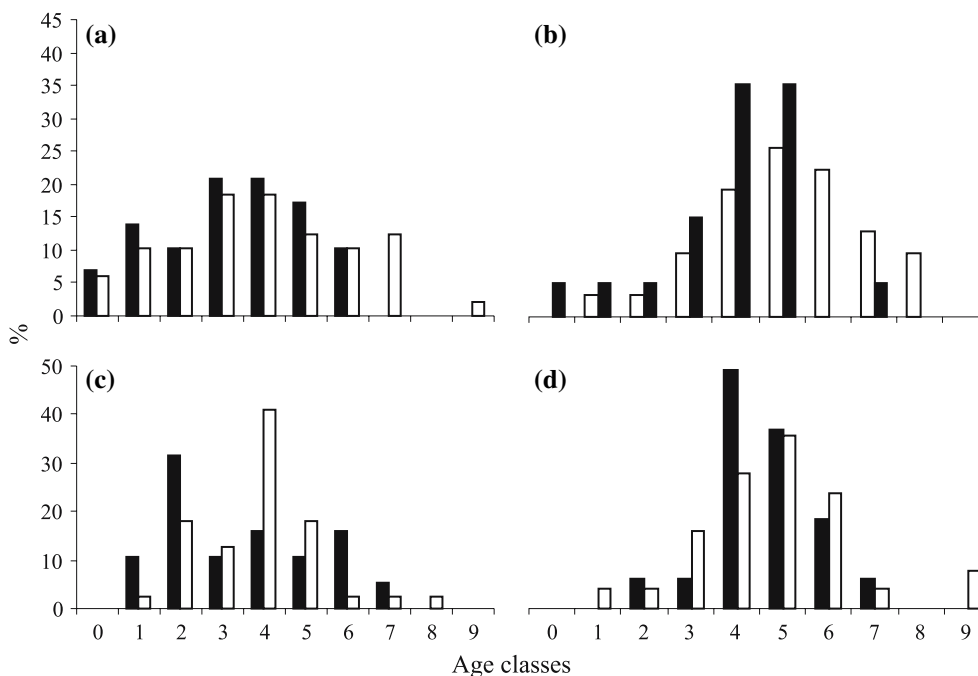


Fig. 5 Age groups of females (white bars) and males (dark bars) of *Etmopterus spinax* in the four seasons (a winter, b spring, c summer, d autumn) from central Tyrrhenian Sea

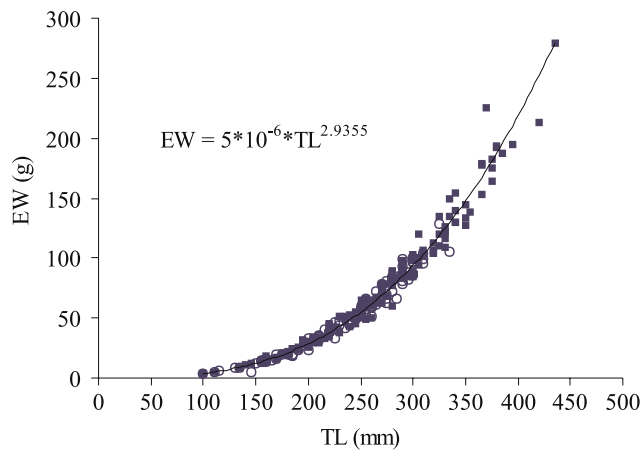


Fig. 6 Relationship between total length (TL) and eviscerated weight (EW) ($F_{1,239} = 15,672.59$, $R^2 = 0.98$, $P < 0.001$) for pooled data (Test for Homogeneity of Slope: $F_{1,237} = 2.65$, $P < 0.10$) of males (open circle) and females (filled square) of *Etmopterus spinax* from central Tyrrhenian Sea

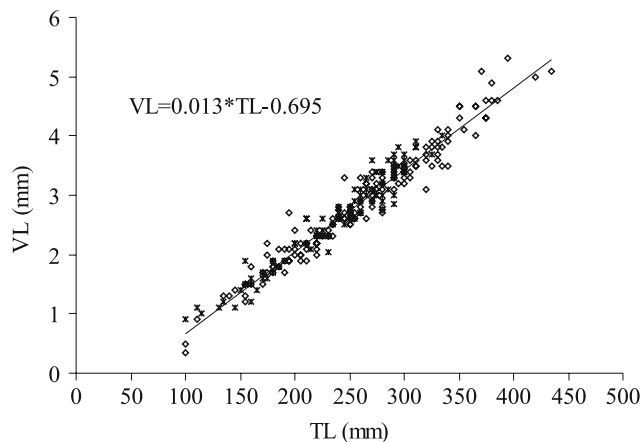


Fig. 7 Relationship between total length (TL) and vertebral length (VL) ($F_{1,239} = 4,201.8$, $R^2 = 0.94$, $P < 0.001$) for pooled data (Test for Homogeneity of Slope: $F_{1,237} = 0.33$, $P < 0.6$) of males (asterisk) and females (diamond) of *Etmopterus spinax* from central Tyrrhenian Sea

Discussion

The proportion of females found in this study increased in the bigger/older size class, as observed in other Mediterranean areas (Punnett 1904; Vacchi and Relini Orsi 1979; Sion et al. 2002; Cecchi et al. 2004).

The dimorphism in size found between sexes is confirmed both by a higher mean weight and a bigger mean vertebral size for females. Nevertheless, the ontogeny of both variables was similar between sexes. Cecchi et al. (2004) described a comparable trend in mean weight for the same species, as Jakobsdóttir (2001) and Cannizzaro et al. (1995) previously reported for the Atlantic co-generic

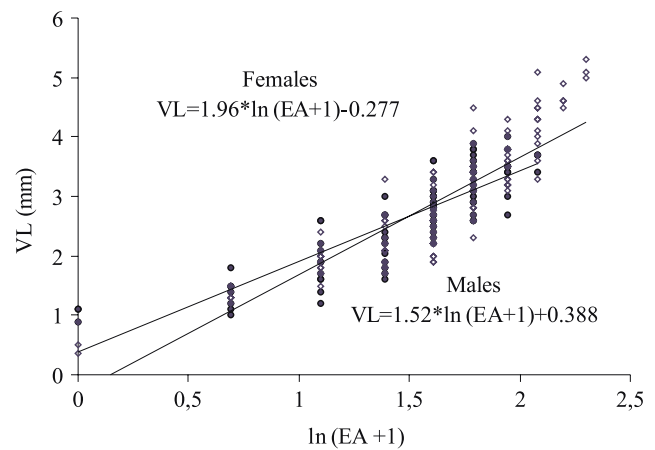


Fig. 8 Relationships (Test for Homogeneity of Slope: $F_{1,237} = 10.25$, $P < 0.01$) between vertebral length (VL) and transformed natural logarithmic of estimated age (EA) in males (filled circle) ($F_{1,87} = 240.76$, $R^2 = 0.73$, $P < 0.001$) and females (diamond) ($F_{1,150} = 469.10$, $R^2 = 0.76$, $P < 0.001$) of *Etmopterus spinax* from central Tyrrhenian Sea

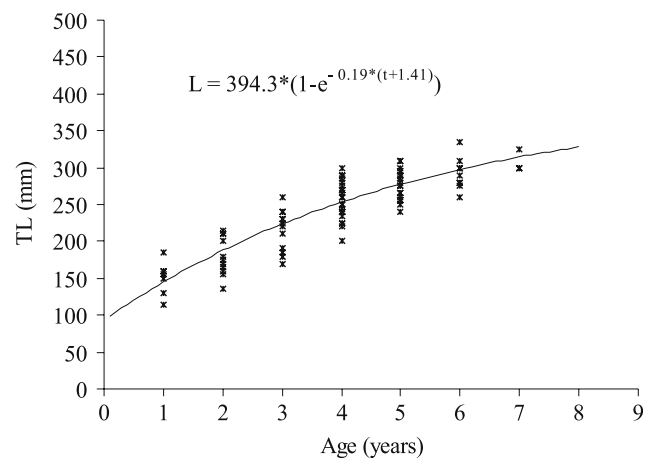


Fig. 9 Von Bertalanffy growth curve in *Etmopterus spinax* males from central Tyrrhenian Sea

E. princeps, and for *Squalus blainvillei*, respectively. All these similar observations confirmed the hypothesis of the latter authors that growth rate is similar between sexes in elasmobranchs until sexual maturation, then rate starts to decrease independently by sex. In fact according to Vacchi and Relini Orsi (1979), *E. spinax* males mature earlier than females (280 vs. 340 mm TL) in the Gulf of Genoa.

The validity of the adopted method was ascertained by the very low level of disagreement found in band count within and between observers and within each age group, according to combined methodology suggested by Calliet et al. (2006). Also a CV of less than 7.6%, corresponding to an IAPE of 5.5%, is considered to be adequate for age estimation precision obtained by band counts in vertebral structures (Campana 2001). The low level of disagreement

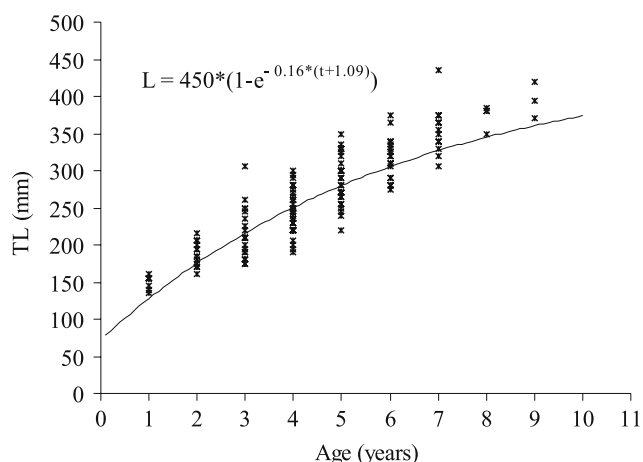


Fig. 10 Von Bertalanffy growth curve in *Etmopterus spinax* females from central Tyrrhenian Sea

Table 2 Asymptotic standard error (ASE), Coefficient of Variation (CV) and estimate for asymptotic length (L_{∞}), rate of growth (k), time (t_0) and length (L_0) at birth in males and females of *Etmopterus spinax* from central Tyrrhenian Sea

Parameter	Females			Males		
	Estimate	ASE	CV	Estimate	ASE	CV
L_{∞} (mm)	450	46.33	0.01	394.3	59.72	0.15
K (years ⁻¹)	0.16	0.04	0.24	0.19	0.07	0.38
t_0 (years)	-1.09	0.39	-0.36	-1.41	0.59	-0.42
L_0 (mm)	72			92.7		

observed in this study was probably due to the high definition digital images utilized for counting. Nevertheless, disagreement between observers increased as fish size increased, and the most common error was plus/minus one band as also Correia and Figueiredo (1997) reported for the deep water blackmouth catshark *Galeus melastomus*.

Misjudgements are usually attributed to many reasons (Campana 2001; Skomal and Natanson 2003): firstly, random error probability among readers could increase because of a greater number of bands to count on larger specimens, since they have more bands than smaller conspecifics. A further source of bias might be originated from the weak separation between distal bands, which is due to reduced growth rate in older individuals (Bertalanffy 1960; Cortés 2000), and therefore bands tend to be deposited at smaller intervals as vertebral length increases. Moreover, such disagreement might be explained since some distal bands remained hidden to observers because in some sharks, for instance *E. spinax*, outer vertebral margins tend to refold on themselves in older specimens as vertebral size increases (Fig. 11); therefore preventing reading and measurements of the latest rings in the older specimens.

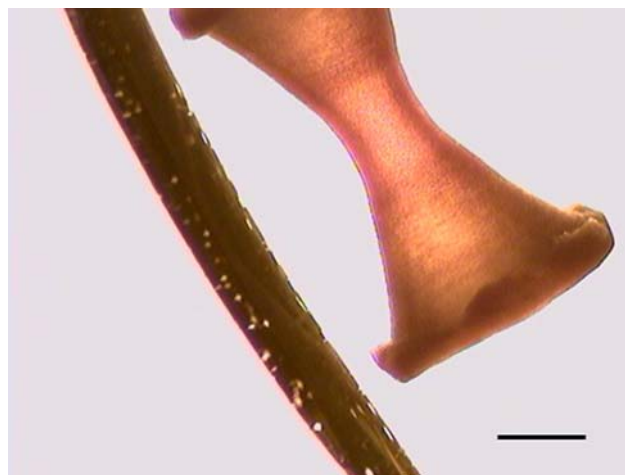


Fig. 11 Example of refolding of larger specimens' vertebrae. Scale bar 1 mm

Vertebral folding is the main reason why increment periodicity was not confirmed in this study, since validating increment periodicity is highly recommended across the entire age range (Beamish and McFarlane 1983; Campana 2001). Even in the co-generic *E. baxteri*, validation was not possible since most traditional techniques are unsuitable for deep water species (Irvine et al. 2006).

Notwithstanding problems in working with so little, fragile and poorly calcified vertebrae, the new methodology proposed in this study was able to successfully enhance vertebral bands. In fact, both calculated asymptotic length and size at birth in this study agree with the data reported for *E. spinax* in the Mediterranean Sea (Fischer et al. 1987), even though size at birth was slightly underestimated for females. Those authors indicated that the maximum reported size is 450 mm TL, and that newborns range between 90–110 mm in size, although some other authors stated the occurrence of 85 mm TL newborns in the Gulf of Genoa (Vacchi and Relini Orsi 1979).

Growth parameters and age groups found in this study are similar to those calculated, through spine age analysis (Sion et al. 2002) and indirect methods (i.e. Bhattacharya, Normsep) (Cecchi et al. 2004), from Ionian and northern Tyrrhenian Sea populations of *E. spinax*, respectively. From the first study, only 7 age classes were found, rather than 9 found in this study. From the second study, both L_{∞} and k were a little different compared to this study, resulting in a slight higher maximum estimated age for both males and females. Such differences may be due either to methodology, or to real differences in comparing natural populations (Tanaka et al. 1990; Skomal and Natanson 2003).

Another important confirmation of the validity of this study, is given by the fact that males result to grow faster and reach a smaller L_{∞} compared to females, which consequently grow older (10 vs. 8 age classes for females and

males, respectively). The higher longevity and size achieved by females is a common feature in several species of elasmobranchs (Cortés 2000): it was ascertained in coastal (Ferreira and Vooren 1991) and deep-water squaliformes (Rey et al. 2002), in large pelagic species (Parker and Stott 1965; Cailliet et al. 1985) and squatiniformes (Natanson 1984). Therefore, since elasmobranch males attain size at first maturity earlier than females (then, their growth rate sharply decreases), differences in maximum length and longevity between sexes are due to a delayed female maturation. In this way, the occurrence of bigger females in mature populations deals with an evolutionary advantage because of the greater fecundity of bigger specimens (Cortés 2000).

Comparing *E. spinax* growth rate with data available for similar-size Mediterranean squaliformes, it grows with a rate more similar to the coastal *S. blainvillei* (Cannizzaro et al. 1995) than to the deep water catshark *G. melastomus* (Correia et al. 1999 for Atlantic waters). In fact, growth rate of *E. spinax* is unusually high within deep water species, if compared, for instance, to *Centrophorus squamosus* (Clarke et al. 2002) and *Squalus mitsukurii* (Taniuchi and Tachikawa 1999) for other areas. In order to explain such a result, three suggestions might be formulated: underestimation age bias, species-specific differences, and nature of inhabited ecosystems;

- Coastal and epipelagic shark species of temperate areas show a more marked alternation of growth increments compared to the poorly calcified vertebrae of deep-water species. It may be due to the pronounced seasonality of such environments as compared to the deep sea (Newton 1999; Randall and Farrell 2002; MacNeil and Campana 2002). Therefore, the fainter nature of band separations within deep-water species can represent a source of underestimation bias in growth increment count. It could reflect the overestimation of growth rates for deep water velvet belly, as well as the difficulties in reading outer bands due to many reasons (folded margin, bands very close to each other, very thin distal margin).
- Species-specific peculiarities among shark species also have to be considered. The high grow rate calculated for the velvet belly might be imputable to specific constrains (i.e. being better opportunistic feeders, higher energy contents of exploited food resources, etc.).
- Coastal and epipelagic ecosystems generally differ from meso-bathypelagic ones by the presence of a marked seasonality, greater food resource availability, and therefore pronounced variability of several biotic and abiotic factors (Cognetti and Sará 1974; Meadows and Campbell 1978). But the peculiar homeothermic condition of warm deep water masses of the Mediterranean Sea (Cognetti and Sará 1974; Meadows and Campbell 1978), reflecting

in a more favourable environment, could play an important role in affecting the growth rate of deep water sharks such as *E. spinax*, which is well adapted to these ecosystems.

Extrapolated longevity shows that, despite its size, *E. spinax* is a long-living animal, with females attaining greater age than males, as it is typical of most elasmobranchs (Cortés 2000). These features, together with late maturity and low fecundity, render such a taxon greatly vulnerable to over fishing (Stevens et al. 2000), both as targeted and by-catch commercial species.

In conclusion, this new technique is able to delineate faint growth bands in the poorly calcified and fragile deep coned vertebrae of *E. spinax*. Therefore this methodology may be used in the future, in order to successfully outline vertebral increments in other difficult species.

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References

- Applegate SP (1967) A survey of shark hard parts. In: Gilbert PW, Mathewson RG, Rall DP (eds) Sharks, skates and rays. Johns Hopkins Press, Maryland, pp 36–67
- Beamish RJ, Fournier DA (1981) A method for comparing the precision of a set of age determinations. Can J Fish Aquat Sci 38:982–983
- Beamish RJ, McFarlane GA (1983) The forgotten requirements for age validation in fisheries biology. T Am Fish Soc 112:735–743
- Bertalanffy L von (1960) Principles and theory of growth. In: Woiniski WW (ed) Fundamental aspects of normal and malignant growth. Elsevier, Amsterdam, pp 137–259
- Bertrand J, Gil De Sola L, Papaconstantinou C, Relini G, Souplet A (2000) Contribution on the distribution of elasmobranchs in the Mediterranean Sea (from the MEDITS surveys). Biol Mar Medit 7(1):385–399
- Cailliet GM (1990) Elasmobranch age determination and verification: an updated review. In: Pratt HL Jr, Gruber SH, Taniuchi T (eds) Elasmobranchs as living resources: advances in the biology, ecology, systematic, and status of the fisheries. US Dep. Commer., NOAA Tech Rep 90 pp 157–165
- Cailliet GM, Martin LK, Kusher D, Wolf P, Welden BA (1983) Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. In: Prince ED, Pulos LM (eds) Proceedings of the international workshop on age determination of oceanic pelagic fishes: tunas, billfishes, and sharks. US Dep. Commer., NOAA Tech Rep NMFS vol 8, pp 157–165
- Cailliet GM, Natanson JL, Welden BA, Ebert DA (1985) In: Preliminary studies on the age and growth of the white shark, *Carcharodon carcharias*, using vertebral bands Memoirs of the Southern California Academy of Science vol 9 pp 49–60
- Cailliet GM, Radtke RL, Welden BA (1986) Elasmobranch age determination and verification: a review. In: Uyeno T, Arai R, Taniuchi T, Matsuura K (eds) Indo-Pacific fish biology: proceedings of the second international conference on Indo-Pacific fishes. Ichthyological Society of Japan, Tokyo, pp 345–360

- Cailliet GM, Smith WD, Mollet HF, KJ Goldman (2006) Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. *Environ Biol Fish* 77:211–228
- Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J Fish Biol* 59:197–242
- Campana SE, Annand CM, McMillan JI (1995) Graphical and statistical methods for determining the consistency of age determination. *T Am Fish Soc* 124:131–138
- Cannizzaro L, Rizzo P, Levi D, Gancitano S (1995) Age determination and growth of *Squalus blainvillei* (Risso, 1826). *Fish Res* 23:113–125
- Cecchi E, Mancusi C, Pajetta R, Serena F (2004) Contributo alla conoscenza della biologia di *Etmopterus spinax* (Linnaeus, 1758) (Chondrichthyes, Etmopteridae). *Biol Mar Medit* 11(2):564–568
- Clarke MW, Connolly PL, Bracken JJ (2002) Age estimation of the exploited deepwater shark *Centrophorus squamosus* from the continental slopes of the Rockall Trough and Porcupine Bank. *J Fish Biol* 60:501–514
- Cognetti G, Sará M (1974) *Biologia marina*. Calderini, Bologna
- Conover WJ (1971) The Wilcoxon signed rank test. In: Conover WJ (ed) *Practical nonparametric statistics*. Wiley, New York pp 206–216
- Correia JP, Figueiredo I (1997) A modified decalcification technique for enhancing growth bands in deep-coned vertebrae of elasmobranchs. *Environ Biol Fish* 50:225–230
- Correia JP, Figueiredo I, Silva A (1999) Age and growth of Blackmouth Catshark, *Galeus melastomus*, from Portuguese waters. In: *Proceedings of American Elasmobranch Society. 1999 Annual Meeting State College, Pennsylvania*
- Cortés E (2000) Life history patterns and correlations in sharks. *Rev Fish Sci* 8(4):299–344
- Fabens AJ (1965) Properties and fitting of the von Bertalanffy growth curve. *Growth* 29:265–289
- Ferreira BP, Vooren CM (1991) Age, growth and structure of vertebra in the school shark, *Galeorhinus galeus* (Linnaeus, 1751), for southern Brazil. *Fish Bull* 89:19–31
- Fischer W, Bauchot ML, Schneider M (1987) *Fiches FAO d'identification des espèces pour les besoins de la pêche. Méditerranée et Mer Noire. Zones de pêche 37 (2) Vertébrés*. FAO, Rome
- Goldman KJ (2004) Age and growth of elasmobranch fishes. In: Musick JA, Bonfil R (eds) *Elasmobranch fisheries management techniques*. Asia Pacific Economic Cooperation, Singapore, vol 370 pp 97–132
- Hoening JM, Brown CA (1988) A simple technique for staining growth bands in elasmobranch vertebrae. *B Mar Sci* 42(2):334–337
- Irvine SB, Stevens JD, Laurenson LJB (2006) Comparing external and internal dorsal-spine bands to interpret the age and growth of the giant lantern shark, *Etmopterus baxteri* (Squaliformes: Etmopteridae). *Environ Biol Fish* 77(3–4):253–264
- Jakobsdóttir KB (2001) Biological aspects of two deep-water squalid sharks: *Centroscyllium fabricii* (Reinhardt, 1825) and *Etmopterus princeps* (Collett, 1904) in Icelandic waters. *Fish Res* 51:247–265
- La Marca MJ (1966) A simple technique for demonstrating calcified annuli in the vertebrae of large elasmobranchs. *Copeia*:351–352
- Linnaeus C (1758) *Systema Naturae*, Ed. X. (Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata) Holmiae. *Systema Nat. ed. 10. i-ii*: 824 pp
- MacNeil MA, Campana SE (2002) Comparison of whole and sectioned vertebrae for determining the age of young blue shark (*Prionace glauca*). *J Northwest Atl Fish Soc* 30:77–82
- Meadows PS, Campbell JI (1978) *Introduction to marine science*. Blackie, Glasgow
- Moss ML (1977) Skeletal tissues of sharks. *Am Zool* 17:335–342
- Natanson LJ (1984) Aspects of age, growth, and reproduction of the Pacific angel shark, *Squatina californica*, off Santa Barbara, California. M.A. thesis, San Jose State Univ., San Jose, pp 71
- Newton GM (1999) The deep-sea environment—earth's final frontier. *Aust Mar Sci Bull* 147:17–21
- Notarbartolo Di Sciara G, Bianchi I (1998) *Guida degli squali e delle razze del Mediterraneo*. Franco Muzzio Editore
- Parker HW, Stott FC (1965) Age, size and vertebral calcification in the basking shark *Cetorhinus maximus* (Gunnerus 1765). *Zool Meded* 40(34):305–319
- Prager MN, Saila SB, Reccsiek CW (1987) FISHPARM: a computer program for parameter estimation of nonlinear models in fishery science. Dep. Oceanogr. Old Dominion University of Norfolk, U. A. Tech Rep, 87(10):1–37
- Punnett RC (1904) Merism and sex in *Spinax niger*. *Biometrika* 3:313–365
- Randall DJ, Farrell AP (2002) Deep-sea fishes. In: Randall DJ, Farrell AP (eds) *Fish physiology series vol 16*. Academic, New York pp 350
- Relini G, Biagi F, Serena F, Belluscio A, Spedicato MT, Rinelli P, Follera MC, Piccinetti C, Ungaro N, Sion L, Levi ED (2000) I Selaci pescati con lo strascico nei mari italiani. *Biol Mar Medit* 7(1):347–384
- Rey J, Massuti E, De Sola LG (2002) Distribution and biology of the blackmouth catshark, *Galeus melastomus*, in the Alboran sea (south-western Mediterranean). NAFO SCR Doc. 02/96. Scientific council meeting, September 2002. Santiago de Compostela, Spain
- Ridewood WG (1921) On the calcification of the vertebral centra in sharks and rays. *Philos Trans R Soc Lond B* 210:311–407
- Sion L, D'Onghia G, Carlucci R (2002) A simple technique for ageing the velvet belly shark, *Etmopterus spinax* (Squalidae). In: Vacchi M, La Mesa G, Serena F, Séret B (eds) *Proceedings of 4th European Elasm Association Meeting, Livorno 2000*. ICRAM, ARPAT & SFI, 2002 pp 135–139
- Skomal GB, Natanson LJ (2003) Age and growth of the blue shark (*Prionace glauca*) in the north Atlantic Ocean. *Fish Bull* 101:627–639
- STATISTICA 6.0 (Electronic Version): StatSoft, Inc. (2006). *Electronic Statistics Textbook*. Tulsa, OK: StatSoft. web: <http://www.statsoft.com/textbook/stathome.html>. (Printed Version): Hill T, Lewicki P (2006). *STATISTICS Methods and Applications*. StatSoft, Tulsa, OK
- Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000) The effects of fishing on sharks, rays, and chimaeras (Chondrichthyans), and the implications for marine ecosystems. *ICES J Mar Sci* 57:476–494
- Tanaka S, Cailliet GM, Yudin KG, (1990) The Status of the fisheries differences in growth of the blue shark, *Prionace glauca*: technique or population? In: Pratt HL Jr, Gruber SH, Taniuchi T (eds) *Elasmobranchs as living resources: advances in the biology, ecology, systematic*. NOAA Tech Rep, NMFS, 90:177–187
- Taniuchi T, Tachikawa H (1999) Geographical variation in age and growth of *Squalus mitsukurii* (Elasmobranchii: Squalidae) in north Pacific. In: Séret B, Sire JY (eds) *Proceedings of 5th Indo-Pacific Fish Conference, Nouméa, 1997*. Paris Soc Fr Ichtyol, pp 321–328
- Tortonese E (1956) *Leptocardia, cyclostomata vel Selachii*. Fauna d'Italia. Calderini, Bologna
- Urist MR (1961) Calcium and phosphorus in the blood and skeleton of the elasmobranchs. *Endocrinology* 69:778–801
- Vacchi M, Relini Orsi L (1979) Aspetti riproduttivi in *Etmopterus spinax* L. (Chondrichthyes, Squalidae). *Quad Civ Staz Idrobiol Milano* 7:63–74