

# Age and Growth Characteristics of Marbled Electric Ray *Torpedo marmorata* (Risso, 1810) Inhabiting Iskenderun Bay, North-eastern Mediterranean Sea

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#### Abstract

The age and growth characteristic of the marbled electric ray (Torpedo marmorata) was determined inhabiting in Iskenderun Bay is located in north-eastern Mediterranean Sea. A total of 117 specimens sampled caught 52% were female and 48% were male. The total length to weight relationship was determined as: W=0.0195×L2.9856. This study is the first known example using known aging methods for this species. The growth parameters that were derived by using the von-Bertalanffy method for this species were: L $\infty$ =57.31cm, k=0.187053 year-1 and to=-0.39231 year-1. According to the Marginal Increment Analysis it was found that age band growth was annual. This is the first and only known information in regards to the age and growth of this species.

Keywords: Marbled electric ray, Torpedinidae, chondrichthyes, staining method, safranin-O.

## Kuzeydoğu Akdeniz, İskenderun Körfezi'ndeki Elektrikli Vatoz (*Torpedo marmorata*)'un Yaş ve Büyüme Özellikleri

## Özet

Bu çalışma, Elektrikli vatoz (*Torpedo marmorata*) 'un yaş ve büyüme özelliklerinin tespit edilmesi amacıyla kuzeydoğu Akdeniz'deki İskenderun Körfezi'nde gerçekleştirilmiştir. Örneklenen toplam 117 bireyden % 52'si dişi ve % 48'inin erkek bireylerden oluştuğu tespit edilmiştir. Boy-Ağırlık ilişkisi TW = W=0.0195×L2.9856 şeklinde hesaplanmıştır. Bu türe ait ilk yaş tayini bu çalışma ile ortaya konulmuş olup, ilk defa Safranin-O boyama yöntemi ile yaş tayini yapılmıştır. Türün von-Bertalanffy büyüme parametreleri L∞=57.31cm, k=0.187053yıl-1 ve to=-0.39231yıl-1 olarak hesaplanmıştır. Ayrıca Kenar Büyüme Analiz'ine göre yaş halkalarındaki büyümenin yıllık olduğu tespit edildi. Böylelikle bu türün yaş ve büyüme parametreleri ile ilgili ilk bilgilere bu çalışma ile ulaşılmıştır.

Anahtar Kelimeler: Elektrikli vatoz, Torpedinidae, kıkırdaklı balıklar, boyama yöntemi safranin-O.

## Introduction

Torpedinidae are commonly known as electric rays. There are three species (*Torpedo marmorata, T. nobiliana* and *T. torpedo*) that have been found near the Turkish coast of the Mediterranean Sea (Başusta and Erdem, 2000; Bilecenoglu *et al.*, 2002; Golani *et al.*, 2006).

There are presently many studies such as: systematic, zoogeographical distribution, reproduction biology, diet composition, age determination, growth parameters, length-weight parameters and histology on the cartilaginaous fishes in the northeastern Mediterranean (Başusta *et al.*, 1998; Başusta, 2002; Ismen, 2003; Başusta *et al.*, 2008; Çek *et al.*, 2009; Yeldan *et al.*, 2009; Bircan-Yıldırım *et al.*, 2011; Başusta *et al.*, 2012a; Başusta *et al.*, 2012b; Başusta and Sulikowski, 2012). Although there are some researchs on Torpedinidae, there is no information about the age and growth for *T. marmorata* inhabiting the Mediterranean Sea (Mellinger, 1971; Capapé, 1979; Abdel-Aziz, 1994; Capapé *et al.*, 2006; Consolvo *et al.*, 2007). Unfortunately, this life history characteristic is lacking for most cartilaginous species within the eastern Mediterranean Sea.

Torpedinid fishes are assessed as data deficient (DD) globally due to lack of biological data on catches and population trends by the *International Union for Conservation of Nature* (IUCN) (Abdul Malak *et al.*, 2011). Age information forms the basis

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for the calculations of growth rate, mortality rate and productivity, making it one of the most influential variables for estimating a population's status and assessing the risks associated with over fishing (Cailliet and Goldman, 2004; Goldman, 2005). However erroneous age estimates can negatively affect management of marine resources (Campana, 2001). Most fish age-estimation studies have assumed that growth increments occur annually but not all test this assumption. Marginal increment analysis (MIA) are among the most frequently employed (Okamura *et al.*, 2013). This study is to estimate for the first time the age and growth parameters of the *T. marmorata* found in the Iskenderun Bay, north-eastern Mediterranean Sea.

## **Materials and Methods**

#### Sample Collection

Marbled electric rays were collected by a commercial fisherman using gill nets at approximate depths ranging from 8 to 20m in Iskenderun Bay (Figure 1), between September 2010 and December 2011. The Total Length (TL in cm) was measured as a straight line from the tip of the rostrum to the end of the tail. The disc width (DW in cm) was measured as a straight line between the tips of the widest portion of the pectoral fins. The total wet weight (in grams) was also recorded.

## **Preparation of Vertebral Samples**

A block of 12 vertebral centres were taken from above the abdominal cavity of 117 *T. marmorata*, labeled, and stored frozen (Turkmen *et al.*, 2005). Soft tissue was removed from the frozen vertebral segments using a scalpel and fine forceps. The individual vertebrae were then cut apart from each other and soaked in warm distilled water. Hypochlorite (6%) was used to remove the last remaining bits of connective tissue from the vertebrae. However, hypochlorite can decalcify cartilage when overused, so soak times were kept to nearly 10 minutes. The vertebrae were then air-dried for no less than 48 hours.

Large centres bigger than 5mm in diameter were sectioned using a gem saw (Ray Tech) with two diamond blades separated by a 0.6 mm spacer (Başusta and Sulikowski, 2012). Smaller centres were sanded with a Dremell <sup>TM</sup> tool to replicate a sagittal cut. Processed vertebrae were mounted horizontally on glass microscope slides and ground with successively finer-grit (400 then 600) wet or dry sandpaper. Each vertebra was then remounted and one side was ground to produce a thin (0.4-0.5mm) sample (Başusta *et al.*, 2008).

## **Staining Method**

This staining method was modified by Kahveci *et al.*, (2000) and Tran *et al.*, (2000). The cartilage stains varied from oranges to reds.

The Weigert's Iron Hematoxylin Solution, Stock Solution A contains: 1g hematoxylin, and 100ml alcohol (95%). Stock Solution B contains: 4ml ferric chloride in water (29%), 95ml distilled water, and 1ml of hydrochloric acid (concentrated). The working solution requires mixing equal parts of stock solutions A and B. The Fast Green (FCF) Solution (0.001%) contains: 0.01g fast green, FCF, C.I. 42053, and 1000ml of distilled water. Acetic Acid Solution (1%), 1ml acetic acid, glacial, and 99ml of distilled water. The Safranin-O Solution (0.1%) contains: 0.1g Safranin-O, C.I. 50240 and 100ml of distilled water.

## Procedure

Hydrate the slides with distilled water. Stain with Weigert's iron hematoxylin working solution for

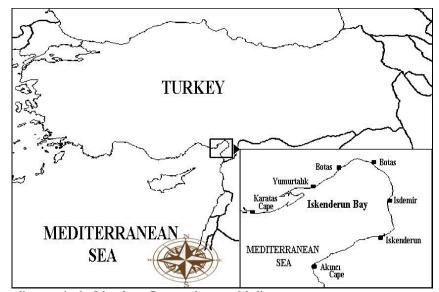


Figure 1. The sampling area in the Iskenderun Bay, northeastern Mediterranean sea.

10 minutes. Wash in running tap water for 10 minutes. Stain with fast green (FCF) solution for 5 minutes. Rinse quickly with acetic acid solution for no more than 6 seconds. Stain in Safranin-O solution for 5 minutes. Dehydrate and clear with ethyl alcohol (95%), absolute ethyl alcohol, using 2 changes each, 2 minutes each.

## **Counts of Annuli**

Vertebral sections were examined under a compound microscope using reflected light (25 to 40X magnifications). One growth band was defined as an opaque and translucent band pair that traversed the intermedialia and clearly extended into the corpus calcareum.

The index of the average percentage error (IAPE) was calculated to assess the precision of the age determinations between 2 independent readers. The equation (Beamish and Fournier 1981) is expressed as follows:

$$IAPE_{j} = \frac{1}{N} \sum_{j=1}^{N} \left[ \left( \frac{1}{R} \sum_{j=1}^{R} \frac{x_{ij} - x_{j}}{x_{j}} \right) \right] * 100\%$$

Where N is the number of fish aged, R is the number of times each fish was aged,  $x_{ij}$  is the *ith* age determination of the *jth* fish, and  $x_j$  is the mean age calculated for the *jth* fish.

A von Bertalanffy growth function (VBGF) was fitted to the data with the following equation (von Bertalanffy, 1938).

$$TL_t = TL_{\infty} \left[ 1 - e^{-k(t-t_0)} \right]$$

Whereas  $TL_t$  is the expected Total Length at age t years.  $TL_{\infty}$  is the asymptotic average maximum Total Length, k is the growth coefficient, and  $t_0$  is the theoretical age at zero length.

Individual values of condition factor were obtained with the formula  $K=(W/TL^b)x100$ , where W is total weight and TL is Total Length; b is the coefficient of allometric of relationship (Bagenal and Tesch, 1978).

#### **Marginal Increment Analysis**

The periodicity of band pair formation was investigated using the marginal increment ratio (MIR)

(Sulikowski *et al.*, 2003). A sub-sample of 50 vertebrae were randomly selected comprising both juvenile and adult specimens collected in every month. The MIR was calculated as the ratio of the distance between the last and penultimate opaque bands as measured with an optical micrometer. The marginal increment ratio calculated by the following equation (Natanson et. al., 1995):

MIR=(VR-Rn)/(Rn-Rn-1)

where R is the vertebral radius, and Rn and Rn-1 are the last and penultimate opaque bands, respectively. The average MIR was plotted by the month of capture to identify trends in band formation (Cailliet, 1990; Simpfendorfer, 2000).

#### **Statistical Analysis**

The significance of regression was assessed by analysis of variance (ANOVA). Equations expressing length-weight relationships were calculated in relation to sex. In order to test differences between the sexes Student's t-test was used for comparison of the two slopes. All statistical analysis was performed using SPSS v.15.0.

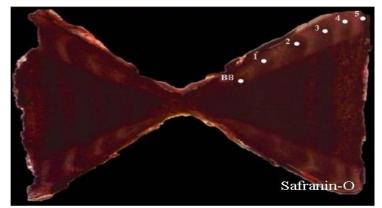
## Results

A total of 117 fish specimens (61 females and 56 males) were collected in this study (Table 1). Males ranged between 9.3 to 30.3cm in total length (TL) and 16.0 to 474.0g total in weight (TW) (W) and females ranged between 15.2 to 40.0cm in TL, and 56.0 to 308.0g in TW. Disc width ranged from 6.2 to 26.5cm for both sexes. Age image, for longitudinal crosssection of vertebral centrum was stained with safranin-O to enhance growth bands are presented in Figure 2. The Total Length-Frequency distribution according to sex is given in Figure 3, the male to female ratio is 1.09/0.91. The results showed that most of the individuals were in age group 3 (Fig. 3). The age of the female and male ranged between 1 to 6 years and 1 to 5 years old respectively.

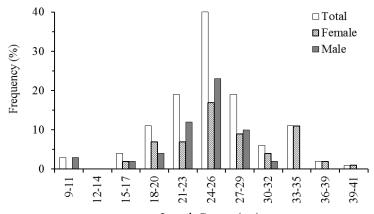
According to the age readings, there are age reading errors based on readings not only in teleost fish but also in all cartilaginous fish. So IAPE is the index of the average percent error found by two independent age readers that were unaware of each other. The credible rates are between 5% and 15% of

Table 1. Average total length and weight at age for Torpedo marmorata, sexes combined

Age Groups	Ν	Average total	Size range	Average total	Weight range
8r-		length(cm)	(cm)	weight(g)	(g)
1	5	12.26	9.3-15.4	40.40	16-90
2	38	21.34	16.5-24.5	192.71	90-248
3	44	26.12	24.4-28.8	321.34	250-436
4	16	30.11	28.8-32	504.95	440-626
5	13	34.15	33-37	896.00	656-1308
6	1	40	40	1062.00	1062



**Figure 2.** Longitudinal cross-section of vertebral centrum from a 36.5 cm TL female *Torpedo marmorata* stained Safranin O and estimated to be 5 years (BB: Birth Band).



Length Groups (cm)

Figure 3. Total length frequency of Torpedo marmorata from Iskenderun Bay, northeastern Mediterranean.

the readings. If not, then there have been mistakes in the readings. The average percentage of error (IAPE) was found as 7.96% for *T. marmorata*.

Length frequency analysis was used for corroboration of the age interpretation belonging to this species. This method is not appropriate for age validation but here as with the other length-based methods, the approach is the most suited for a young specimen and a fast growing fish where the length modes for each age group are easily distinguished (Campana, 2001; Campana, 2013). Length frequency distribution and age classes are shown in Figure 4.

#### Marginal Increment Ratio (MIR)

The age band observed during the age readings is the first year. In researching cartilaginous fish it was found that the formation process does not equal 1 therefore a marginal increment ratio is used (Natanson *et al.*, 1995). Monthly variations of MIR values in *T. marmorata* are given in Figure 5. Data is missing or unavailable for some months. In the chart prepared in accordance with the existing data, MIR started increasing from February and reached its highest level in summer, then started to decrease. Under these conditions, it can be stated that the formulation of bands tend to happen in autumn and winter.

#### **Growth in Age**

In this research the von Bertanffly Growth Equation (VBGE) was found as Lt= $57.317[1 - e^{-0.187(t+} 0.392)]$  in all specimens by considering the length of fish based on the population of *T. marmorata* found in the Iskenderun Bay (Table 2). By using the von Bertalanffy equation, calculated length values were found similar to measured length values in all ages and this situation is the indication of correct evaluation of age readings and length assessments.

The age-weight relationship of the *T. marmorata* population for both sexes in Iskenderun Bay is presented in Figure 6. Age-weight relationships were found to be:  $y=50.182e^{0.5859x} R^2=0.7388$  for all specimens;  $y=34.782e^{0.7149x}$ ,  $R^2=0.598$  for males and  $y=64.452e^{0.5206x}$ ,  $R^2=0.8391$  for females.

The age-total length relationship for *T. marmorata* for both sexes is given in Figure 7. The length-weight relationship for *T. marmorata* for both sexes is presented in Figure 8. The total length-weight relationships of the marbled electric ray are an exponential relationship using the following equations:  $W=0.0195 \times L^{2.9856}$ ,  $R^2=0.9263$  for both

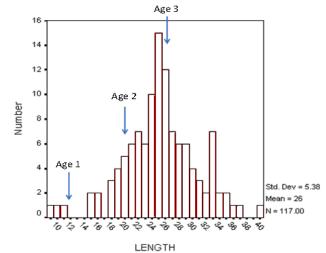


Figure 4. Length Frequency analysis of Torpedo marmorata.

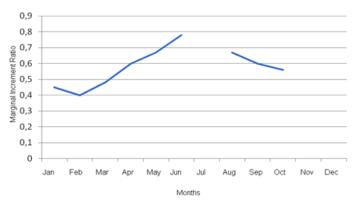


Figure 5. Monthly variation values of Marginal Increment Ratio in Torpedo marmorata.

Table 2. von Bertalanffy growth parameters	for Torpedo marmorata se	xes combined
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L∞	W∞	K	to	n
57.317	3702.668	0.187	-0.392	117
t	Lt (Calculated length) (cm)	Measured Length (cm)	W <sub>t</sub> (Calculated weight) (g)	Measured weight (g)
1	13.14	12.26	39.44	40.40
2	20.67	21.34	159.60	192.71
3	26.93	26.12	360.40	321.34
4	32.11	30.11	620.29	507.63
5	36.41	34.15	913.89	844.00
6	39.97	40.00	1219.08	1062.00

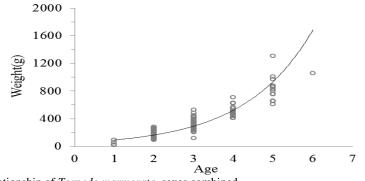


Figure 6. Age-weight relationship of Torpedo marmorata, sexes combined

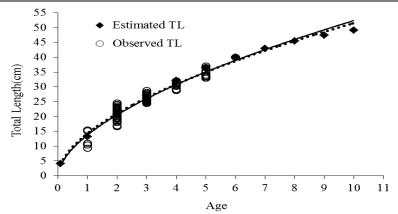


Figure 7. Age-total length relationship of *Torpedo marmorata* sexes combined.

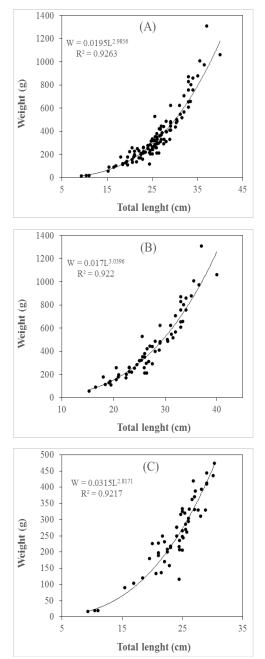


Figure 8. Length-weight relationships of Torpedo marmorata (A) Both sexes, (B) Females, (C) Males.

sexes, W=  $0.017 \times L^{3.0396} R^2 = 0.922$  for females and W =  $0.0315 \times L^{2.8171}$ , R<sup>2</sup>=0.9217 for males.

# Condition Factor (K)

Condition factors calculated for all age groups are presented in Table 3. Average condition factor value of the population was calculated as 2.209 and the highest condition factor value was found as 3.776 in age group 3.

## Discussion

The amount of research on T. marmorata is rather limited and there is no known study on the age determination in this species. This is the first known research made on age reading of T. marmorata. Also the Safranin-O staining technique was used for the first time in studying this fish species. It was determined that the typical age of the samples ranged between 1 and 6 years. The average length values of these marbled electric rays were between 24.4 and 28.8cm. The longest male and female measured 30.3 and 40.0cm in TL, respectively. Females were larger and heavier than the males. Similar patterns were observed for T. marmorata from off Tunisian coast (Capapé 1979), coast of Senegal (Capapé et al. 2001), off Italian coast (Consalvo et al. 2007) and Lagoon of Bizerte, Tunisia (Kamel et al., 2009). Consolvo et al. (2007) reported maximum lengths of 364mm for males and 553mm for females. According to Capapé (1979) maximum lengths for male and female were 390mm and 500mm respectively. These past research studies have shown that females were generally larger than males in T. marmorata. Neer et al. (2001) calculated a K value of 0.073 for females and 0.137 for males of the Pacific Electric Ray (T. californica) from the central and southern California areas. These estimates were less similar to the K value for T. marmorata in our study.

From the length-weight relationship values; the "b"value is 2.9856 for both sexes, 3.0396 for females and 2.8171 for males. According to the "b"values obtained for both sexes, the female of the species showed a isometric growth characteristic. Males showed negative allometric growth. Although it is determined differences between growth characteristic of female and males, no differences in slope were found between sexes (P < 0.05).

According to Consolvo *et al.* (2007) *b* values for males and females were 2.7156 and 2.8547 respectively. This finding was similar with ours for the males, but different for females. The reason for this may depend on the sample size, different habitat and reproduction season.

Various factors may be responsible for the differences in parameters of length-weight relationships among seasons and years, such as temperature, salinity, food (quantity and quality), sex and maturity stage (Bello Olusoji *et al.*, 2009).

Table 3. Average condition values of Torpedo marmorata

Age Groups	Condition Factors
1	1.866
2	2.013
3	3.766
4	1.853
5	2.098
6	1.659
Average	$2.209 \pm 0.413$

According to the Marginal Increment Analysis it was found that age band growth was annual. Assessment and management of marine fisheries can be negatively affected by misspecification of ages (Okamura et al., 2013). There are various approaches for verifying age estimation methods of which edge analysis (EA) and marginal increment analysis (MIA) are among the most frequently employed. These methods focus on incremental patterns of growthband pairs throughout the year. In this study MIR was calculated. These methods focus on incremental patterns of growth-band pairs throughout the year. They assume that the width or the density of the outermost increment will exhibit a yearly sinusoidal cycle when plotted against the month of capture if growth bands are formed annually (Okamura et al., 2013).

The average condition factor of *T. marmorata* was found as 2.209 and the highest condition factor was found as 3.776 for age group 3.

Research on the marbled electric ray is very limited and more studies should be done to establish their stock availability. Procreation areas should be detected and fishing should be banned in these areas. This species typically lives in shallow water and this is why they are caught in deep gill nets. This fishing technique and the fishing areas should be reviewed.

It is also suggested that the Safranin-O staining technique should be used for age determination in new research and this staining approach would be useful in studies of other torpedinids.

## Acknowledgements

The authors thank N. Gayır for their assistance for obtaining the fish sample; warmly thank Prof. Erdoğan Çiçek, for his valuable advice and constructive criticism. An experimental fishing permit was granted by the General Directorate of Fisheries and Aquaculture, Ministry of Food, Agriculture and Livestock of Turkish Republic. Funding was provided by The Scientific and Technological Research Council of Turkey (TUBITAK), Project No: TOVAG 109O634.

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