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A study on age and growth of juvenile and semi adult *Torpedo nobiliana* Bonaparte, 1835 inhabiting Iskenderun Bay, northeastern Mediterranean Sea

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Abstract: In this study, age and growth of juvenile and semi adult Atlantic electric ray were reported for the first time and safranin-o method was also used to stain sectioned vertebrea of this species. A total of 93 *Torpedo nobiliana* that are 12.0 to 35.5 cm were collected as by-catch by trawl fishermen between September 2010 and December 2011. There was no adult specimen. Of the juvenile and semi adult 42% was female and 58% was male. It was identified that population dispersed from 1 to 5 age groups. The length and weight relationship was expressed as $W=0.0151\times L^{3.0611}$ for combined sexes. The growth parameters of the von-Bertalanffy equations were: L_∞=74.47 cm TL, K=0.1089 year^{-1} and to=-1.058 year^{-1}. Age and growth characteristics of juvenile and semi adult Atlantic electric ray were found out first time with this study.

Keywords: Electric ray, Aging method, Growth, Safranin-o, Mediterranean Sea.

Introduction

The Atlantic electric ray, *Torpedo nobiliana*, is found in the Atlantic Ocean, from Nova Scotia to Brazil in the west and from Scotland to Morocco and off southern Africa in the east, including the Mediterranean Sea, occurring at depths of up to 800 m (Capapé et al., 2006a; Golani et al., 2006). Specimens captured in MEDITS surveys in the Mediterranean Sea were present throughout the depth range surveyed (10-800 m) but found mostly between 200-500 m (Baino et al., 2001). This species reaches a maximum size about 180 cm in total length (McEachran and Carvalho, 2002). Reproduction is yolk-sac viviparity. Up to 60 embryos have been reported in large females, gestation period is about 12 months (Whitehead et al., 1984; McEachran and Carvalho, 2002). This species is assessed as data deficient (DD) globally due to lack of biological data on catches and population trends by the IUCN (Abdul Malak et al., 2011).

There has not been any information about age and growth for *T. nobiliana*. Unfortunately, this life history characteristic is lacking for most cartilaginous species within the Mediterranean Sea, despite of there are some studies such as systematic, zoogeographical distribution and reproduction biology, diet composition, age determination, growth parameters, length-weight parameters and histology in the eastern Mediterranean Sea (Abdel-Aziz, 1994; Başusta et al., 1998; Başusta and Erdem, 2000; Başusta, 2002; Ismen, 2003; Capapé et al., 2006b; Başusta et al., 2008; Çek et al., 2009; Yeldan et al., 2009; Bircan-Yildirim et al., 2011; Başusta et al., 2012a, b; Başusta and Sulikowski, 2012). Since, the lack of age and growth parameters is a limiting factor in the development of elasmobranch management action plans (Hoff and Musick, 1990; McFarlane and King, 2006). Therefore, this study was aimed to determine the age and growth parameters of juvenile and semi adult Atlantic electric ray inhabiting Iskenderun Bay, northeastern Mediterranean Sea.

Materials and Methods

Sample collection: The samples were collected as discard by commercial trawl fishermen approximate depths ranged from 80 to 200 m in Iskenderun Bay (Fig. 1), between September 2010 and December 2011 by monthly intervals. Total length (TL, in cm) was measured as a straight line distance from the tip of the rostrum to the end of the tail, and disc with (DW, in cm) as a straight line distance between the tips of the widest portion of pectoral

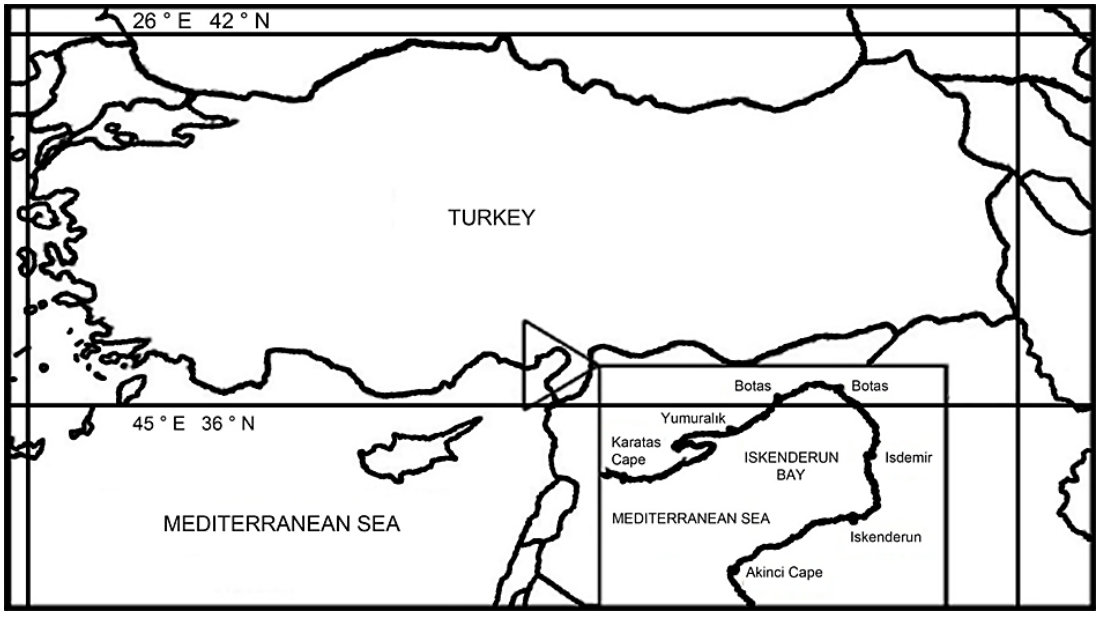

Figure 1. Sampling area in the Iskenderun Bay.

fins. Total weight (g) was also recorded.

Preparation of vertebral samples: A block of 12 vertebral centra were taken from above the abdominal cavity of 93 specimens, labelled, 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 over used, so soak times were kept to nearly 10 minutes. The vertebrae were then air-dried for no less than 48 hours.

Large centra bigger than 5 mm 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 centra were sanded with a Dremell^{TM} 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: In this study, safranin-o staining method was applied. 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: 1 gr haematoxylin, and 100 ml alcohol (95%). Stock Solution B contains: 4 ml ferric chloride in water (29%), 95 ml distilled water, and 1 ml 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: 0.01 gr fast green, FCF, C.I. 42053, and 1000ml of distilled water. Acetic Acid Solution (1%), 1 ml acetic acid, glacial, and 99 ml of distilled water. The safranin-o solution (0.1%) contains: 0.1 gr safranin-o, C.I. 50240, and 100 ml of distilled water.

Procedure: Hydrate the slides with distilled water. Stain with Weigert's iron haematoxylin working solution for 10 min. Wash in running tap water for 10 minutes. Stain with fast green (FCF) solution for 5 min. Rinse quickly with acetic acid solution for no more than 6 seconds. Stain in safranin-o solution for 5 min. 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

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

Age Groups	N	Average total length (cm)	Size range (cm)	Average total weight (g)	Weight range (g)
1	6	14.05	12-17	59.33	14-126
2	32	21.42	16.8-30	192.81	104-386
3	37	25.16	25-29	284.43	162-492
4	17	29.4	28-32	496.70	372-704
5	1	35.5	35.5	850	850

equation (Beamish and Fournier 1981) is expressed as follows:

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

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):

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

Where, TL_t is the expected total length at age t years. TL_∞ 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:

$$K=(W/TL^b)*100$$

Where W is total weight and TL is total length; b is the coefficient of allometric of relationship (Bagenal and Tesch, 1978; Tirasin, 1993).

All total lengths and weights were fitted to the length-weight equation: $W=aL^b$, by using least square methods with Statistica software. The test of normality was conducted by using SPSS v.21.0.

Results and Discussion

A total of 93 specimens (54 females and 39 males) were examined in this study (Table 1). Age image, for longitudinal cross-section of vertebral centrum was stained with safranin-o to enhance growth bands are presented in Figure 2. Total length of females ranged from 17 to 35.5 cm, body weight varied from 14.0 to 980.0 gr, whereas males ranged between 15.3 and 32.0 cm and 12.0-546.0 gr. Total length was reported as 17 to 77 cm for males and 19.5 to 120 cm for females from coasts of Tunisia and southern France (Capapé et al., 2006a). The

Figure 2. Longitudinal cross-section of vertebral centrum from a 25.5 cm TL female *Torpedo nobiliana* stained safranin-o and estimated to be 3 years (BB: Birth Band).

largest specimen reported by Bigelow and Schroeder (1953) off the eastern coast of the United States, total length was 170 cm and body weight was 90 kg. These values are higher than our data. The reason for this difference could be the use of different fishing technique and depth of sampling area. The samples were collected up to 200 m depth in Iskenderun Bay. Bigger specimens of demersal fish generally distributed in the deepest ground. Indeed positive size-depth relationships was reported for many fish species (Macpherson and Duarte, 1991; Labropoulou and Papaconstantinou, 2000).

Total length-frequency distribution by sex of this species was given Figure 3 and sex ratio was 1.28/0.72. When the data was examined, it is seen that lots of individuals are in the 3th age group (Figure 3). It was identified that population dispersed from 1 to 5 age groups. According to the age readings, the index of the average percentage error (IAPE) was found 3% and the credible rates are between 5% and 15 of the readings so this result indicated that our aging method represents a precise approach to the age assessment of *T. nobiliana*

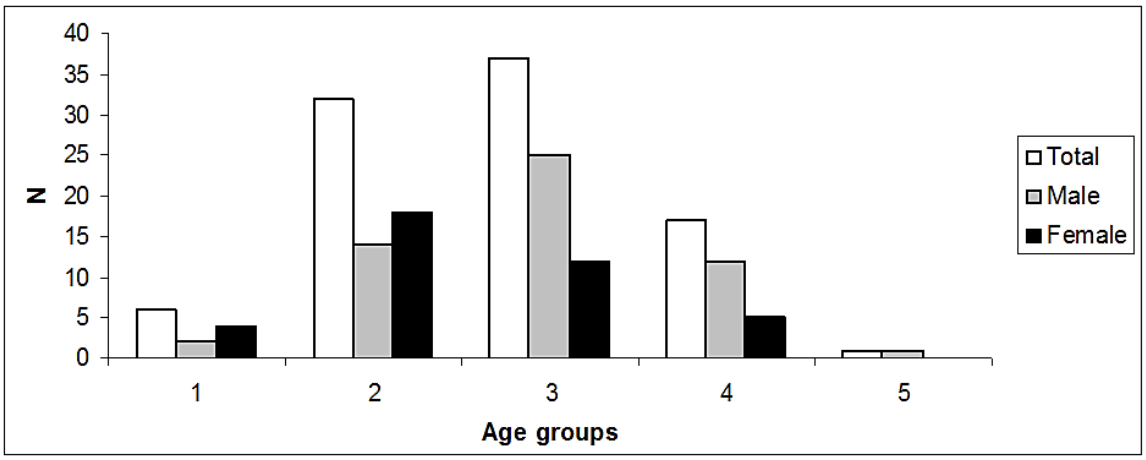

Figure 3. Age groups-frequency distribution of *Torpedo nobiliana* for each sex.

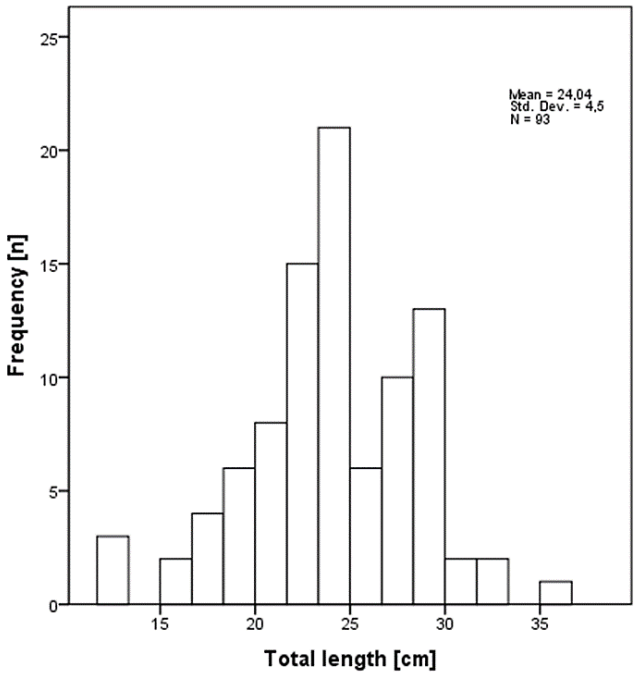

Figure 4. Total length frequency analysis of *Torpedo nobiliana*.

(Campana, 2001; Sulikowski et al., 2003; Duman and Başusta, 2013).

The length-frequency distribution of the samples is shown in Figure 4. The data showed a normal distribution pattern according to Kolmogorov-Smirnov significance value (*P*=0.2) (Fig. 4).

The parameters of the von Bertalanffy Growth Equation (VBGE) were found as L_{∞}=74.47 cm TL, W_{∞}=3453.57 g, *K*=0.108922 year^{-1} and t_0=-1.05828 year for combined sexes (Table 2). By using the von Bertalanffy equation, calculated length values were found similar to measured length values in all ages and this

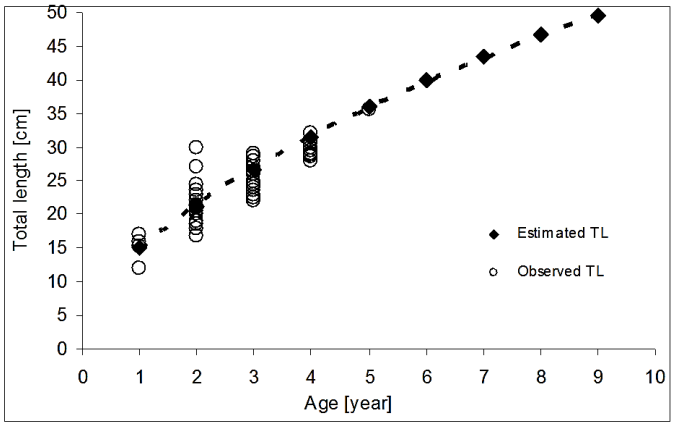

Figure 5. Age-total length relationship of *Torpedo nobiliana* for combined sexes.

situation is the indication of correct evaluation of age readings and length assessments (Fig. 5). Duman and Başusta (2013) calculated a *K* value of 0.187, t_0= -0.39231 for marbled electric ray (*T. marmorata*) from the Iskenderun Bay. These estimates were less similar to the *K* and t_0 value for *T. nobiliana* in our study. Normally, Atlantic electric ray has 180 cm total length in nature but this L_{∞} value is normal for juvenile and semi adult individuals for this species.

The age-total length relationship for *T. nobiliana* for combined sexes is given in Figure 5. The total length-weight relationships of the Atlantic electric ray is an exponential relationship using the following equations: $W=0.0151*L^{3.0611}$ (R^2= 0.8958) for combined sexes, $W=0.0078*L^{3.2713}$ (R^2=0.9128) for females and $W=0.042*L^{2.7434}$ (R^2=0.8684) for males (Fig. 6). The length-weight relationship for *T. nobiliana* for both sexes, females and males are presented in Figure 6 (A, B, C). In

Table 2. von Bertalanffy growth parameters of *Torpedo nobiliana* for combined sexes.

L_∞	W_∞	K	t_o	n
74.47	3453.57	0.108	-1.058	93
Age	**L_t (Calculated length) (cm)**	**Measured Length (cm)**	**W_t (Calculated weight) (g)**	**Measured weight (g)**
1	14.95	14.05	63.16	59.33
2	21.09	21.42	176.00	192.81
3	26.60	25.16	351.21	284.43
4	31.54	29.40	583.25	496.70
5	35.97	35.50	862.65	850.00

Table 3. Average condition values of *Torpedo nobiliana*.

Age Groups	Condition
1	2.112
2	1.956
3	1.778
4	1.961
5	1.899
Average	1.94±0.12

the equation of Length-weight "*b*" values are 3.06 for sexes combined, and that's why characteristic of isometric growth was indicate in this species (Ricker, 1968). There were no significant differences between males and females of *T. nobiliana* (T-test, $P>0.05$).

Average condition values calculated for all age groups and examples from the research is presented in Table 3. Average condition value of the population was calculated as 1.94 and based on the ages the highest condition factor was found as 1 age with 2.112.

This is the first time a research was made on the age reading of *T. nobiliana* with this paper and also first time the safranin-o staining technique was used for this species.

Researches on the biology of *T. nobiliana* are rather limited and there is no study on the fishery of this species. As a result studies should be done to establish their stock availability. Especially breeding and nursery areas should be detected and fishing should be banned in this area, if it is necessary. This species is living generally in shallow water and that's why they are caught through deep gill net and this fishing technic and area should be reviewed.

With this research age and growth values were examined and it is suggested that safranin-o staining technique should be used in the new researches.

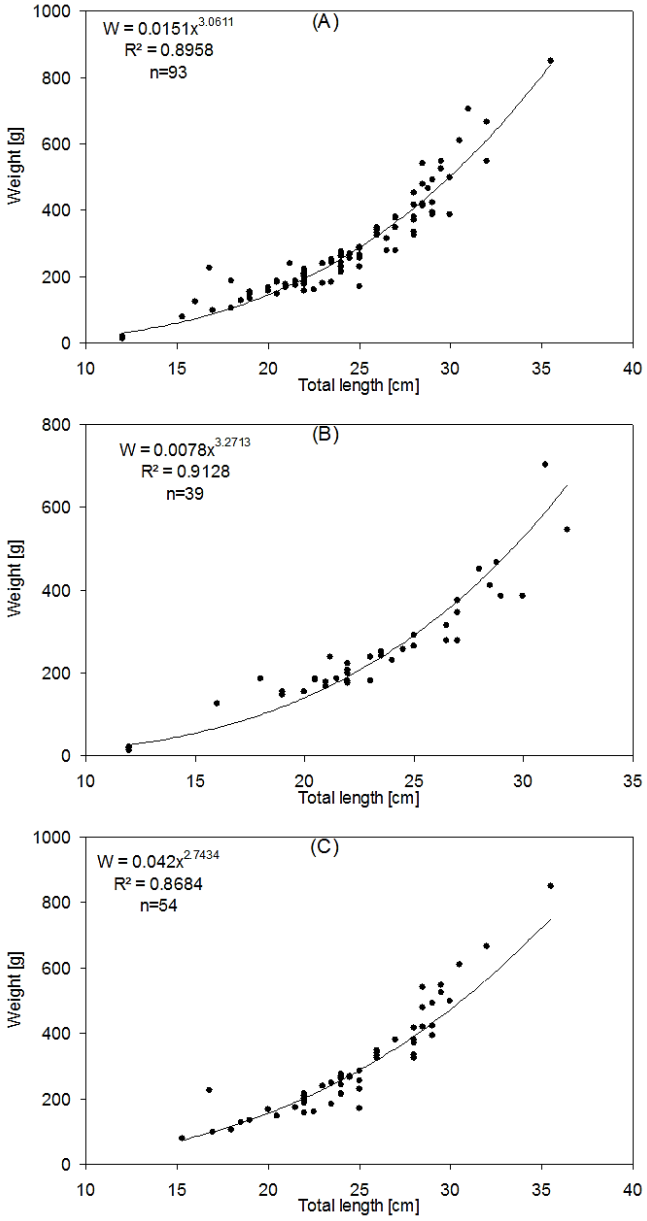

Figure 6. Length- weight relationships of *Torpedo nobiliana* from northeastern Mediterranean for each sex, (A) combined sexes; (B) females; (C) males.

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