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Biological Aspects of the Brown Ray (*Raja miraletus* Linnaeus, 1758) in the Saros Bay, the Northern Aegean Sea

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Abstract: The present study reports the age, growth, and reproduction parameters of 52 brown rays (*Raja miraletus*) captured from the Saros Bay (Northern Aegean Sea) between September 2006 -2008. The males and females accounted for 44.2% and 55.8% of the sample, respectively. The males measured 10.5-53.5 cm (disc width, 6.5-32 cm) and the females ranged 25.5-47.7 cm (disc width, 18-30.5 cm) in total length (TL). The equations $TW=0.0017*TL^{3.27}$ and $TW=0.0017*DW^{3.38}$ were used to describe the total length-weight and disc width-weight relationships, respectively. The length-at-age data on vertebrae were used to estimate the von Bertalanffy growth equation: $L_\infty = 62.43$ cm, $K = 0.28$ year^{-1}, $t_0 = -0.54$ year. The maximum age was found to be 4 years for both the males and females.

Keywords: *Raja miraletus*, Age, Growth, Reproduction, Saros Bay, Aegean Sea

Saros Körfezi'nde (Kuzey Ege Denizi) Kahverengi Vatozun (*Raja miraletus* Linnaeus) 1758 Biyolojik Yönleri

Özet: Bu çalışmada, Kuzey Ege Denizi, Saros Körfezi'nde, Eylül 2006 ve 2008 tarihleri arasında, 52 adet kahverengi vatozun (*Raja miraletus*) yaş, büyüme ve üremesi çalışılmıştır. Cinsiyet kompozisyonu %44,2 erkeklerden ve %55,8 dişilerden oluşmuştur. Erkeklerin toplam boy aralıkları 10,5'den 53,5 cm'e (disk genişliği, 6,5-32 cm), ve dişilerin 25,5'den 47,7 cm'e (disk genişliği, 18-30,5 cm) olarak değişmiştir. Boy-ağırlık ve disk genişliği-ağırlık ilişkileri sırasıyla, $TA=0,0017*TB^{3,27}$ and $TA=0,0055*DG^{3,38}$ olarak tanımlanmıştır. Omur okumalarından boy-yaş verilerine dayanılarak hesaplanan von Bertalanffy denklemindeki büyüme parametreleri: $L_\infty = 62,43$ cm, $K = 0,28$ yıl^{-1}, $t_0 = -0,54$ yıl olarak hesaplanmıştır. Maksimum yaşının 4 yıl olduğu tespit edilmiştir.

Anahtar Kelimeler: *Raja miraletus*, yaş, büyüme, üreme, Saros Körfezi, Ege Denizi

Introduction

The brown ray, *Raja miraletus* Linnaeus, 1758 is a small cartilaginous fish found on the continental shelf and distributed from shallower waters down to 530 m with typical depths of 50-150 m (Relini *et al*., 1999; Serena, 2005; Hemida *et al*., 2007). It can inhabit a wide range of substrates, including sandy-muddy seabeds to *Posidonia* meadows (Relini *et al*. 1999). They are very common in the eastern Atlantic Ocean, Mediterranean Sea, and Western Indian Ocean in which they gather in masses (McEachran *et al*., 1989; Compagno *et al*., 1991). *R. miraletus* is not an intended target at commercial fisheries; however, it is unintentionally captured by demersal trawls, gillnets, trammel nets, and bottom longline fisheries in the North Aegean Sea. Even though it has suffered from increasing fishing pressure, little is known about its life history; therefore, it is reported as "Least Concern" in the IUCN Red List assessments (Cavanagh & Gibson, 2007; Kadri *et al*., 2014a)

Previous research reports data on age, growth, reproductive biology, feeding, and distribution of *R. miraletus* from the Mediterranean – e.g., Tunisia – (Capapé & Quignard 1974, 1977; Kadri *et al*., 2012; Kadri *et al*. 2014a; Kadri *et al*., 2014b; Marongiu *et al*., 2015), Egypt (Abdel-Aziz, 1987; 1992), Adriatic waters (Zupanovic, 1961; Jardas, 1973; Ungaro, 2004), and Eastern Atlantic (coast of Senegal) (Capapé *et al*., 2010). There is some data on the morphological characteristics and distribution of *R. miraletus* in Turkish waters, (Torcu & Aka, 2000; Filiz & Mater, 2002; Filiz & Bilge, 2004; Ismen *et al*., 2007a; İlkyaz *et al*., 2008; Yıgın & Ismen, 2009; Gurbet *et al*., 2013; Akyol *et al*., 2017; Bilge *et al*., 2014). Therefore, elucidation of basic life history of this species in Turkish waters is important for developing conservation and management strategies. as previous studies offer no data on this species' age, growth and reproductive biology in the Northern Aegean Sea. Thus, the present study provides data concerning age, growth, and age at sexual maturity of *R. miraletus* in the Saros Bay, the North Aegean Sea.

Material and Methods

In this study, 23 male and 29 female *R. miraletus* were captured by bottom trawls (with a stretched mesh size of 44 mm at the cod-end) at depths ranging from 50 - 200 m in the Northern Aegean Sea (Figure 1). Trawl operations were performed monthly during September 2006 - September 2008. Samples were transferred to the laboratory the same day and total length (TL), disc width (DW), and body weight (W) were measured. The individuals' sexes were determined based on the presence and absence of clasper (Hara *et al*., 2018). Length-weight relationships were defined by allometric equations (Sparre *et al*., 1989): $W=aL^b$, where W refers to the total body weight (g), L to the total length (cm), and a and b are employed as constants. The Length-weight relationships between sexes were compared using t-tests to determine statistical differences. The slopes of the regression equations were corrected to follow a geometric mean regression as defined by Ricker (1973). Two sample t-test was used to test the hypothesis of isometric relationship (H0: slope=3; H1: slope≠3) (Zar, 1999).

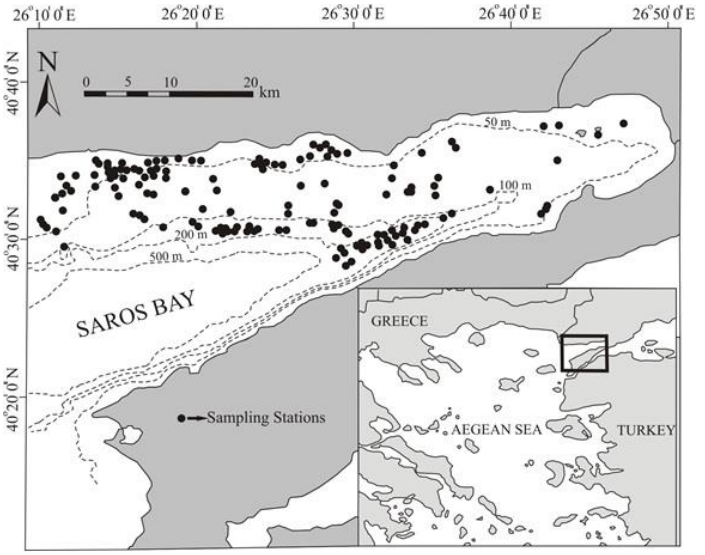

Figure 1. Locations of trawl sampling stations in Saros Bay, the North Aegean Sea.

Age determination was performed by counting the rings in the vertebral centra after usingthe silver nitrate staining method (Kusher *et al*., 1992; Ismen *et al*., 2007b; Yeldan *et al*., 2009; Yıgın & Ismen, 2010). This method briefly included the following steps: Ten vertebrae (located between approximately the 15th and 25th vertebrae) were obtained from the posterior portion of each specimens' body cavity. The vertebrae were prepared for aging by first cleaning and then staining with silver nitrate. The connective tissues were cleaned with 5–25% sodium hypochloride solution for 1h. After burning in 88% formic acid for 2–4 min, the centra surfaces were washed in distilled water for 5 min. They were then stained in 1% silver nitrate solution for 10–15 min and exposed to ultraviolet light for 15 min. The obtained sections were rinsed in distilled water and placed in 5% isopropyl alcohol for 1 min to eliminate excess material (Schwartz, 1983; Serena *et al*., 2005). The stained vertebrae were examined with a light microscope (Olympus SZX16). Three maturity stages (immature, maturing and mature) were determined with respect to size, shape and structure of the clasper, vas deferens, and alar thorns for the male individuals and of the ovaries, uteri, and oviductal glands for the females (Table 1) (Zeiner & Wolf, 1993; Hara *et al*., 2018).

Table 1. Characteristics of each maturity stage for male and female *Raja miraletus* individuals (Zeiner & Wolf, 1993; Hara *et al*., 2018)

Stage	Male	Female
Immature	Vas deferens uncoiled. Claspers uncalcified and not reaching posterior edge of pelvic fin. Alar thornsabsent.	Ovaries small and undeveloped. Differentiated ova absent. Shell glands undeveloped. Uteri thin.
Maturing	Vas deferens coiling. Claspers extending to posterior edge of pelvic fin and partially calcified. Alar thorns developing	Ovaries enlarging and differentiating. Small, whitish ova distinguishable. Shell glands and uteri enlarging.
Mature	Vas deferens almost completely coiled. Claspers fully calcified. Alar thorns fully grown.	Ova fully developed. Shell glands large and heart-shaped. Uteri thick and fully developed

The growth was calculated by the von Bertalanffy growth equation (Beverton & Holt, 1957): $L_t = L_\infty(1-e^{-K(t-to)})$, where L_∞ signifies the asymptotic total length, L_t the total length at age t, K the growth curvature parameter, and t_0 the theoretical age of fish with zero total length. The growth parameters were estimated by the nonlinear method employing a software package, (FISAT; FAO-ICLARM Stock Assessment Tools) (Sparre *et al.*, 1989). The gonadosomatic index (GSI) was assessed monthly by the equation: GSI = (gonad weight/gonad-free fish weight)*100. Oocytes were removed from the ovaries and measured and weighted to the nearest gram. The diameter of oocytes (D_0, cm) were measured to the nearest centimeter. The total weight of each oocytes (W_0, g) were weighed to the nearest 0.01 g. The oocyte diameter-weight relationships were calculated for *R. miraletus* females (Yigin & Ismen, 2013).

Results

Morphological measurements

The TL ranged between 10.5 and 53.5 cm (DW 6.5-32 cm) for the males (n=23) and between 25.5 and 47.7 cm (DW 18-30.5 cm) for the females (n=29) (Figure 2). A sex-based assessment of the relationship between TL-TW and DW-TW is given in Figure 3. The differences in the TL-TW and DW-TW relationships between the males and females were not found to be statistically significant (P>0.05).

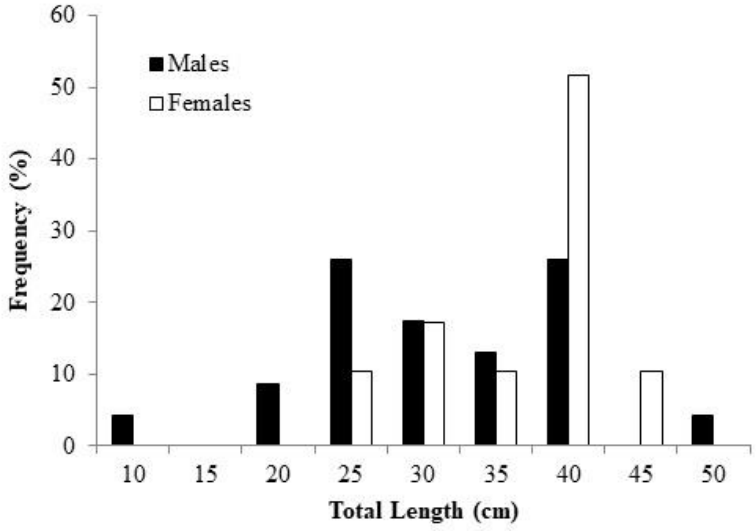

Figure 2. Sex-based length-frequency distribution of the brown ray, in Saros Bay, North Aegean Sea.

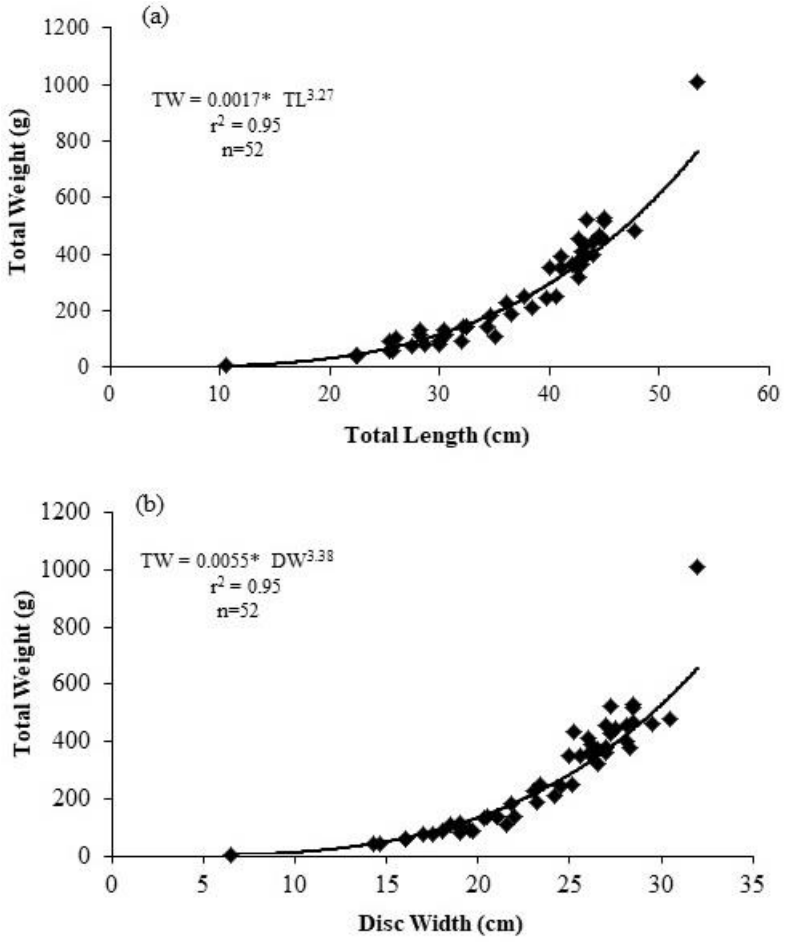

Figure 3. (a) Total length–weight and (b) disc width-weight relationships of *R. miraletus*, (both sexes combined).

Age and growth

Age distribution of the males and females ranged from 0-4 and 1-4 years, respectively. The dominant mode for ages were 2-3 years for the males and 3 for the females, (Table 2). Growth-in-length of the males, females, and both sexes combined, are given in Table 3.

Table 2. Mean length (cm) in each age group of brown ray, *R. miraletus*

Age	N	Range (cm)	Mean Length (cm)
0	1	10.5	10.5 (±0.000)
1	5	22.4-25.7	24.3 (±0.753)
2	15	26.0-36.5	30.6 (±0.713)
3	21	35.0-43.4	41.1 (±0.558)
4	8	44.0-47.7	44.9 (±0.418)

Table 3. von Bertalanffy growth parameters of brown ray, *R. miraletus*

Sex	L_∞ (cm)	K (year^{-1})	t_0 (year)
Males	59.75	0.32	-0.58
Females	58.50	0.34	-0.50
Combined	62.43	0.28	-0.54

Gonadosomatic Index (GSI) and Maturity

Figure 4 shows monthly changes in the mean GSI values of all samples. The GSI values for females are relatively high in March, August and November. Figure 5 presents the percentage of each individual's gonadal development stage. Findings indicated that 32% of eggs were immature (I), 40% maturing (II), and 28% mature (III). Data on maturity stages suggested that spawning occurs between February - October (Figure 5). Based on GSI values and maturity stages of eggs, *R. miraletus* is expected to have a year-round spawning period with a peak in the spring and summer.

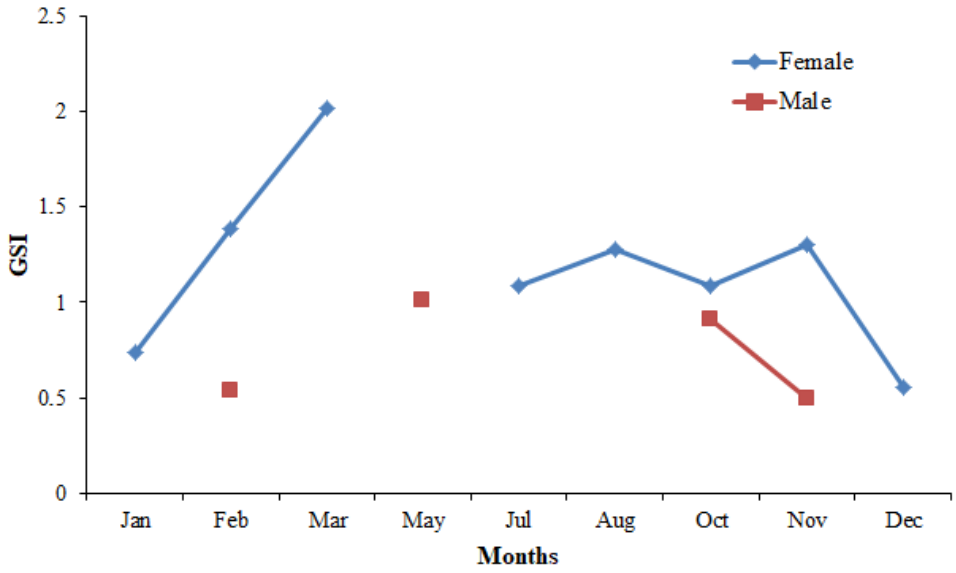

Figure 4. Gonadosomatic index (GSI) values of *R. miraletus.*

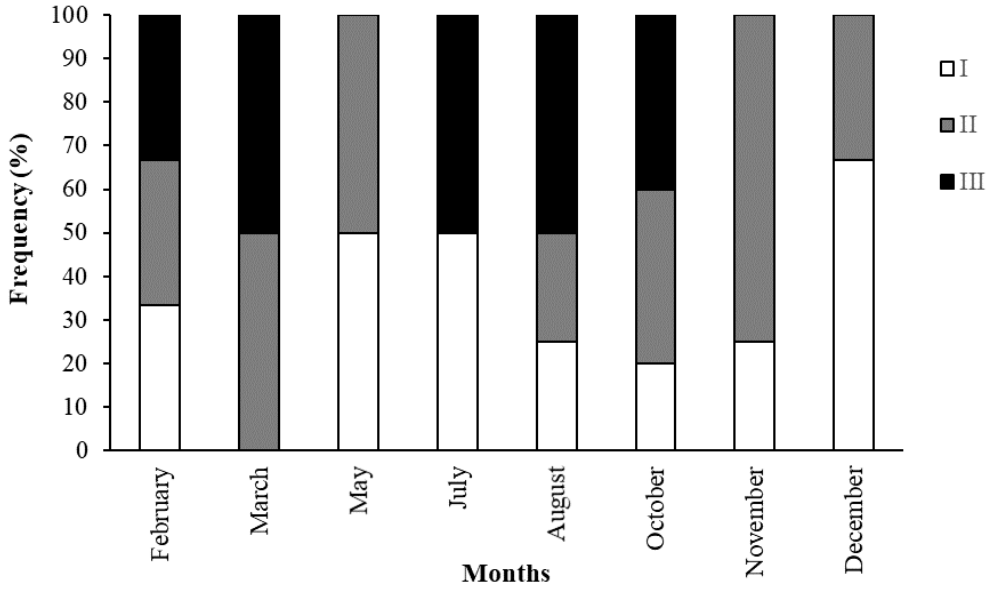

Figure 5. Monthly variations of maturity stages in *R. miraletus* females (I: immature; II: maturing; III: mature).

Figure 6 shows a strong relationship between oocyte diameter (D_0) and weight (W_0). A higher variation in oocyte weight was observed for oocytes larger than 0.6 cm.

Two different phases in clasper growth was detected. An abrupt change in the clasper length-total length relationship begins at ~37.7 cm TL (Figure 7).

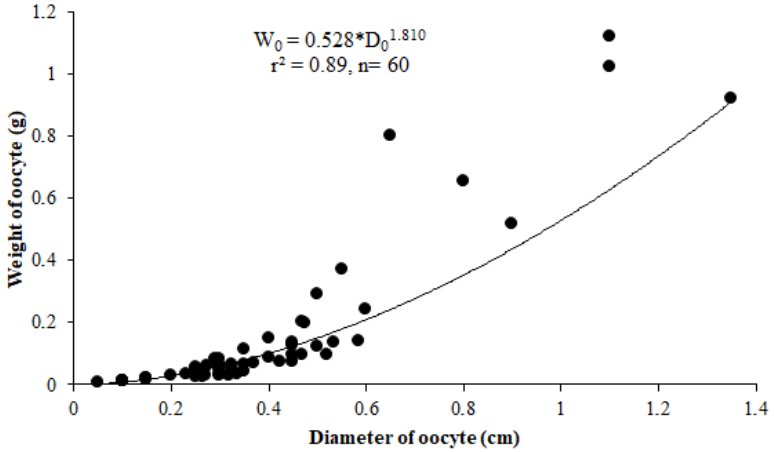

Figure 6. Diameter-weight relationship of oocytes for female *R. miraletus*.

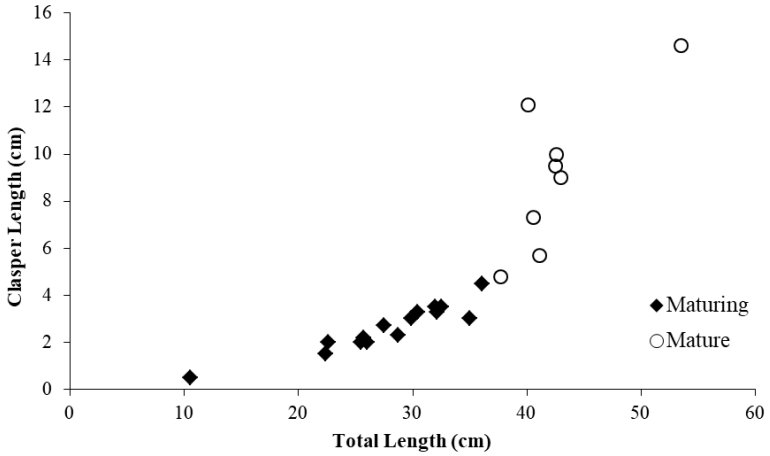

Figure 7. Relative growth of claspers in males.

Discussion

In this study, meristic measurements of the brown ray were similar to those reported from other studies. The specimens collected from the Gulf of Gabès (Kadri *et al*., 2014b) ranged from 13.5 -58 cm in total length, 8.5 - 37 cm in disc width, and 7 to 980 g in weight. Capapé and Quignard (1974) reported 32-33 cm disc width and 54-57 cm total length for male and female brown rays, respectively, with weights ranging from 151 - 1338 g. Ungaro (2004) found that Total length (TL) ranged from 16.5-51.0 cm and 15.0-49.5 cm for females and males, respectively, and the weight for *R. miraletus* to vary between 20-750 g in the southern Adriatic basin. In Senegale coasts Capapé *et al*. (2007) Reported that the weight for *R. miraletus* was 1205-1336 g.

Ungaro (2004) reported a positive allometric growth pattern for *R. miraletus* from the Southern Adriatic basin. Kadri *et al*. (2014b) estimated the b-values as 3.38 and 3.33 for the female and male brown rays, respectively, in the Gulf of Gabès. Establisment of the weight-length relationships allow estimation of condition factors and geographical comparison of life histories, and therefore, is critical for fisheries ecology and stock assessment (Petrakis & Stergiou, 1995; Gonçalves *et al*., 1997; Froese & Pauly, 2004).

The studied individuals' growth model suggests that males are capable of reaching a larger asymptotic length (59.75 cm) than females (58.50 cm). The von Bertalanffy growth model produced an L_∞ of 62.43 cm for both sexes. The age and growth rates for the male and female *R. miraletus* reported in this study differed slightly from those given in the previous research. Kadri *et al*. (2012) and Abdel-Aziz (1992) reported a higher growth rate *k* for male brown rays than for the females in the Gulf of Gabès and on the Egyptian Mediterranean coasts. The estimated von Bertalanffy growth parameters of *R. miraletus* in different areas are given in Table 4. The differences in growth model parameters between the present research and previous studies may result from several factors, such as sampling method, location, and age

classes included in the models (Neer & Thompson, 2005; Yigin & Ismen, 2012). Although *R. miraletus* males attain larger sizes than females, this difference between sexes were not significant ($p>0.05$). Similarly, previous studies reported a low degree of sexual dimorphism in several skate species (Frisk & Miller, 2006; Natanson *et al*., 2007; Sulikowski *et al*., 2005; Hara *et al*., 2018). In addition, skate species are known to exhibit single oviparity or they can simultaneously carry two egg capsules (Wyffels, 2009; Hara *et al*., 2018). Developing large embryos may not necessarily require a large body size in *R. miraletus* and this may explain similar body sizes between males and females and lower degree of sexual dimorphism observed in the present study.

Table 4. Region-based comparison of von Bertalanffy growth parameters for *R. miraletus*.

Region	Sex	L_∞ (cm)	K (year^{-1})	t_0 (year)	Source
Egyptian Mediterranean Coasts	F	69.2	0.18	0.11	Abdel-Aziz (1992)
	M	67.0	0.22	1.01	
Gulf of Gabès	F	91.92	0.17	0.25	Kadri et al. (2012)
	M	87.87	0.19	0.50	
Saros Bay, North Aegean Sea	F	59.75	0.32	-0.58	Present study
	M	58.50	0.34	-0.50	

The GSI of *R. miraletus* were reported to vary between females and males, which is associated with the production of countless large and heavy yolky vitellogenic follicles and incessant reproductive activity throughout the year (Capapé *et al*., 2007). Hence, the GSI values for males and females showed monthly changes during the year. Similar patterns were reported for *R. miraletus* from the Senegal coasts (Capapé *et al*., 2010) and the Gulf of Gabès (Kadri *et al*., 2012), for *Raja clavata* from the Tunisian coast (Capapé, 1979), and for *Atlantoraja cyclophora* from the SW Atlantic Ocean (Oddone & Velasco, 2006; Oddone *et al*., 2008a). Fluctuating GSI values throughout the year indicate continuous reproductive activity for both male and female *R. miraletus*. . Likewise, the Rio skate, *Rioraja agassizi*, in Southeastern Brazil showed variations in testicular lobule diameters and mature vitellogenic follicle diameters for males and females, respectively (Oddone *et al*., 2007), Seasonal GSI changes typical of oviparous species were also reported for other species, such as *Scyliorhinus canicula* from the northern coast of Tunisia (Capapé, 1977), *Psammobatis extenta* in the Southwestren Atlantic (Braccini & Chiaramonte, 2002), and *Raja undulata* in Southern Portugal (Coelho & Erzini, 2006). Although oocyte diameter and oocyte weight relationship has been reported for other chondrichthyes species in earlier studies (Oddone & Vooren, 2005; Demirhan & Seyhan, 2006; Oddone *et al*., 2008b; Kousteni & Megalofonou, 2011; Kadri *et al*., 2014) this study reports such relationship for *R. miraletus* for the first time.

Currently there is limited information on many aspects of the life history of *R. miraletus*, such as stock structure, mortality rates, spawning grounds and vertical distributions to develop successful management plans for *R. miraletus* populations. The present research provides the first data on age and growth estimates for brown rays in the Saros Bay. The brown rays exhibit similarities to other elasmobranchs with respect to their relatively longer life, lower fecundity, and older age at sexual maturity. The present work will be an important reference for future studies on age validation, breeding season reproductive cycle, and growth patterns of *R. miraletus*.

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Conflict of Interests

The author declares no conflict of interest.

Author contribution

A. İşmen and C.Ç.Yığın planned and designed the research. A. İşmen and C.Ç.Yığın performed the sample collection and analysis. C.C.Yığın contributed in writing the manuscript. All authors read and approved the final manuscript.

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