



## Reproductive Biology of Spiny Dogfish *Squalus acanthias*, in the North Aegean Sea

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### Abstract

A total number of 620 *Squalus acanthias* was sampled from the bottom trawl catches in the North Aegean Sea, Saros Bay from February 2005 to September 2008. The overall F/M ratio was 1.26:1. Females ranged from 17.1 cm to 117.5 cm in total length (TL) and males from 20.8 cm to 121.6 cm. Females showed positive allometric growth ( $b>3$ ) while males indicated negative allometric growth ( $b<3$ ). There were statistically significant differences in the relationship for total mass versus TL, and liver mass versus TL between males and females. The smallest sexually mature female was 54.5 cm in TL, while the smallest sexually mature male was 52 cm in TL. Mean length at 50% maturity was estimated at 56.4 cm for females and 52.8 cm for males. The diameter of the yolky oocytes ranged between 0.5 mm and 46 mm, while the mass ranged between 0.01 and 36.24 g. Hepatosomatic index (HSI) and gonadosomatic index (GSI) significantly increased with size in both males and females. While the ranges of HSI and GSI values for males ranged between 1.93-14.09% and 0.01-34.35%, those for females ranged between 1.63-17.17% and 0.01-96.50%, respectively. HSI reached the highest values in both sub-adult and adult specimens, reflecting the role of the liver in the gonadal production.

**Keywords:** Elasmobranchii, Saros Bay, maturity, reproductive cycle.

### Kuzey Ege Denizi'ndeki Mahmuzlu Camgöz (*Squalus acanthias*)'ün Üreme Biyolojisi

#### Özet

Kuzey Ege Denizi, Saros Körfezi'nde, Şubat 2005-Eylül 2008 tarihleri arasında, dip trolü ile toplam 620 adet *Squalus acanthias* örneklenmiştir. Örneklerin tümünde cinsiyet oranları 1.26:1'dir. Dişilerin toplam boyları 17,1'den 117,5 cm'e, erkeklerin ise 20,8'den 121,6 cm'e değişim göstermiştir. Dişiler pozitif allometrik büyüme ( $b>3$ ), erkekler negatif allometrik büyüme göstermiştir ( $b<3$ ). Erkekler ve dişiler arasında, toplam boy-toplam ağırlık ve karaciğer ağırlığı-toplam boy ilişkileri istatistiksel olarak önemli derecede bulunmuştur. Cinsi olgunluğa erişen en küçük dişi 54,5 cm toplam boya sahipken, cinsi olgunluğa erişen en küçük erkek 52 cm toplam boya sahiptir. Bireylerin %50'de ulaştığı ilk cinsi olgunluk boyu dişilerde 56,4 cm iken, erkeklerde 52,8 cm'dir. Oositlerin ağırlıkları 0,01 ile 36,24 g arasında değişim gösterirken, çapları 0,5 mm ve 46 mm arasında olduğu belirlenmiştir. Hepatosomatik indeks (HSI) ve gonadosomatik indeks (GSI) değerleri, hem dişi hem erkeklerin boyları ile önemli derecede artış göstermiştir. HSI ve GSI değerleri sırasıyla, erkeklerde 1,93-14,09% ve 0,01-34,35% arasında değişirken, dişilerde 1,63-17,17% ve 0,01-96,50% arasında değişim göstermiştir. Karaciğerin üreme dönemine etkisinden dolayı, HSI değerleri hem yetişmekte olan hem de yetişkin bireylerde en yüksek değerine ulaşmıştır.

**Anahtar Kelimeler:** Kıkırdaklı balıklar, Saros Körfezi, olgunluk, üreme zamanı.

#### Introduction

The spiny dogfish (*Squalus acanthias*) has a worldwide distribution except for the tropical and polar regions (Compagno, 1984) and it is known from Northwest Atlantic (Cuba to Greenland), Northeast Atlantic (Barent Sea to Morocco, Mediterranean and the Black Sea), Southwest Atlantic (Uruguay to Argentina) and Southeast Atlantic (South Africa

(Avsar, 2001; Demirhan and Seyhan, 2006; Capapé and Reynaud, 2011). It also occurs in the North Pacific (Japan to Mexico, including the Bering Sea), in the South Pacific (Chile, New Zealand and southern Australia) (Compagno, 1984; Hammond and Ellis, 2005). *S. acanthias* have found from the intertidal zone to depths of 900 m, but mostly <200 m in the Mediterranean (Compagno, 1984).

*S. acanthias* was the focus of several studies

concerning distribution, population, size-structure and reproductive biology for specimens from the Atlantic (Ketchen, 1972; Nammack *et al.*, 1985; Saunders and McFarlane, 1993; Jones and Ugland, 2001; Cortès, 2002; Henderson *et al.*, 2002; Hammond and Ellis, 2005) and the Pacific (Hanchet, 1988; McFarlane and King, 2003; Tribuzio *et al.*, 2010; Orlov *et al.*, 2011). Off the Mediterranean coast, Chatzisprou and Megalofonou (2005) and Capapé and Reynaud (2011) were studied maturity, reproductive cycle and fecundity of *S. acanthias* in the eastern and northern regions. Avsar (2001), Düzgüneş *et al.* (2005) and Demirhan and Seyhan (2007) were examined population parameters, reproduction and embryonic growth of the specimens from the Eastern Black Sea.

The spiny dogfish is an extremely long-lived, slow-growing species whose reproductive capacity is limited by late maturity, long gestation period and low fecundity. It is reported to reach 160 cm in total length (TL) and 9100 g weight (Compagno, 1984; Chatzisprou and Megalofonou, 2005). *S. acanthias* is an ovoviparous species, where the embryos develop inside the uteri of their mother, in the absence of placenta (Wourms, 1977).

The large size of preovulatory follicles suggests lengthy vitellogenesis because the energy demands to develop such large oocytes are high. Many reproductive cycles are biennial or triennial (Carrier *et al.*, 2010). Tanaka *et al.* (1990) suggested a 3.5 year gestation period for frilled shark *Chlamydoselachus anguineus* based on growth rates of embryos held in artificial conditions. Braccini, Gillanders and Walker (2006) demonstrated that *S. megalops* off Australia has an ovarian cycle and gestation period of two years. The results of Braccini, Gillanders and Walker (2006), as well as data from the shelf-occurring *S. acanthias* (Ketchen, 1972; Nammack *et al.*, 1985;

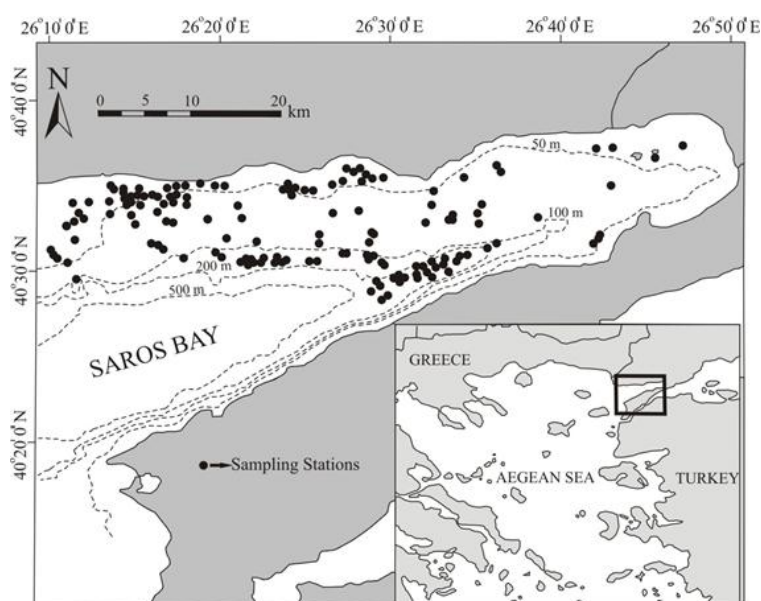
Chatzisprou and Megalofonou, 2005), suggest that many other squalids exhibit biennial reproductive cycles. Despite the knowledge obtained from the extensive studies on the species, limited information exists from the North Aegean Sea (Bilecenoglu *et al.*, 2002; Filiz and Mater, 2002; Filiz and Bilge, 2004; Kabasakal and Kabasakal, 2004; Altuğ *et al.*, 2007; Ismen *et al.*, 2009) on the distribution and length-weight relationships of the species, and no information from the Saros Bay.

The objective of the present study is to provide some basic biological information concerning reproduction of spiny dogfish from the North Aegean Sea and to compare the data with that available from other regions. The focus of the study are length-weight relationships and size at first sexual maturity of the species. Two biological indices, the gonadosomatic index (GSI) and hepatosomatic index (HSI), were examined to test how they change during the year and the reproductive cycle.

## Materials and Methods

A total of 620 spiny dogfish was obtained from commercial bottom trawls, between February 2005 and September 2008. Sampling was carried out in the Saros Bay, the North Aegean Sea in depths from 5 to 500 m (Figure 1). Trawl times lasted for approximately 30 minutes with trawl speeds averaging 2.5 knots.

Total length (TL, cm) of each specimen was measured to the nearest centimetre, from the tip of the snout to the tip of the upper lobe of the caudal fin. Total mass (TM, g) was measured to the nearest gram. Clasper length (CL, cm) was measured according to Collenot (1969), from the forward rim of pelvic girdle to tip of clasper. Oocytes and embryos were removed



**Figure 1.** Map of the study area; Saros Bay, in the North Aegean Sea.

from the ovaries and the uterus, respectively and then measured and weighed to the nearest gram. The length of individual embryos ( $TL_e$ , cm) and diameter of eggs ( $D_o$ , cm) were measured to the nearest centimeter. The total mass of each embryos ( $M_e$ , g) and eggs ( $M_o$ , g) were weighed to the nearest 0.01 g.

The onset sexual maturity was determined in males from the condition and the length of claspers following Holden and Raitt (1974), Avsar (2001) and Capapé and Reynaud (2011). The claspers of juveniles are shorter than pelvic fin, flexible and not calcified; those of sub-adults are longer than pelvic fin, flexible but slightly calcified. In adults, claspers are elongated, longer than pelvic fins length, rigid and calcified. Size at sexual maturity was determined in females from the condition of ovaries and the morphology of the reproductive tract (Henderson *et al.*, 2006). In both males and females, specimens were divided into three categories: juveniles, sub-adults and adults. Length at 50% maturity was determined by fitting maturity ogives. The percentages of mature individuals per length class (1 cm) were estimated for males and females. A logistic curve was fitted to the data and total length at which 50% of individuals are sexually mature was calculated (King, 1995) using the equation:  $P_i = 1/[1 + e^{-(a+bL)}]$ , where  $P_i$  is the proportion of mature individuals in length class  $i$  and  $a$  and  $b$  are fitted parameters which can change during the life cycle. The mean length at sexual maturity was calculated as  $L_{50} = a/b$  (Kousteni and Megalagonou, 2011). The lower and upper limits of the 95% confidence intervals (CI) were calculated for each estimated parameter.

Liver and gonads were removed from the body cavity, along with the expanded uteri in the case of gravid females. The weight of the organs were measured to the nearest gram. Both the gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated to examine how they fluctuate during the year and reproductive cycle (Chatzisyprou and Megalofonou, 2005). HSI and GSI were calculated for both males and females, as  $HSI = (LM/TM) * 100$ ,  $GSI = (GM/TM) * 100$  where LM is liver mass in g and GM is gonad mass of fish in g

(Capapé and Reynaud, 2011). Variations in HSI and GSI related to size were considered in all categories of specimens in both sexes. Normality and homogeneity of variance of the variables were tested by the Shapiro-Wilk test and Levene's test, respectively. Test for significance ( $P < 0.05$ ) were performed by using one-way Anova for comparing HSI and GSI values between juveniles, sub-adults and adults. The Student's t-test was used for differences between embryos in the left and right uterus. Parameters of the TM-TL and LM-TL relationships were estimated by the least-squares method applied to the log-transformed data for male and females as  $\log M = \log a + b * \log L$ , where  $M =$  mass (g),  $L =$  length (cm),  $a =$  is the intercept, and  $b =$  is the slope of the linear regression. In the relationship mass versus total length and liver mass versus total length, comparisons of curves were carried out by analysis of covariance (Zar, 1999).

## Results

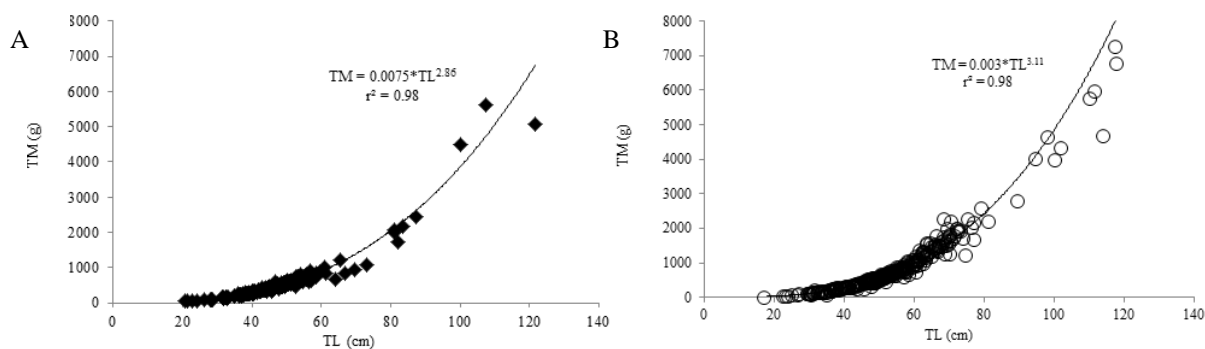
### Length-weight Relationships

A total of 620 (346 females and 274 males) spiny dogfish was sexed. The range of TL was from 17.1 cm to 117.5 cm (mean=52.6±7.9) for females and from 20.8 to 121.6 cm (mean=49.1±6.8) for males. Total mass was found to vary between 20 and 7262 g (mean=848±48.8) in female spiny dogfish and between 36 and 5626 g (mean=582.9±35.2) in male.

The relationship between TL and total mass (TM), plotted in Figure 2, showed significant differences between males and females ( $F = 26.41$ ,  $P < 0.05$ ,  $df = 1$ ). The relationship between TL and liver mass (LM) differed significantly between males and females ( $F = 15.62$ ,  $P < 0.05$ ,  $df = 1$ ).

### Reproduction of Male *S. acanthias*

The observed juveniles ranged from 20.8 to 46.5 cm TL and weighed from 36 to 462.96 g. Testes and genital ducts were inconspicuously developed and thread-like. Juveniles were not recorded in April and



**Figure 2.** Relationships for total mass (TM) versus total length (TL) for male (A) and for female (B) *Squalus acanthias*.

August; in contrast, a peak of 23 specimens was observed in October. The observed sub-adults ranged between 44 and 57.8 cm TL, and the mass was between 320 and 770 g. During the sub-adult stage, the claspers developed, they were slightly longer than pelvic fins. The testes increased in mass, but had no spermatocysts externally visible; no sperm was observed in the seminal vesicles (Figure 3a, b). The genital duct was slightly convoluted anteriorly. Sub-adults were recorded in all months, with peak in October. During the adult stage, the claspers were elongated, calcified and rigid, and are slightly longer

than the pelvic fins. Testes were well-developed and exhibited spermatocysts externally visible. The genital duct was twisted and sperm occurred in seminal vesicles. Size at first maturity was recorded 52.8 cm (95% CI, 52.5-53.1 cm) for males (Figure 4). Adult males were collected throughout year, with a peak in October. The HSI of males showed high values in the the smallest specimens, and decreased from total length (Figure 5a). Then, HSI increased when males entered maturation stage and became sub-adults; HSI reached the highest values in adult specimens. However, significant differences in HSI

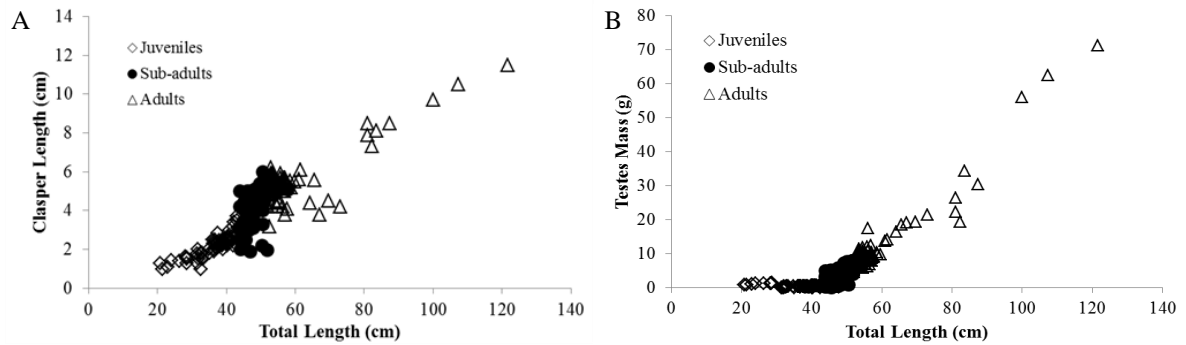


Figure 3. Clasper length (a) and testes mass (b) versus total length in male *Squalus acanthias*.

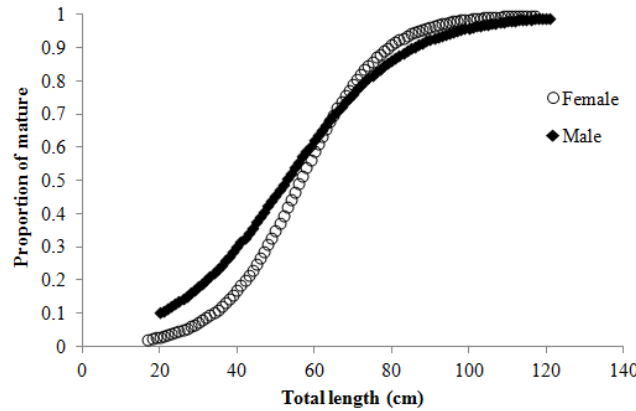


Figure 4. Maturity ogives (length at 50%) by sex of Spiny dogfish (*Squalus acanthias*) from the North Aegean Sea.

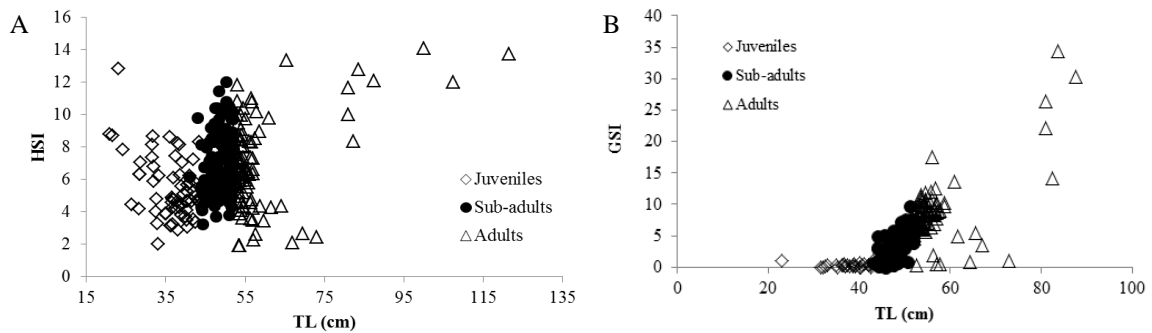


Figure 5. Variations in hepatosomatic index (HSI) (a) and gonadosomatic index (GSI) (b) versus total length in male *Squalus acanthias*.

values between juveniles and sub-adults ( $df=1$ ,  $F=29.25$ ,  $P<0.05$ ) and between sub-adults and adults ( $df=1$ ,  $F=0.18$ ,  $P>0.05$ ) did not appear differences among males. The male GSI values increased with TL of about 80 cm onward (Figure 5b); additionally, they showed significant differences between juveniles and sub-adults ( $df=1$ ,  $F=60.67$ ,  $P<0.05$ ) and between sub-adults and adults ( $df=1$ ,  $F=4.08$ ,  $P<0.05$ ).

### Reproduction of Female *S. acanthias*

The observed juveniles ranged from 22.5 to 68.5 cm TL and weighed between 45.1 and 1279 g, had whitish and undeveloped ovaries, thread-like oviducts and inconspicuous oviducal glands. Captures occurred especially from January to December. The observed sub-adults ranged from 45 to 59.5 cm TL and weighed from 422 to 1020 g, exhibiting primarily white translucent follicles and a well-differentiated genital duct. The oviducal glands were visible and slightly rounded. Rare specimens were caught year round; in contrast a peak of captures was recorded in June. The adult females exhibited a single functional ovary containing batches of yolky oocytes and exhibited fully developed genital ducts. The oviducal glands were conspicuously rounded and the mass considerably increased in adults. The number of embryos varied between 1 and 9 per female. One female contained candles in May. This had 5 eggs that were mean 14 mm big and 4 embryo that was mean

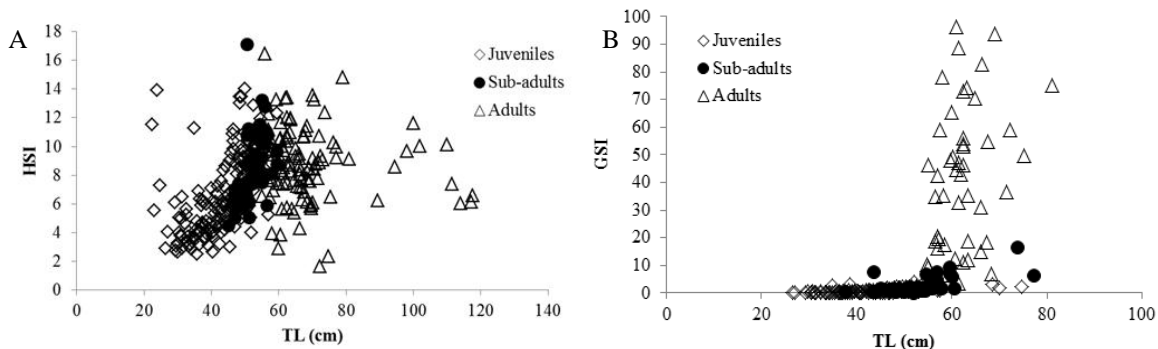
43 mm long *S. acanthias* produces large yolky oocytes. Size at first maturity was recorded 56.4 cm (95% CI, 56.0-56.8 cm) TL were adult (Figure 4). In all, 101 adult females were collected, mostly in October and November.

Female HSI showed significant differences between juveniles and sub-adults ( $df=1$ ,  $F=24.62$ ,  $P<0.05$ ) and between sub-adults and adults didn't appear differences ( $df=1$ ,  $F=0.18$ ,  $P>0.05$ ) (Figure 6a). The female GSI values significant differences between juveniles and sub-adults ( $df=1$ ,  $F=40.61$ ,  $P<0.05$ ) and between sub-adults and adults ( $df=1$ ,  $F=57.37$ ,  $P<0.05$ ) (Figure 6b).

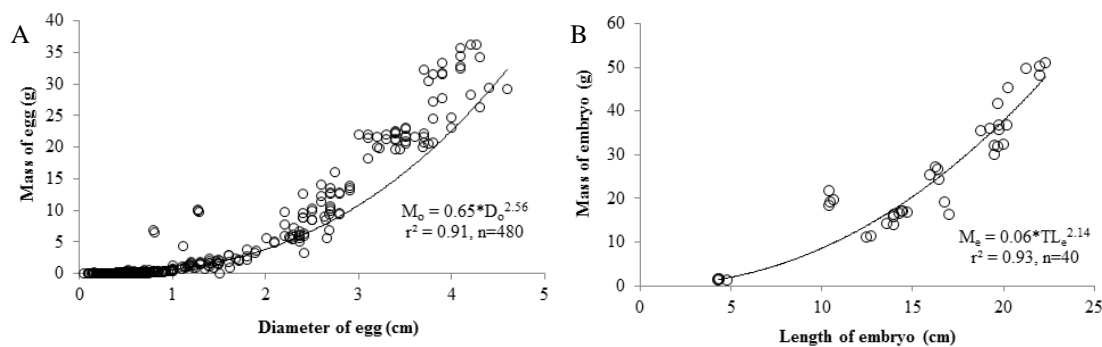
A total of 480 embryos were obtained from *S. acanthias* individuals and the length range of embryos were measured between 4.3-22.3 cm. The length ( $TL_e$ )-mass ( $M_e$ ) relationship was also studied for all embryos available. Differences were not statistically significant between embryos from the left and right uterus ( $P>0.05$ ). Therefore, all data were combined. The relationship between egg diameter ( $D_o$ ) and mass ( $M_o$ ) was also determined and it was found that eggs increase in weight exponentially (Figures 7a, b).

### Discussion

The present study reports for the first time basic information on the reproductive biology of the species *S. acanthias* from Saros Bay, in the North Aegean Sea. Previous reports on the maximum length of the



**Figure 6.** Variations in hepatosomatic index (HSI) (a) and gonadosomatic index (GSI) (b) versus total length in female *Squalus acanthias*.



**Figure 7.** Diameter-weight relationship of ovarian eggs (a) and length-weight relationship of embryos of *S. acanthias* (b).

spiny dogfish is the similar with the sizes recorded in the present study. The maximum length recorded in the Atlantic Ocean was 110 cm (Jones and Ugland, 2001), whereas in the Pacific it was 130 cm (Saunders and McFarlane, 1993) and in the South-eastern Black Sea it was 136 cm (Avsar, 2001). Compagno (1984) expressed that *S. acanthias* grows up to 160 cm, while Fischer *et al.* (1987) reported a maximum length of 200 cm in the Mediterranean Sea. In our study, the highest values of fish sampled in the North Aegean Sea were 121.6 cm its capture depths between 206-384 m for males and 117.5 cm its capture depths between 263-304 m for females. Capture depth of spiny dogfish differed from regions due to specific environmental characteristics of the area (Capapé and Reynaud, 2011). *S. acanthias* is known to be a highly migratory species that swims towards deeper waters as it grows or migrates offshore when mating occurs (Jones and Ugland, 2001). Mean length in females was found to be greater than that in males probably due to the fact that different sizes and sex occupy different depths of the water column. Chatzisyrou and Megalofonou (2005) noted the occurrence of capture in depths between 350-418 m in the eastern Mediterranean and found that similar results of size differences between females and males.

The relationships of TL-TM for females and males were significantly different. The functional regression b-values for females and males were 3.11 and 2.86; correlation coefficients 0.977 and 0.976, respectively. The confidence intervals for the b-values of males (2.80-2.91) and females (3.06-3.16) mean that the body shape of the females show positive allometric growth characteristics and the males display negative allometric growth (Figure 2a, b). Similar results were found by Avsar (2001) in the South-eastern Black Sea, Chatzisyrou and

Megalofonou (2005) in the eastern Mediterranean Sea, Demirhan and Seyhan (2007) in the southern Black Sea. Filiz and Mater (2002) found that b-values for females and males were 2.78 and 3.18 for the Northern Aegean population which not coincide with the our findings. This differences may be due to differences in sampling times and size distribution.

Size at first maturity was recorded 56.4 for females and 52.8 for males. Size at sexual maturity is relatively well known, the available data on maturity sizes reported in Table 1 for *S. acanthias*. The difference between at first maturity for the spiny dogfish in the present study and other studies may be caused by the difference in regions of these studies and therefore different geographical distributions and environmental conditions, growth characteristics, also differences exploitation and abundance for each stock (Avsar, 2001; Ismen, 2003; Yigin and Ismen, 2012). However, the spiny dogfish from the eastern Mediterranean Sea (Chatzisyrou and Megalofonou, 2005) matures earlier than spiny dogfish from the Saros Bay, the North Aegean Sea.

In spiny dogfish, it clearly appeared that vitellogenesis continued with embryonic development and could explain why the relationship of liver mass versus total mass also showed significant differences between males and females. Also, statistically significant differences were found in mean GSI and HSI values in both sexes from one stage of maturity to the next. Eventually, it suggests that liver is a key organ in reproduction, especially in the case of females because it is involved in yolk production through production of vitellogenin (Koob and Callard, 1999; Lucifora *et al.*, 2002; Kousteni and Megalofonou, 2011). Generally, the liver is larger in females than in males which may be related to the increased energy expenditure during vitellogenesis,

**Table 1.** Size at maturity of *S. acanthias* obtained by other authors

TL <sub>50</sub> (cm)	Sex	Region	Author
75	F	Southwest Ireland	Marques da Silva and Ross, 1993
92	F	Northeast Pacific	Marques da Silva and Ross, 1993
74-88	F	Northwest Atlantic	Saunders and McFarlane, 1993
73-95	F	Northeast Atlantic	Saunders and McFarlane, 1993
76-85	F	USA, Northeast coast	Saunders and McFarlane, 1993
71-93	F	Scottish Norwegian Stock	Saunders and McFarlane, 1993
59	M	Australia	Last and Stevens, 1994
77	F	Swedish waters	Stenberg, 2005
59.5	M	USA, Northeast coast	He and Stewart, 2001
82	M	Southeastern Black Sea	Avsar, 2001
88	F		
57.5	M	West coast of Ireland	Henderson <i>et al.</i> , 2002
78.2	F		
55	M	Northeast Atlantic	Pawson and Ellis, 2005
69-73	F		
47	M	Eastern Mediterranean Sea	Chatzisyrou and Megalofonou, 2005
51.8	F		
63.5-70	M	Languedocian coast,	Capapé and Reynaud, 2011
86-88	F	Southern France, Northern Mediterranean	

oocyte maturation and gestation; females store large quantities of lipids in the liver during the reproductive cycle (Lucifora *et al.*, 2005; Capapé and Reynaud, 2011).

The largest yolky oocyte recorded in the eastern Mediterranean had 5.1 cm in diameter and weighed 29.32 g (Chatzisprou and Megalofonou, 2005). Capapé and Reynaud (2011) recorded from 4.3 to 4.7 cm in diameter, and weighing from 29.5 to 37.4 g in fully yolked oocytes of spiny dogfish caught off the Languedocian coast. In this study, the largest yolky oocytes had 4.6 cm in diameter and weighed 36.24 g (Figure 7a). Intraspecific variations didn't appear in diameter and mass of yolky oocytes of female *S. acanthias* according to region (Capapé and Reynaud, 2011).

Shark species displaying an annual reproductive cycle assume concomitantly vitellogenesis and embryonic development (Capapé *et al.*, 2006). Embryos developed in uteri, a crop of fully yolked oocytes was enlarging and receiving yolk in the ovary, and soon after parturition, the female ovulated and conceived again (Capapé and Reynaud, 2011). The results obtained on the breeding cycle of *S. acanthias* need to be interpreted carefully, since there is no information about the gestation period of the spiny dogfish in the Saros Bay, the North Aegean Sea, nor for the parturition-fertilization interval. Capapé and Reynaud (2011) reported that near-term females had observed throughout the year suggests that the length of the breeding cycle of spiny dogfish was not clearly delineated from the northern Mediterranean. Ford (1921) noted that embryos ready for birth remained in the uterus for an extended period of time; it could explain that females occurred in different months of the year. Compagno (1984) reported that spiny dogfish had a 22 month gestation period. Henderson *et al.* (2002), nevertheless a wider range of gestation from 18 to 24 months and more, could be considered. Eventually, the present study indicated that the spiny dogfish in the Saros Bay, the North Aegean Sea reaches smaller sizes than spiny dogfish in other areas. However, prior reported estimates of body size at sexual maturity for spiny dogfish in the Eastern Mediterranean Sea were slightly larger compared with ours (Table 1).

In order to defined the reproductive biology of *S. acanthias* in the North Aegean Sea, further investigation must take place on the biology of the species by using the first results recorded and presented in this study. Also, these findings increase the life history data available for *S. acanthias* and form a valuable contribution to the implementation of basic management measures to ensure the sustainability of catches of this by-catch species.

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