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Reproduction Biology of the Garfish, *Belone euxini* Günther, 1866 (Belonidae: Belone) in the Southeast Black Sea

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Abstract

To describe the reproduction biology of the garfish, *Belone euxini* and differences in parameters from other populations, monthly sampling were conducted between December 2011 and July 2013 in the southeast Black Sea. Absolute fecundity ranged between 4015 and 32453 (mean: 14365±1049) and mature eggs that was left for each spawning portion (batch fecundity) ranged between 560 and 9713 (mean: 2338±243). Correlation coefficient of fecundity and total length relationships were significantly different from zero. Mean diameter of immature eggs in ovary was significantly lower than mature eggs (*t*-test: *P* = 1.54E-230). The proportion of mature eggs in the ovary ranged between 8% and 34% (mean: 16%±0.7) and the number of batches was obtained between 3 and 12 (mean: 6.8±0.3) times in a year. Monthly proportion of gonad maturity stages and monthly *GSI* values showed that the spawning period of garfish was between May and September. The size at first sexual maturity (TL_{50}) was estimated as 34.4 cm *TL* for females and 33.3 cm *TL* for males. The results of this study were offered as biological input parameters regarded as a reference for management of Black Sea stocks of the garfish species.

Keywords: Garfish, *Belone euxini*, spawning, fecundity, size at sexual maturity, Black Sea

Güneydoğu Karadeniz'de Zargana Balığının, *Belone euxini* Günther, 1866 (Belonidae: Belone) Üreme Biyolojisi

Özet

Zargana balığının (*Belone euxini*) üreme biyolojisini belirlemek ve bu özelliklerin diğer popülasyonlardan farkını tanımlamak için Güneydoğu Karadeniz'de Aralık 2011 ve Temmuz 2013 tarihleri arasına aylık örneklemeler yapılmıştır. Zargana balığının toplam yumurta verimi 4015 ve 32453 adet/birey arasında değiştiği (ortalama: 14365±1049) ve bir batın için bırakılan ovaryumlardaki olgun yumurta sayısının ise 560 ve 9713 adet/birey arasında değiştiği (ortalama: 2338±243) belirlenmiştir. Yumurta verimi ve toplam boy arasındaki korelasyon katsayısı (r) istatistiksel olarak sıfırdan farklı hesaplanmıştır. Ovaryumlardaki küçük yumurtaların ortalama çapı büyük yumurtaların (olgun yumurtar) ortalama çapından istatistiksel olarak daha küçük hesaplanmıştır (*t*-test: *P* = 1.54E-230). Ovaryumlardaki olgun yumurta sayısının toplam yumurta sayısına oranı %8 ve %34 arasında değiştiği (ortalama: %16±0,7) ve bir yıl içerisindeki batın sayısının ise 3 ve 12 arasında değiştiği (ortalama: 6,8±0,3) belirlenmiştir. Araştırma bölgesinde zargana balığının gonat olgunluk safhaları ve GSI değerlerinin aylık seyrine göre Mayıs ve Eylül ayları arasında yumurtladığı tespit edilmiştir. İlk cinsi olgunluk boyu (TL_{50}) dişiler için 34,4 cm *TL* ve erkekler için ise 33,3 cm *TL* şeklinde hesaplanmıştır. Bu çalışmanın sonuçları Karadeniz'de zargana balığı stok yönetimi için biyolojik girdi parametreleri olarak önerilmiştir.

Anahtar Kelimeler: Zargana balığı, *Belone euxini*, yumurtlama, yumurta verimi, cinsi olgunluk boyu, Karadeniz.

Introduction

Four garfish species, *Belone acus* Risso, 1827, *Belone euxini* Günther, 1866, *Belone svetovidovi* Collette and Parin, 1970 and *Tylosurus imperialis* (Rafinesque-Schmaltz, 1810), belong to Belonidae family were reported in the Turkish seas (Dalyan and Eryılmaz, 2006; Fricke *et al*., 2007). Garfish species was reported in previous studies as *B. belone euxini* by several authors (Collette and Parin, 1970; Salekhova *et al*., 1988; Zaitsev and Mamaev, 1997; Samsun *et al*., 2006) in the Black Sea and Azov Sea. Recently, in Froese and Pauly (2014), catalog of fishes and Fricke *et al*. (2007), the garfish species in the Black sea was reported valid endemic species as *B. euxini* to Black Sea, Sea of Azov and Sea of

Marmara. Above mentioned four garfish species' average annual catch is about 442.2±34.12 tons (between 232-661 tons) (TUIK, 2001-2012) for last decades in Turkey.

The first detailed studies on reproduction properties of *B. belone* such as spawning time and gonad maturity stages was carried out by Dorman in Courtmacsherry Bay in the Southern Ireland (Dorman, 1989) and from Strömstad on the west coast to Västervick in the Baltic Sea (Dorman, 1991). After the studies of Dorman, reproduction biology of *B. belone* such as spawning time, size at sexual maturity and fecundity were studied between January 2003 and December 2008 in the eastern part of the middle Adriatic Sea (Zorica *et al*., 2011). Embryonic and larval development of *B. belone* was also studied under laboratory conditions (Dulčić *et al*., 2009; Kužir *et al*., 2009). Spawning time based on gonadosomatic index and fecundity of *B. belone* was studied in the Turkey's Aegean Sea coasts by Uçkun *et al*. (2004) and in the Bosporus by Yüce (1975). Moreover, only one study of *B. euxini* spawning time and absolute fecundity features was carried out for population in the middle Black Sea (Samsun *et al*., 2006). However, no knowledge existed on size at first maturity of *B. euxini* in the Black Sea. The objective of the present study was to provide new findings on annual number of spawning of this species, also to present some details estimation on the size at sexual maturity necessary for the introduction of suitable management plans for garfish small scale fisheries in the Anatolian coast of the Black Sea and to assess the differences in these parameters in other populations of the different geographical region.

Materials and Methods

Study Area and Sampling

Samplings were conducted monthly between December 2011 and July 2013 except for August from the Rize coasts, in the southeast Black Sea (Figure 1). 17 sampling were conducted some months by garfish encircling nets with 21 mm mesh size during the study period (2011: 2 sampling in December, 2012: 2 sampling in January and October, 1 sampling in February, September, November and December, 2013: 2 sampling in January and March, 3 sapling in February). In other months, garfish specimens were obtained from garfish fishermen fishing with commercial garfish encircling nets with 21, 22 and 23 mm mesh sizes. In the warmer part of the year (May - July) due to absent of garfish species which are not creating schools in this area at that time, no garfish catches were obtained. Furthermore, because of the fact that the commercial garfish fishing was completely finished in summer months in the study area, and so garfish specimens for this study

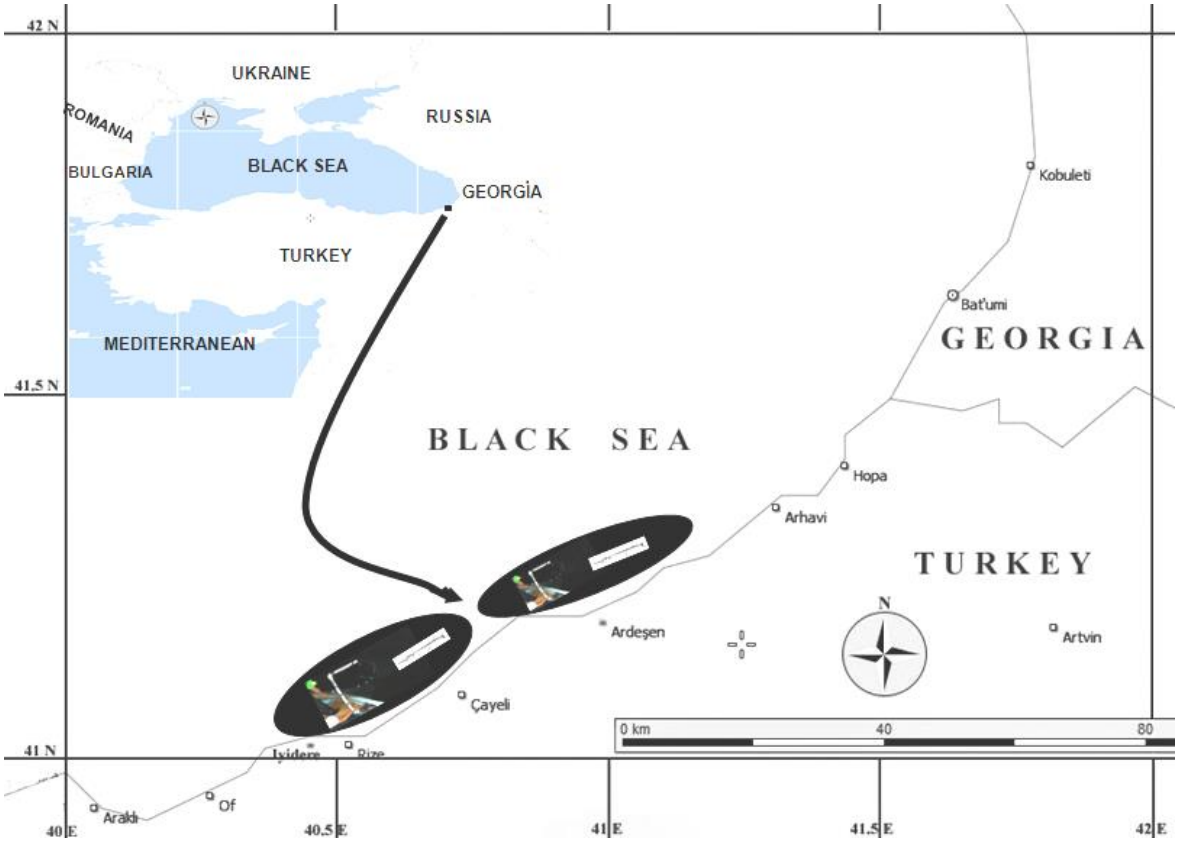

Figure 1. Study site off Rize coast, Black Sea. Shaded areas indicate the sampling sites.

were obtained from fisheries of the red mullet and whiting bottom set trammel nets in May 2012, May, June and July 2013. Moreover, no garfish specimens were obtained in August during the study period.

Reproduction Biology

Gonad Maturity and Spawning Time

The maturity stages of *B. euxini* specimens were determined within five categories, based on morphological characteristics of the ovaries and testes, modified by Dorman (1989): stage 1: virgin or immature, stage 2: early developing and resting, stage 3: mature, stage 4: ripe, and stage 5: recently spent.

The spawning period was graphically determined for both sexes by the monthly variation of mean values of the gonadosomatic index (*GSI*) as:

$$\mathrm{GSI} = \frac{Wg}{W} \times 100 ,$$

where, W_g is gonad weight (g), W is total garfish weight (g).

Fecundity

A total of 46 mature ovaries with stage 3 and 4 were used for fecundity estimation. The mature ovaries were cut longitudinally and fixed separately in Gilson's fluid (Davenport, 1960). The ovaries were regularly vigorously shaken about 60 days to remove the eggs from the membranes. To calculate of total (absolute) fecundity and batch fecundity, the ovaries were than processed as described by Dorman (1991).

Total fecundity was calculated using gravimetric methods as follows:

$$F = \frac{n * G}{g} ,$$

where, F is total fecundity, n is eggs number of fish gonad subsample, G is fish gonat weight (g) and g is subsample gonad weight of fish gonad (g).

The average number of eggs (absolute fecundity) that are left for each spawning portion was calculated (Avşar, 2005) as:

$$\overline{N} = \sum_{i=1}^{m} ((\frac{n_i * G_i}{g_i}) * (\frac{1}{m})) ,$$

where, $\overline{N}$ is the average number of eggs that are left for each spawning portion, n_i is eggs number of i th fish gonad subsample, G_i is i th fish gonat weight (g), g_i is subsample gonad weight of i th fish gonad, m is number of examined garfish gonads.

The average number of spawning frequency was calculated (Avşar, 2005) as follows:

$$\overline{NB} = \sum_{i=1}^{m} \left(\frac{TF_i}{ME_i} \right) * \left(\frac{1}{m} \right) ,$$

where, $\overline{NB}$ is the average number of spawning in a year, TF_i is the total number of eggs in the *i th* fish ovary (number of small and large sized eggs) and ME_i is the total number of matured eggs in the *i th* fish ovary (number of large sized eggs) and m is number of examined gonads.

Percent of small sized eggs (*PS*) and large sized eggs (*PL*) in ovary was calculated as:

$$PS = \frac{F_s}{F_{s+l}} * 100 , \qquad PS = \frac{F_l}{F_{s+l}} * 100 ,$$

where, F_{s+l} is number of small plus large sized eggs in ovary, F_s is number of small sized eggs in ovary and F_l is the number of large sized eggs in ovary.

Comparison of the difference of correlation coefficient from zero *t*-test (Snedecor and Cochran, 1989) was calculated as:

$$t = \frac{r * \sqrt{(n-2)}}{\sqrt{(1-r^2)}} ,$$

where, n is the number of garfish used in the computation and r is correlation coefficient. The value of correlation coefficient is different from zero if t value is greater than the tabled t values for n-2 degrees of freedom.

Egg size diameter of 5-15 eggs for each ovary was measured using a Nikon SMZ1000 mark stereomicroscope with a Nikon DSFI1 digital camera connected to a computer. Eggs were separated into two groups according to size (small: immature and large: mature) and these eggs was performed for fecundity analyzes. Size frequency distributions analyze for small and large sized eggs were conducted using Kolmogorov-Smirnov two-sample test. Comparison of the mean diameter of eggs between two groups was also performed using t-test performed with the software package PAST version 2.14 (Hammer *et al*., 2001).

Fecundity-total length relationship (Avşar, 2005) was estimated as:

$$F = a * TL^b ,$$

where, F is the fecundity, TL is the total length (cm), a is the intercept, and b is the slope of the regression line.

Size at Sexual Maturity

Size at sexual maturity was determined from female and male by calculating the proportion of

mature female and male in 2 cm size classes in the breading period. Individuals with stage 3, 4 and 5 in the gonad development stage were considered to be mature (Dorman, 1989; Zorica *et al*., 2011). The proportion of mature female and male by size were fitted to the logistic equation:

$$P = \frac{1}{1+e^{a+bTL}},$$

where, P is the proportion of mature female or male, a and b are the coefficients of the equation, and TL is the total length. Size at sexual maturity (TL_{50}), corresponding to 50% of males and females that have reached first sexual maturity, was calculated from -(a/b).

Results

Length Structure

A total of 1211 garfish (618 female, 593 male) were examined between January 2012 and July 2013. The total length of female ranged 24.7 and 65.1 cm and the total length of male ranged between 22.2 and 55.3 cm. The mean total length of female (mean 39.10±0.248 cm) was significantly (t test: P = 9.41E-31) greater than the mean total length of male (mean 35.2±0.209 cm). Size frequency distribution were also significantly different (Kolmogorov-Smirnov two-sample test: d = 0.29178, P = 3.95E-23) between female and male.

Fecundity

Number of small, large sized and combined eggs in ovary (fecundity) and total length relationships were showed in Figure 2, separately. A total of 46 garfish total length ranged between 33.4 and 62 cm (mean: 43.0±0.9 cm) were used for fecundity estimation. Total fecundity (small plus large sized eggs) ranged between 4015 and 32453 (mean: 14365±1049). Small sized eggs ranged between 3447 and 27527 (mean: 12037±860) and large sized eggs that was left for each spawning portion ranged between 560 and 9713 (mean: 2338±243). Correlation coefficients of the fecundity and total length relationships were significantly different from zero. Fecundity and total length relationships were estimated as follows:

Small plus large sized eggs: $F_{s+l}=53.431TL^{1.4598}$ (r^2=0.1673, n = 46, t = 3.2581, P<0.01)

Small sized eggs: $F_s = 69.381TL^{1.344}$ (r^2 = 0.1453, n = 46, t = 2.9585, P<0.01)

Large sized eggs: $F_l = 1.2314TL^{1.9635}$ (r^2 = 0.2207, n = 46, t = 3.9989, P<0.01).

Egg Size

Diameter of small sized eggs ranged between 380 and 1310 µm (mean: 1820.2±14.81 µm) and diameter of large sized eggs ranged between 1420 and 3410 µm (mean: 1720.9±319.30 µm). Mean diameter of small sized eggs was significantly lower than large sized eggs (t-test: P = 1.54E-230) and size frequency distributions were also significantly different between small and large sized eggs (Kolmogorov-Smirnov two-sample test; d = 1, P = 2.593E-120).

Annual Number of Batches

The proportion of small sized eggs in the ovary ranged between 66% and 92% (mean: 84%±0.7) and the proportion of large sized eggs in the ovary ranged between 8% and 34% (mean: 16%±0.7) (Figure 3). The number of batches was also calculated between 3 and 12 (mean: 6.8±0.3) times in a year during the spawning period.

Spawning Time and Gonad Maturity

Monthly proportions of gonad maturity stages are showed in Figure 4. A total of 1211 garfish (618 female and 593 male) were used for gonadosomatic index and maturity stages estimation. The stages 1 and 2 appeared generally between September and April for both sexes and stages 3 and 4 appeared generally between March and July for female and mostly between February and April with a small ratio in June and July for male. Recently spent stage was observed in two female individuals collected in July.

Looking at the monthly changes in *GSI* values variation (Figure 5), one peak of *GSI* values were clearly exhibited in May 2012 and 2013 and then *GSI* values decreased until July 2013 and reached the lowest level in September 2012 for both female and male. This monthly fluctuation of *GSI* values showed that the spawning period of garfish was between May and September in the study area.

Size at Sexual Maturity

Size at sexual maturity was estimated from 176 female of which 131 were mature and from 100 male of which 51 were mature. Total length of mature females ranged between 29.1 and 62.1 cm (mean: 41.6±0.61 cm) and between 29.7 and 55.3 cm (mean: 39.1±1.14 cm) for males. Length at first maturity for males and females are shown in Figure 6. The relationship between total length and proportion of mature males and females were estimated as follows:

$$P = \frac{1}{1+e^{14.668-0.4409*TL}} \text{ (males)}$$

$$P = \frac{1}{1+e^{8.7456-0.2547*TL}} \text{ (females).}$$

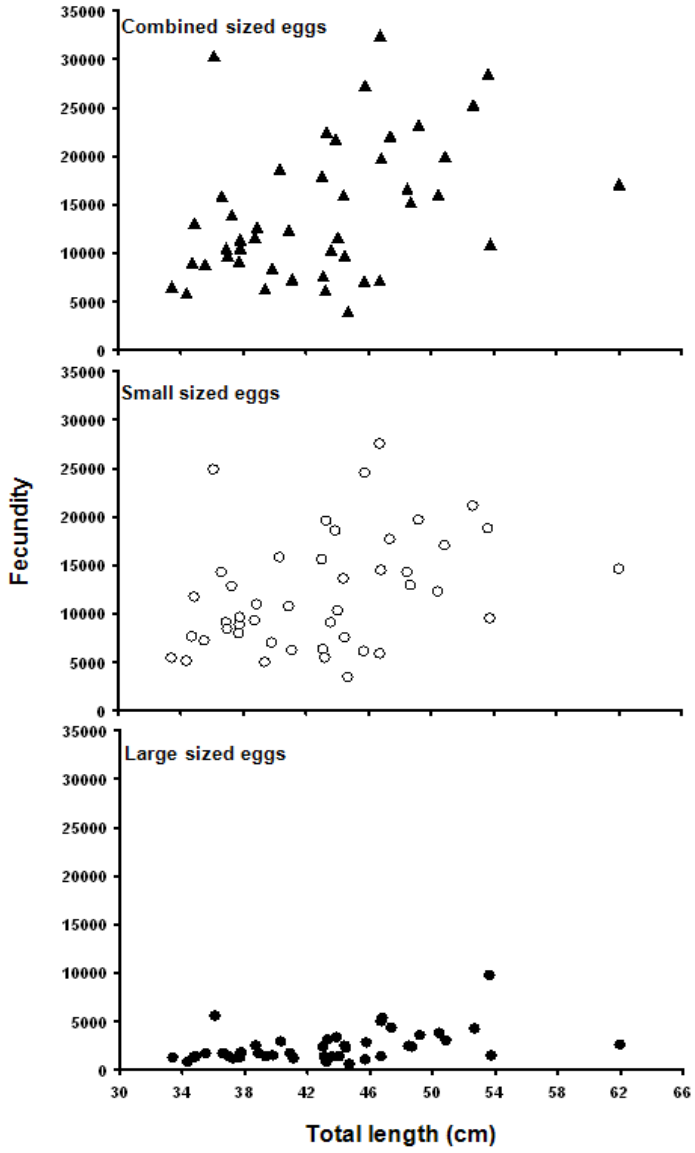

Figure 2. Fecundity and total length relationships of garfish in the Black Sea.

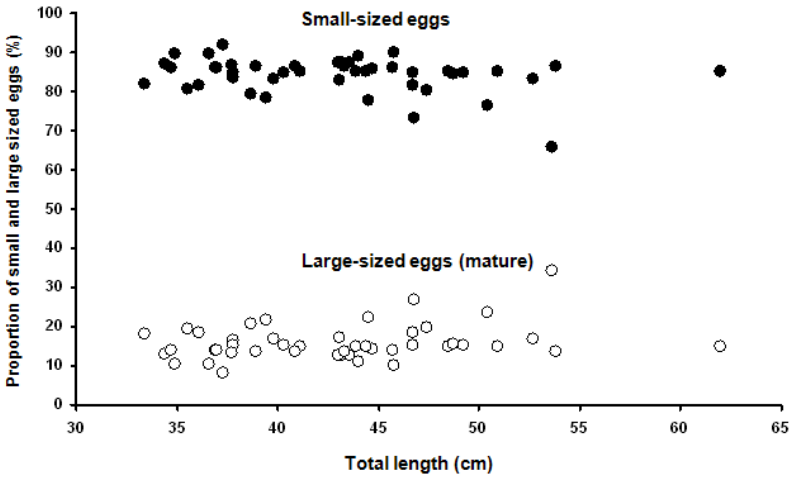

Figure 3. The proportion of small (immature) and large (mature) sized eggs in the ovary and total length relationships of garfish specimens in the Black Sea.

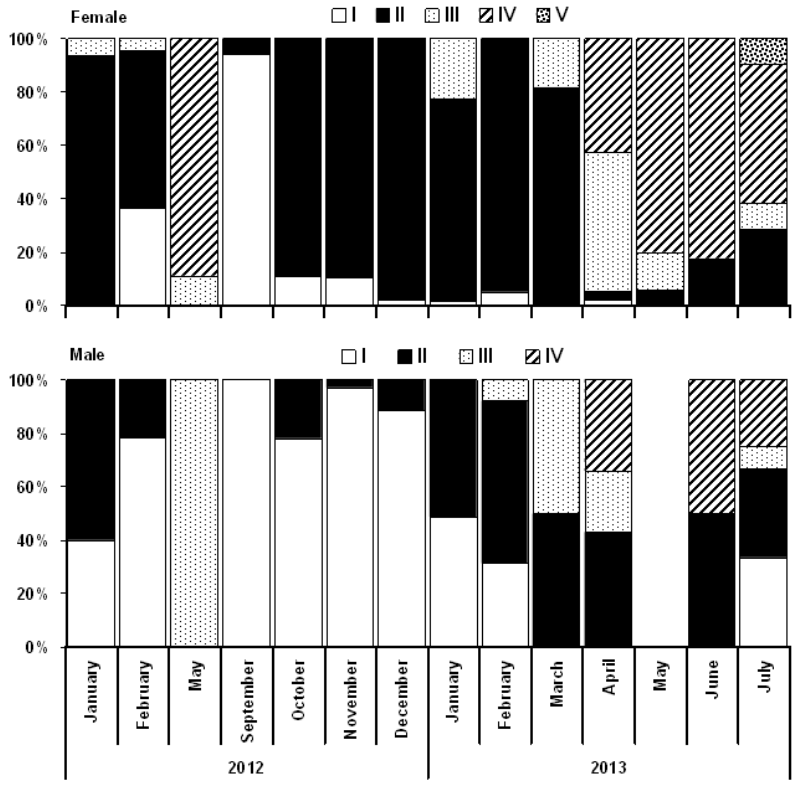

Figure 4. Monthly variations of ovary (female) and testis (male) maturity stages of *Belone euxini* between January 2012 and July 2013 in the Black Sea.

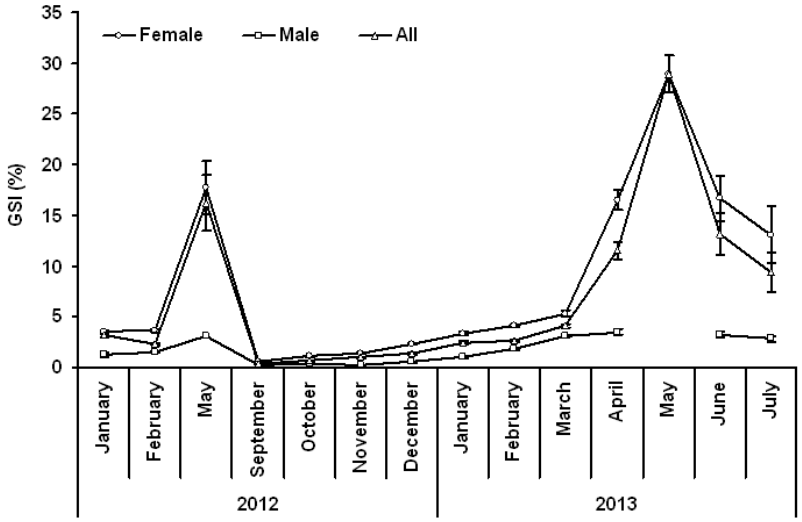

Figure 5. Monthly variations of gonadosomatic index of female, male and combined garfish specimens between January 2012 and July 2013 in the Black Sea.

So, females reached its first maturity at total body length of 34.4 cm and males at 33.3 cm (Figure 6).

Discussion

Data concerning population dynamics details such as length and age structure, sex ratio, growth and mortality rate of *B. euxini* have been published (Bilgin *et al*., 2014), while data describing this species' reproduction biology such as fecundity and egg size, fist maturity size, spawning time and annual number of spawning are presented here.

Fecundity and Egg Size

Total fecundity, batch fecundity and eggs diameters of garfish species obtained different geographical regions are showed in Table 1. The largest egg diameter for *B. belone* was reported as

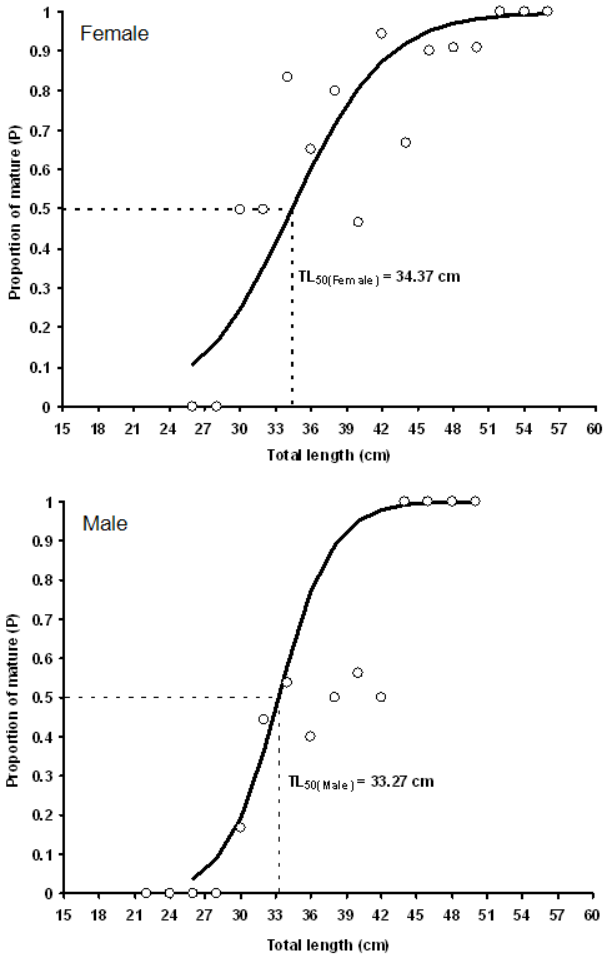

Figure 6. Size at 50% sexual maturity for female and male garfish specimens in the Black Sea.

Table 1. Comparison of fecundity and egg size (minimum-maximum and mean ± standard error values) of *Belone belone* and *B. euxini* from different geographical areas. Total fecundity: total number of eggs (small and large sized eggs) in the ovary. Batch fecundity: the number of eggs spawned (mature eggs) in each batch

Author	Locality	Species	Total fecundity	Batch fecundity	Egg diameter (µm)	
					Small and large sized eggs	Large sized eggs
Uçkun *et al*. (2004)	Aegean Sea	*B. belone*	1066-20446 (7780)		400-4000 (1840)	
Dorman (1991)	Atlantic Sea	*B. belone*	2193-10804			
Zorica *et al*. (2011)	Adriatic Sea	*B. belone*	8319-53534 (23595)			1223-4283 (2269±332)
Samsun *et al*. (2006)	Black sea	*B. euxini*	8460-51694 (24088±184)	353-4711 (1242±843)		
Present study	Black Sea	*B. euxini*	4015-32453 (14365±1049)	560-9713 (2338±243)		1420-3410 (1721±320)

4000 µm (mean: 1840 µm) in the Aegean Sea (Uçkun *et al*., 2004) and 4283 µm (mean: 2269±332 µm) in the Adriatic Sea (Zorica *et al*., 2011). The average diameters of newly spawned eggs of *B. belone* caught in the Karin Sea (middle Adriatic Sea) was also reported as 3071.9±75.73 µm at 19.4ºC and 22.3ºC temperature (Dulčić *et al*., 2009), this result was naturally higher than average size of mature egg yet not spawned. In the present study, the largest egg diameter for *B. euxini* was recorded as 3410 µm (mean: 1721±320 µm). The egg size results indicating that *B. euxini* had lower egg diameter than *B. belone*. Fecundity for *B. belone* was reported between 1066 and 20446 oocytes per ovary (mean: 7780) in the Aegean Sea (Uçkun *et al*., 2004), between 2193 and 10804 oocytes per ovary in the Atlantic Sea (Dorman, 1991) and from 8319 to 53534 oocytes per ovary (mean: 23595) in the Adriatic Sea (Zorica *et al*.,

2011). Moreover, fecundity for *B. euxini* was reported as 51694 oocytes per ovary in the Black Sea (Samsun *et al*., 2006). Fecundity is a phenotypic character that is affected in different ways and intensities by specific features of different environments (Hines, 1991) and its variation among species may enable species coexistence (Nazari *et al*., 2003). Egg size is also an important diverse life history characteristic of species. On the one hand, eggs size and fecundity can be change due to size and age of brood stock individuals. As fecundity is a size dependent parameter, it is possible that the differences concerning fecundity among the geographical regions could be explained by the size of the garfish in each region (Zorica *et al*., 2011).

Our data on egg size and fecundity showed that individual female produce 3 and 12 (mean: 6.8±0.3) batches of eggs during the spawning period and the proportion of large sized eggs (mature eggs) in the ovary ranged between 8% and 34% (mean: 16±0.7%). When total fecundity of *B. belone* divided by batch fecundity, female individuals produce 11.32 and 23.6 (mean 18.99) batches of eggs during the spawning period in the Adriatic Sea (Zorica *et al*., 2011). These results may be indicating that garfish species such as *B. euxini* and *B. belone* are batch spawner and spawning continuous during the spawning season. These results also should be supported by eggs and larvae surveys studies on the garfish species in the Black Sea. Also, Gürcan (2012) firstly reported 10 mm total length a garfish larvae in June 2012 off Sinop coast in the Black Sea that supported our results.

Spawning Season

Temperature is one of the most important environmental factors that influence the reproduction activity, embryonic and larval development and growth of fish (Avşar, 2005). Seasonal change in temperature has a profound effect on reproduction in fish species and increasing temperatures provoke reproductive development in spring spawning species, and falling temperatures stimulate reproduction in autumn spawners (Pankhurst and Munday, 2011). The spawning season of *B. belone* was reported over 6 months in the early winter-spring period between December and May in the eastern part of the middle Adriatic Sea (Zorica *et al*., 2011), between May and June in Courtmacsherry Bay in the Southern Ireland from Strömstad on the west coast to Västervick in the Baltic Sea (Dorman, 1989; 1991), between April and August in the Turkey's Aegean Sea coasts (Uçkun *et al*., 2004). It was also reported for *B. euxini* over 5 months between April and August in the Bosporus (Yüce, 1975) and between May and September off Sinop peninsula in the Black Sea (Samsun *et al*., 2006). According to our results, monthly changes of *GSI* and maturity stages of female and male showed that annual spawning cycle of *B. euxini* was between May and September off Rize coast in the Southeast Black Sea. The differences of garfish spawning season among different geographical regions may be due to differences environmental factors especially temperature and salinity (Zorica *et al*., 2011).

Size at Sexual Maturity

Size at sexual maturity is one of the important parameters that can be used in defining minimum mesh and landing size. In the previous studies there are no findings include size at sexual maturity for the Black Sea garfish stocks. But, Samsun *et al*. (2006) speculated that, size at sexual maturity of *B. euxini* was 38.6 cm TL off Sinop peninsula in the middle Black Sea. Moreover, it was reported for *B. belone* as 31.5 cm TL for female and 28.0 cm TL for male in the Adriatic Sea (Zorica *et al*., 2011). Our results were higher than Adriatic Sea and slightly lower than middle Black Sea. Different results on size at sexual maturity may be due to different length composition which used to size at maturity calculation and different fishing pressure levels among the researcher areas.

The results of this study could be used as biological input parameters regarded as a reference (e.g., minimum fish size: 35 cm total length, closed season: between May and July) for management of Black Sea stocks of this species.

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