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## Reproductive performance of turbot (*Psetta maxima*) in the southeastern Black Sea

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**Abstract:** Spawning time, total fecundity, fertilization, and hatching rates of turbot (*Psetta maxima*) were investigated in 10 wild female broodstock (mean wt: 3809.7 ± 1238.4 g). Spawning lasted 37 days, from April 17 to May 23. Mean total fecundity was 2329 ± 1260 × 10<sup>3</sup> eggs per female. Fertilization and hatching rates were 30.6 ± 25.4% and 17.9 ± 16.1%, respectively. A significant positive correlation was observed between body weight and total fecundity (r = 0.637, P < 0.05). The timing and frequency of hand stripping, fecundity, fertilization, and hatching rates in turbot are presented.

**Key words:** Turbot, *Psetta maxima*, Black Sea, reproduction, fecundity, fertilization, hatching rate

### Güneydoğu Karadeniz'deki kalkan balığı (*Psetta maxima*)'nın üreme performansı

**Özet:** Doğadan yakalanan 10 dişi kalkan, *Psetta maxima*, anacında (ortalama ağırlık 3809,7 ± 1238,4 g) yumurtlama zamanı, toplam yumurta verimi, döllenme ve çıkış oranı belirlendi. Yumurtlama 17 Nisan 23 Mayıs arasında 37 gün devam etti. Ortalama toplam yumurta verimi 2329 ± 1260 × 10<sup>3</sup> yumurta/anaç, döllenme ve çıkış oranları sırası ile %30,6 ± 25,4 ve %17,9 ± 16,1 olarak belirlendi. Ağırlık ile toplam yumurta verimi arasında önemli pozitif korelasyon (r = 0,637; P < 0,05) saptanmıştır. Sonuç olarak, kalkan balığının sağım zamanı ve aralığı, yumurta verimi, döllenme ve çıkış oranları ortaya konuldu.

**Anahtar sözcükler:** Kalkan balığı, *Psetta maxima*, Karadeniz, üreme, yumurta verimi, döllenme, çıkış oranı

### Introduction

Turbot (*Psetta maxima*) is a very large, broad-bodied left-eyed flatfish that belongs to the family Scophthalmidae. Its geographical range extends from Icelandic seas to the Mediterranean, including the Sea of Marmara, and the Black Sea (Blanquer et al., 1992). This species is commercially fished using beach

seines, gill nets, and trammel nets in coastal waters, and beam and otter trawl nets in deeper nearshore waters.

Various aspects of the biology of turbot have been investigated along the Black Sea coast of Turkey, including the identification of flatfish in the Black Sea and its adjacent waters (Amaoka et al., 2001), stock

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assessment (Zengin, 2000; Suzuki et al., 2001; Samsun et al., 2007), broodstock management (Hara et al., 2002; Başaran and Samsun, 2004), larval and juvenile development (Şahin, 2001a, 2001b; Kohno et al., 2001; Moteki et al., 2001; Şahin and Üstündağ, 2003; Türker et al., 2005; Türker, 2006), and some commercial fishing aspects (Samsun, 1995; Samsun and Kalaycı, 2004).

Although turbot has recently attracted interest as a candidate for stock enhancement and aquaculture in Turkey, and is among the major components of demersal fish communities in many coastal areas of the Black Sea, data on the reproductive biology of this species are limited. The present study aimed to clarify the reproductive biology of turbot along the southeastern Black Sea coast of Turkey.

## Materials and methods

Ten females (length: 47.4-66.7 cm; mean wt: 3809.7 ± 1238.4 g) and 10 males (length: 45.2-57.2 cm; mean wt: 2407.6 ± 695.1 g) were collected with a trawl net by the research vessel of the Central Fisheries Research Institute (CFRI) during a survey conducted once a week at a depth of 5-70 m off the coast of Trabzon, Turkey, just prior to spawning in April 2003. Upon arrival at the CFRI, the fish were examined and put into a quarantine tank for observation and disease treatment. Fish were bathed in formaldehyde for 1 h (100 ppm) and furazolidone for 1 h (20 ppm) to prevent bacterial disease.

After 2 weeks of acclimation the fish were individually tagged and stocked at 3-4 fish per m<sup>2</sup> in a maturation tank (1 × 2 × 0.5 m). Light intensity was maintained at around 100 lux using fluorescent bulbs at night and ambient conditions during the daytime. Daily water exchange averaged 900%. Aeration was provided by 2 air stones with a total output of 4 L/min. During spawning water temperature was maintained at 15.0 °C. The fish were not fed during spawning.

Gonad maturation in males was examined microscopically. One drop of seawater was placed on a glass slide, to which a small amount of milt was added. Sperm activity was examined under a microscope at 100× magnification. Viable sperm were characterized by whirl-like movement after the seawater and milt were stirred. Gonad maturation in

females was evaluated by sampling a small number of eggs with a cannula. These eggs were transferred to a glass slide and measured under 40× magnifications. Females with at least 100 oocytes larger than 0.4 mm in mean diameter were considered ready to spawn (Çiftçi et al., 2002).

Males with viable milt and females ready for spawning received hormonal injections. Males were injected with a mixture of human chorionic gonadotropin (HCG) and white salmon pituitary gland (WSPG: 100 IU/kg fish). Females were injected with a pelletized luteinizing hormone-releasing analogue (LHRH-a: 100 µg/kg fish) (Berlinsky et al., 1996).

Eggs were stripped into a clean plastic container filled an adequate amount of seawater. The wet method was used for fertilization. Eggs from individual fish were fertilized in seawater by gently mixing with newly stripped milt from 2 males. Eggs were kept in the mixing container for about 10 min to ensure fertilization. Ten minutes after fertilization the eggs were rinsed with filtered seawater. The seawater used for fertilization and egg rinsing were the same temperature as the seawater in which the broodstock were maintained. Thereafter, the eggs were disinfected for 5 min in 50 ppm PVP iodine and rinsed thoroughly in filtered seawater. No attempt was made to separate viable and nonviable eggs. Unfertilized eggs did not quickly settle out of suspension because of low salinity; therefore, fertilization success was calculated by counting the proportion of eggs that started cell division. Eggs were incubated as described by Şahin (2001b).

The weight and length of spawners, spawning time, total fecundity (number of eggs/female), relative fecundity (eggs/kg female), fertilization rate (at the 4-cell stage 4 h after fertilization), and hatching rate (ratio of hatched larvae to total number of eggs) were recorded. Data were analyzed using Minitab statistical software, and means and differences at the 5% level were considered significant.

## Results

The spawning season commenced on April 17 and continued until May 23 (37 days), and spawning during the first 10 days was high, peaking on April 24

(Figure 1). Biometric and reproductive parameters in the broods used in this study are summarized in the Table. Figure 2 shows the number of eggs produced per body weight of female turbot. The egg batches were stripped at 24-h intervals. During 119 hand-stripping trials 23,761,770 eggs from 10 females were obtained. Female A was stripped 7 times and produced 1,610,000 eggs, and female J was stripped 16 times and produced 3,499,000 eggs. The highest fecundity was observed in female G (weight: 5361 g), which was stripped 14 times. Total fecundity exhibited a significant positive correlation with female body weight ( $r = 0.637$ ,  $P < 0.05$ ) (Figure 3). The egg fertilization rate varied between 9.1% and 97.7% (mean:  $30.6 \pm 25.4\%$ ), and the hatching rate ranged from 1.9% to 58.5% (mean:  $17.9 \pm 16.1\%$ ).

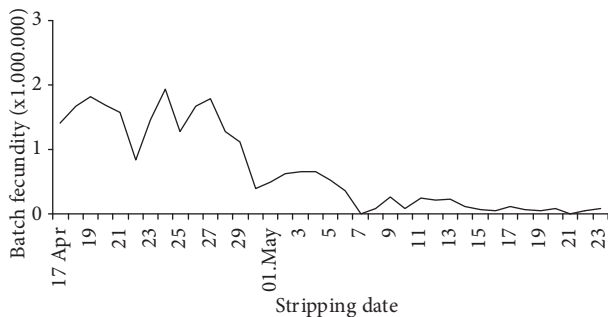


Figure 1. Total egg number obtained daily from different batches at 24 h intervals (from April 17 to May 23) in 10 females of turbot (*P. maxima*).

## Discussion

Female fecundity is a valuable parameter for estimating the number of available eggs per spawning season (Bambill et al., 2006). According to Zengin

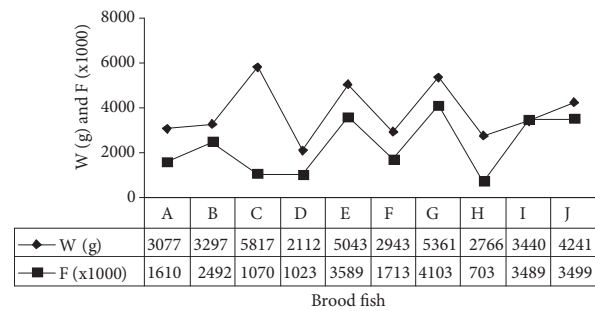


Figure 2. Number of eggs produced (F) per body weight (W) of 10 female turbot (*P. maxima*).

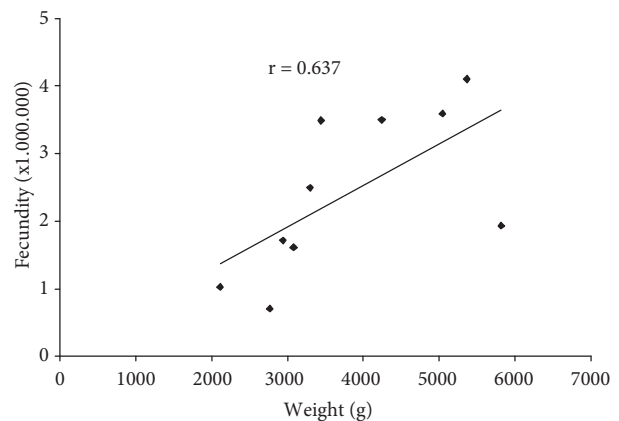


Figure 3. Correlation between total fecundity and brood weight in turbot (*P. maxima*).

(2000) and Hara (2001), turbot can produce up to 400,000-500,000 eggs/kg of female body weight. Colman (1973) reported that the mean fecundity of sand flounder (*R. plebeia*) varied from 100,000 eggs to 500,000 eggs for fish 18-30 cm long, and that of yellow-belly flounder (*R. leporina*) varied from 250,000 eggs to 1.25 million for fish 30-45 cm long.

Table. Reproductive variables of turbot (*P. maxima*) broodstock.

Variable	n	Minimum	Maximum	Mean $\pm$ SD
Total length (cm)	10	47.4	66.7	$56.3 \pm 5.47$
Body weight (g)	10	2111.8	5817.2	$3809.7 \pm 1238.4$
Total fecundity ( $10^3$ )	10	703	4103	$2329 \pm 1260$
Relative fecundity ( $10^3$ )	10	184	1014	$610 \pm 257$
Fertilization rate (%)	10	9.1	97.7	$30.6 \pm 25.4$
Hatching rate (%)	10	1.9	58.5	$17.9 \pm 16.1$

The fecundity of *P. orbignyana* was reported as 240,000-280,000 eggs/kg for fish weighing 1.78 kg and 2.86 kg by Cerqueira et al. (1997), but Bambill et al. (2006) reported a lower number for the same species (185.951-399.118 eggs/kg). Mean fecundity in southern flounder (*P. lethostigma*) reported by Watanabe et al. (2000) was 735,432 eggs per female for females weighing 1.12 kg, while Smith and Denson (2000) reported 230,000-1,000,000 eggs per female for females with a mean weight of 1.2-2.9 kg. The fecundity observed in the present study is higher than that in the other flatfish species. These differences are not contradictory, considering that broodstock size, age, and genotype, as well as daily and seasonal feeding rates can influence the number of eggs produced (Bromage, 1996). There is a general positive exponential relationship between fish size and the number of eggs (Jenning et al., 2001). In most flatfish species fecundity is positively related to age (Bagenal, 1966). The fecundity of *P. maxima* varied greatly with body weight, but the average total and relative fecundity obtained in the present study had a linear relationship with weight.

Turbot is a batch-spawning flatfish. Estimates of the ovulatory periodicity in turbot indicate that a 1-day interval may characterize the regular ovulation pattern. According to the present findings, females produced a mean number of 7-16 batches for about 1 month, and batch fecundity usually remained within

a range of 30,000-750,000 eggs. Mean fertilization and hatching rates recorded in the present study were 30.6% and 17.9%, respectively. As observed during this strip spawning study, there was variability in fertilization success, which may have been related to egg quality. According to Bromage et al. (1994), over-ripening is a significant determinant of egg quality in many fish species. The fertilization rate depends primarily on the time after ovulation (Koya et al., 1994). Maslova (2002) reported that fertilization rates in Black Sea turbot eggs decreased from 90%-95% to 0% following a 24-h delay between the ovulation and stripping time. In the present study the minimum time between the 2 strippings was 24 h; therefore, over-ripening and low fertilization rates would be expected.

In conclusion, the present study describes the management of turbot (*P. maxima*) for use as broodstock. The timing and frequency of hand stripping, and fecundity, fertilization, and hatching are presented. Further research that focuses on larval production and on-growing on a commercial scale is needed.

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