Turkish Journal of Zoology

Volume 35 | Number 6 Article 4

1-1-2011

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TÜBİTAK

Research Article

Turk J Zool
2011; 35(6) 811-817
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doi:10.3906/zoo-1004-1

The reproductive performance of wild and hatchery-reared flounder, *Platichthys flesus luscus*, in the southern Black Sea coast

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Received: 01.04.2010

Abstract: The spawning period, total fecundity, egg size, and fertilization and hatching rates of wild and hatchery-reared flounder, *Platichthys flesus luscus*, are investigated in this report. Wild and hatchery-reared broodstock consisted of 18 females (452.8 ± 193.2 g) and 17 females (138.9 ± 47.4 g), respectively. The spawning period lasted 79 days, from 22 January to 10 April. Mean total fecundity was 285 ± 157.9 × 10^3 per female for wild broodstock while it was 115.6 ± 53.6 × 10^3 eggs for hatchery-reared broodstock. The fertilization and hatching rates were 38.2 ± 17.0% and 62.3 ± 24.1% for wild and 45.3 ± 22.7% and 54.8 ± 26.5% for hatchery-reared broodstock, respectively. The reproductive parameters of wild and hatchery-reared flounder in the Black Sea were similar.

Key words: Flounder, *Platichthys flesus luscus*, reproduction, fecundity, fertilization, hatching rate

Güney-doğu Karadeniz'deki doğal ve kültür pisisi (*Platichthys flesus luscus*)'nin üreme performansı

Özet: Doğadan yakalanan ve kültürü yapılan pisi, *Platichthys flesus luscus* anaçlarında yumurtlama zamanı, toplam yumurta verimi, yumurta çapı, döllenme ve çıkış oranı belirlenmiştir. Doğadan yakalanan 18 balığın ortalama ağırlığı 452,8 ± 193,2 g dır. On sekiz adet kültür balığının ortalama ağırlığı ise 138,9 ± 47,4 g dır. Yumurtlama 22 Ocak 10 Nisan arasında 79 gün devam etmiştir. Ortalama toplam yumurta verimi doğadan yakalanan balıklar için 285 ± 157,9 × 10^3 ve kültür balıkları için ise 115,6 ± 53,6 × 10^3 yumurta/anaçtır. Döllenme ve çıkış oranları sırası ile doğadan yakalanan grup için % 38,2 ± 17,0, % 62,3 ± 24,1 ve kültür balıkları için % 45,3 ± 22,7, % 54,8 ± 26,5 olarak belirlenmiştir. Sonuç olarak, doğadan yakalanan ve kültürü yapılan pisisinin üreme performansı benzer olduğu görülmüştür.

Anahtar sözcükler: Pisi, *Platichthys flesus luscus*, üreme, yumurta verimi, döllenme, çıkış oranı

Introduction

With the world-wide decline in stocks of important wild fish species, aquaculture is now becoming of increasing importance as a major source of dietary protein. In Turkey, aquaculture began with salmonids and nowadays species such as sea bass and

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gilthead sea bream are also being cultured on a large scale. The intensive culture of marine fish in Turkey has experienced rapid growth during the last decade and has become an important source of income for the country. This has occurred not only as a result of advantageous environmental conditions for this type of industry in Turkey, but also as a result of the successful transfer of culture technology.

In any primary production system, reliance on a narrow range of species can increase the risk of failure as a result of causes such as adverse climatic effects or attacks from diseases and pests (Oglend and Tveteras, 2009). Increasing the range of species produced improves the security of the industry by broadening the opportunities for new markets. Fish farmers need to develop farming techniques for new fish commodities as the market has become saturated with farmed sea bass and gilthead sea bream (Çiftçi et al., 2002). These farmers have recently achieved successful reproduction of the common pandora (*Pagellus erythrinus*), dentex (*Dentex dentex*), sharpsnout sea bream (*Puntazzo puntazzo*), and common sea bream (*Pagrus pagrus*) (Başçınar, 2004). Further research is underway to develop the technology to farm new species such as turbot (*Psetta maxima*) and flounder (*Platichthys flesus luscus*).

The European flounder, *Platichthys flesus* (Linnaeus 1758), is one of the marine flatfish species using estuarine systems as nurseries (Van der Veer et al., 1991) and it is one of the few flatfish species which can also use rivers as nursery areas (Andersen et al., 2005). *Platichthys flesus* is a winter spawner commonly found in the shallow waters of the Eastern Atlantic, from the North Sea to the Mediterranean and Black Sea (Cabral et al., 2007).

The flounder is an important commercial flatfish species and is currently considered a likely candidate for Turkey's developing aquaculture industry. However, available research on aquaculture of the flounder is limited to larvae rearing (Şahin, 2000), adaptation and feeding of wild-caught flounder juveniles in aquaculture conditions (Ergün and Yalçın, 2006), and reproductive and egg development in the Black Sea (Şahin et al., 2008). The objective of this study was to investigate the spawning season, fecundity, and egg size of wild and hatchery-reared flounder in the southern Black Sea.

Materials and methods

Data were collected from the Central Fisheries Research Institute Hatchery in Trabzon during the 2009 fish breeding season. Hatchery-reared broodstock were raised from eggs that hatched in February 2007 and consisted of 17 mature females (18.0-25.8 cm in total length; mean weight 138.9 ± 47.40 g). Wild broodstock consisted of 18 females (24.1-42.0 cm in total length; mean weight 452.8 ± 193.20 g). Wild specimens were collected in January and February 2009 using a trawl net on board a research vessel from the Central Fisheries Research Institute (CFRI); research surveys were conducted once a week at a depth of 5-70 m at a location off the coast of Trabzon (southeastern Black Sea region; 40°59′N; 39°50′E).

Brood fish were individually tagged and stocked at 5-6 fish per m^2 in a maturation tank (1 × 2 × 0.5 m). The daily water exchange averaged 9 times a day. Aeration was provided by 2 air stones with a total output of 4 L/min. During the period of spawning, the water temperature was maintained at 10 °C. The broodstock were fed whiting cut into 2 or 3 pieces. Feed was provided gradually until satiation.

Gonad maturation in females was evaluated by sampling a small amount of eggs with a cannula. These samples were transferred to a glass slide and measured at a magnification of 40×. Females with at least 100 oocytes larger than 0.4 mm in mean diameter were considered ready to spawn (Çiftçi et al., 2002). Females were then 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 with an adequate amount of seawater. 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 approximately 5 min to ensure fertilization. Next, 5 min after fertilization, the eggs were rinsed with filtered seawater. The seawater used for fertilization and rinsing the eggs was at the same temperature as the seawater in which the broodstock were maintained. Afterward, the eggs were disinfected with 50 mg/L iodine for 5 min and rinsed thoroughly with filtered seawater. Fertilization success was calculated by counting the proportion of eggs that had started cell

division. Eggs were incubated as described by Şahin (2000).

Data were recorded for weight and length of spawners, spawning time, total fecundity (number of eggs/female), and relative fecundity (eggs/kg female) as well as for the fertilization rate (at the 4-cell stage, 3 h after fertilization) and hatching rate (ratio of hatched larvae to total number of eggs). Data were analyzed using Minitab statistical software and means and differences at the 5% level were considered significant.

Results

Spawning lasted 49 days for wild broodstock (22 January to 11 March) and 43 days for hatchery-reared broodstock (27 February to 10 April).

Figure 1 shows the weight of eggs in each 24-h batch from each female. The total fecundity differed significantly between stocks (Table 1) and was linearly related to body weight (Figure 2) and total length (Figure 3). The difference in relative fecundity between stocks was not found to be significant. The

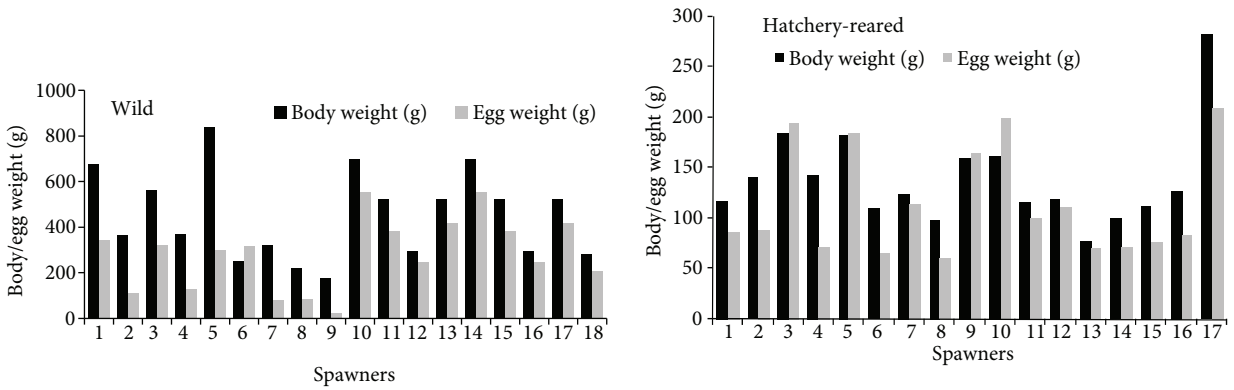

Figure 1. Body weight and egg weight of female flounders, *Platichthys flesus luscus*.

Table 1. Biometrics and reproductive variables of *Platichthys flesus luscus* broodstock (SD: Standard deviation).

	Broodstock				
	Wild[1]		Hatchery-Reared[2]		
Variables	Means ± SD	Range	Means ± SD	Range	t-test
Total length (cm)	31.5 ± 5.05	24.1-42.0	20.5 ± 1.88	18.0-25.8	*
Body weight (g)	452.8 ± 193.20	177.0-840.6	138.9 ± 47.40	78.0-283.3	*
Total fecundity (10^3)	285 ± 157.9	22-556	115.6 ± 53.60	60-210	*
Relative fecundity (10^3)	622 ± 282.7	123-1262	816 ± 200.8	507-1240	NS
Egg weight (g)	280.4 ± 153.7	21.7-553.3	38.9 ± 20.64	12.0-72.0	*
Egg diameter (mm)	1.15 ± 0.049	1.05-1.30	1.10 ± 0.041	0.97-1.21	NS
Fertilization rate (%)	38.2± 17.0	12.1-85.7	45.3 ± 22.7	7.6-88.9	NS
Hatching rate (%)	62.3 ± 24.1	10.3-100.0	54.8 ± 26.5	8.5-98.5	NS
Spawning time	22 Jan-11 March		27 Feb-10 April		

*: significantly different at a level of $P < 0.05$, NS: not significantly different
[1] Data derived from 19 fish.
[2] Data derived from 17 fish.

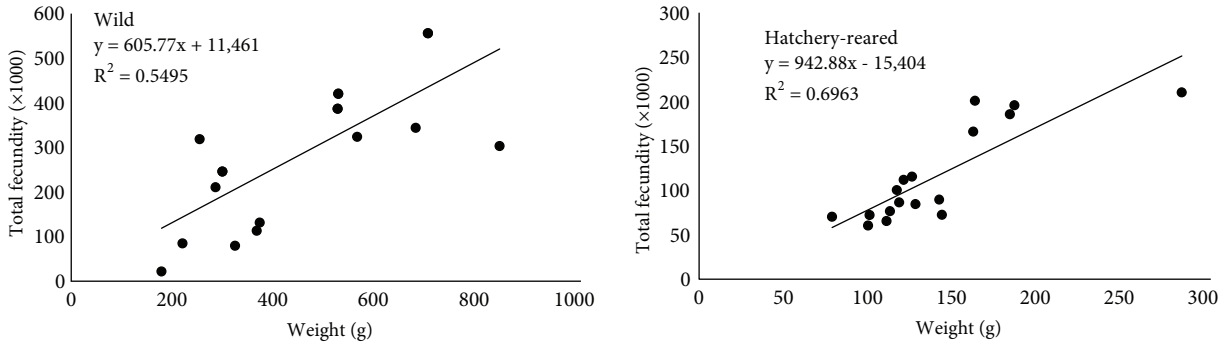

Figure 2. The relationship between total fecundity and the weight of spawners.

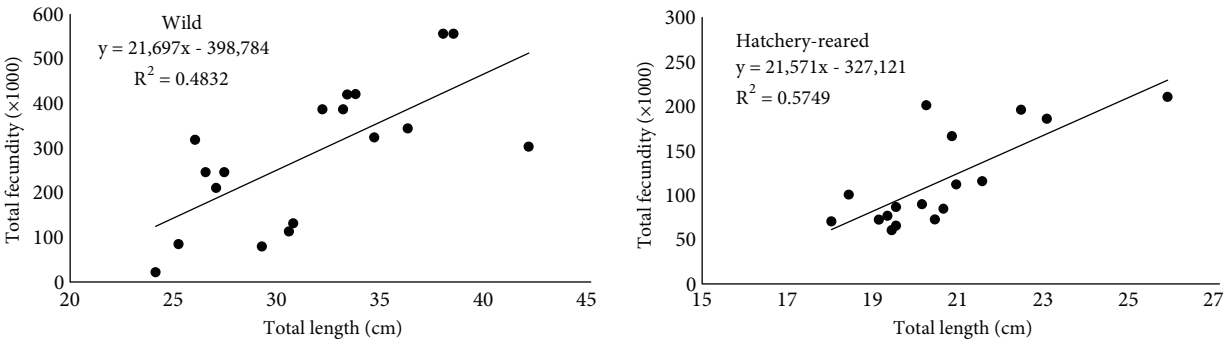

Figure 3. The relationship between total fecundity and the length of spawners.

correlation between relative fecundity (RF) and body weight (W) was low; it was positive for wild broodstock (RF = 569303 + 116.41 W; $r^2 = 0.006$; $P < 0.05$) and for hatchery-reared broodstock (RF = 588226 + 1071 W; $r^2 = 0.001$; $P < 0.05$).

Egg diameter, fertilization, and hatching rates were similar in the 2 stocks. The mean egg fertilization and hatching rates obtained in this study were 38.2 ± 17.0% and 62.3 ± 24.1% for wild and 45.3 ± 22.7% and 54.8 ± 26.5% for hatchery-reared broodstocks, respectively. A significantly positive linear correlation was observed between the fertilization and hatching rates ($P < 0.05$) (Figure 4).

Discussion

The reproductive cycle of fish is regulated by environmental factors, mainly photoperiod, water temperature, water quality (e.g., dissolved oxygen, pH, hardness, salinity, alkalinity), flooding, and water current, as well as the tides and cycles of the moon, weather cycles (e.g., atmospheric pressure, rainfall), nutrition, disease, parasites, and the presence of other fish (Peter and Crim, 1979; Rottmann et al., 1991). Reproduction of the flounder off the southern Black Sea coast took place from December to March but peaked in December (Çiloğlu, 2005; Şahin et al., 2008). In this study, viable embryos were produced from late January until mid-April.

Female fecundity is a valuable parameter for estimating the number of available eggs per spawning season. The mean total and relative fecundity obtained in this study were 285,000 eggs and 622,000 eggs/kg for a fish with a mean total length of 31.5 cm and a mean weight of 452.8 g in wild broods and 115,600 eggs and 816,000 eggs/kg for a fish with a mean total length of 20.5 cm and a mean weight of 138.9 g in hatchery-reared broods, respectively. The study also revealed that, although the older fish was more fecund, it is the younger fish that produces more ova per gram weight of ovary. An increase in fecundity was observed to occur along with an

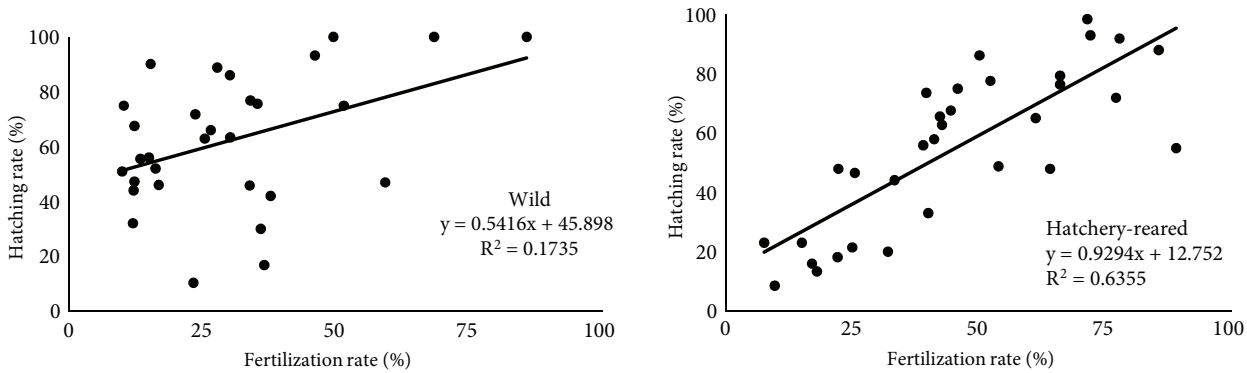

Figure 4. The relationship between hatching rates and fertilization rates.

increase in the size of the fish. The fecundity obtained in this study appears to be lower than those of the other flounders (Table 2). The different fecundities recorded are not contradictory considering that broodstock size, age, and genotype, as well as the 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 number of eggs (Jenning et al., 2001). In most flatfish species, fecundity is positively related to age (Bagenal, 1966). The fecundity of *P. flesus luscus* showed large variation with size but the total fecundity obtained in this study had a linear relationship with weight and total length.

P. flesus luscus eggs present the basic patterns of most marine fishes: they are pelagic and float individually near the surface; are spherical, with a diameter around 1 mm; and hatch into undeveloped yolk-sac larvae (Cerqueira, 2005). The average egg diameter was slightly higher than those of other flounder species (0.818 mm for *Paralichthys orbignyanus*, Bambill et al., 2006; 0.62 mm for *Rhombosolea plebeia*, Colman, 1973), but within the ranges described by Zaharia et al. (2000), Şahin (2000), and Şahin et al. (2008) for the same species (1.04-1.30, 1.15, and 1.156 mm, respectively). Broodstock size, husbandry conditions, and the quality and quantity of food also influence egg dimensions, as stated by Bromage (1996).

The flounder is a batch-spawning flatfish. Studies of estimates of the ovulatory periodicity of the flounder indicate that a 1-day interval may characterize regular ovulation patterns (Şahin et al., 2008). Although wild females produced a mean number of 11-14 batches and batch fecundity usually remained within a range of 2000-122,200 eggs, hatchery-reared females examined in this study produced a mean number of 2-11 batches with a batch fecundity that varied

Table 2. Total and relative fecundities of different flounder species.

Species	Size	Fecundity ($\times 10^3$)	Author(s)
P. flesus luscus	25.2-42.0 cm	79.1-318.1 eggs/fish	Şahin et al., 2008
Rhombosolea plebeia	18-30 cm	100-500 eggs/fish	Colman, 1973
Rhombosolea leporina	30-45 cm	250-1250 eggs/fish	Colman, 1973
Paralichthys orbignyanus	1.78-2.86 kg	240-280 eggs/kg	Cerqueira et al., 1997
Paralichthys orbignyanus	-	185-399 eggs/kg	Bambill et al., 2006
Paralichthys lethostigma	1.12 kg	735 eggs/fish	Watanabe et al., 2000
Paralichthys lethostigma	1.2-2.9 kg	230-1000 eggs/fish	Smith and Denson, 2000

between 9246 and 43,215 eggs during the spawning season. Şahin et al. (2008) reported a mean number of 6-21 batches and fecundity of 2000-44,000 eggs for the same species.

Şahin et al. (2008) reported the fertilization and hatching rate of *P. flesus luscus* as 17.2 ± 15.7% and 51.5 ± 27.6%, respectively. The mean fertilization and hatching rate recorded in the present study were higher than those of the findings reported by Şahin et al. (2008). As observed during this strip spawning study, there was variability in fertilization success and this may be 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) found that fertilization rates of turbot eggs decreased from 90%-95% to 0% following a 24 h delay between the ovulation and stripping time. In this study, the minimum time between the 2 strippings was 24 h. For this reason, the rates of over-ripening and low fertilization would be expected.

In summary, the present study demonstrated that adult flounder could be obtained from the wild and broodstock management and artificial spawning in captivity could be achieved successfully. This experimental-scale information will be the key to the further development of this species for aquaculture. However, additional research is needed to identify the performance of different life stages in different rearing systems.

Acknowledgements

The authors thank E. Küçük for assistance with field studies. This study was supported by the Ministry of Agriculture and Rural Affairs and the Central Fisheries Research Institute, under the project title "Research on Rearing Possibilities of Flounder (*Platichthys flesus luscus* L.)."

References

Andersen, A.K., Schou, J., Sparrevohn, C.R.N. and Støttrup, J.G. 2005. The quality of release habitat for reared juvenile flounder, *Platichthys flesus*, with respect to salinity and depth. Fisheries Manag. and Ecol. 2: 211-219.

Bagenal, T.B. 1966. The ecological and geographical aspects of the fecundity of the plaice. J. Mar. Biol. Assoc. UK. 46: 161-186.

Bambill, G.A., Oka, M., Radonic, M., Lopez, A.V., Muller, M.I., Bocanfuso, J.J. and Bianca, F.A. 2006. Broodstock management and induced spawning of flounder *Paralichthys orbignyanus* (Valenciennes, 1839) under a closed recirculated system. Rev. Biol. Mar. Oceanog. 41(1): 45-55.

Başçınar, N. 2004. Aquaculture in the world and overview to the future of Turkey. Sümae Yunus Research Bulletin. 4(4): 1-3.

Berlinsky, D.L., King, W.V., Smith, T.I.J., Hamilton II, R.D., Holloway Jr., J. and Sullivan, C.V. 1996. Induced ovulation of southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. J. World Aquac. Soc. 27:143-152.

Bromage, N.R. 1996. Broodstock management and seed quality: General consideration. In: Broodstock Management and Egg and Larval Quality (eds. N.R. Bromage and R.J. Roberts), Blackwell Sci. Ltd., England, pp. 1-24.

Bromage, N.R., Bruce, M., Basavaraja, N., Rana, K., Shields, R, Young, C., Dye, J., Smith, P., Gillespie, M. and Gamble, J. 1994. Egg quality determinants in fish: The role of over ripening with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus hippoglossus*. J. World Aquac. Soc. 25(1): 13-21.

Cabral, H.N., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos, P., Lopes, M., Ruano, M., Campos, J., Freitas, V., Santos, P.T. and Costa, M.J. 2007. Relative importance of estuarine flatfish nurseries along the Portuguese coast. J. Sea Res. 57: 209-217.

Cerqueira, V.R., Mioso, R., Macchiavello, J.A.G. and Bruegger, A.M. 1997. Ensaios de inducao desova do linguado (*Paralichthys orbignyanus* Valenciennes, 1839). Boletim do Instituto de Pesca Sao Paulo. 24:247-254.

Cerqueira, V.R. 2005. Egg development of *Paralichthys orbignyanus* (Valenciennes, 1839). Brazilian Arch. Biol. Technol. 48(3): 459-465.

Colman, J.A. 1973. Spawning and fecundity of two flounder species in the Hauraki Gulf, New Zealand. NZ J. Mar. Freshw. Res. 7(1-2): 21-43.

Çiftçi, Y., Üstündağ, C., Erteken, A., Özongun, M., Ceylan, B., Haşimoğlu, A., Güneş, E., Yoseda, K., Sakamoto, F., Nezaki, G. and Hara, S. 2002. Manual for the Seed Production of Turbot, Psetta maxima in the Black Sea. Special Publication No. 2, Central Fisheries Research Institute, Trabzon.

Çiloğlu, E. 2005. Trabzon-Rize Sahillerinde Pisi Balığının (*Platichthys flesus luscus* Pallas, 1811) Çeşitli Av Kompozisyonları İçindeki Oranı ve Birim Alandaki Yoğunluğu. E.Ü. Su Ürünleri Dergisi 22 (1-2): 35–38.

Ergün, S. and Yalçın, M. 2006. A study on the adaptation and feeding of wild-caught flounder juveniles in aquaculture conditions. EU J. Fish. Aquat. Sci. 23(1/2): 215-218.

Jennings, S., Kaiser, M.J. and Reynolds, J.D. 2001. Marine Fisheries Ecology. Blackwell Publish. Oxford.

Koya, Y., Matsubara, T. and Nakagawa, T. 1994. Efficient artificial fertilization method based on the ovulation cycle in barfin flounder *Verasper moseri*. Fish. Sci. 60(5): 537-540.

Maslova, O.N. 2002. Problems and achievements in seed production of the Black Sea turbot in Russia. Turkish J. Fish. Aquat. Sci. 2: 23-27.

Oglend, A. and Tveteras, R. 2009. Spatial diversification in Norwegian aquaculture. Aquaculture Econ. Manage. 13: 94-111.

Peter, R.E. and Crim, L.W. 1979. Reproductive endocrinology of fishes: gonadal cycles and gonadotropin in teleosts. Annual Review of Physiology. 40: 323-346.

Rottmann, R.W., Shireman, J.V. and Chapman, F.A. 1991. Introduction to hormone-induced spawning of fish. Southern Regional Aquaculture Center, Publication No. 421, Mississippi.

Smith, T.I.J. and Denson, M.R. 2000. Controlled spawning of southern flounder *Paralichthys lethostigma*: Issues and progress. In: Proceedings of the 28th UJNR Aquatic Panel on Spawning and Maturation of Aquaculture Species (ed. C. Tamaru), Hawaii. pp. 97-108.

Şahin, T. 2000 Larval rearing of flounder *Pleuronectes flesus luscus* under laboratory conditions. Turkish Journal of Marine Sciences 6: 263-270.

Şahin, T., Güneş, E., Aydın, İ. and Polat, H. 2008. Reproductive characteristics and egg development in Flounder (*Pleuronectes flesus luscus*) in the southern Black Sea. The Israeli Journal of Aquaculture – Bamidgeh 60(1): 20-26.

Van der Veer, H.W., Bergman, M.J.N., Dapper, R. and Witte, J.I.J. 1991. Population dynamics of an intertidal 0-group flounder *Platichthys flesus* population in the western Dutch Wadden Sea. Mar. Ecol-Prog. Ser. 73: 141-148.

Watanabe, W.O., Carroll, P.M. and Daniels, H.V. 2000. Recent progress in controlled reproduction of southern flounder *Paralichthys lethostigma.* In: Proceedings of the 28th UJNR Aquatic Panel on Spawning and Maturation of Aquaculture Species (ed. C. Tamaru), Hawaii. pp. 141-148.

Zaharia, T., Alexandrov L., Zaharia, M. and Bilal, I. 2000. The results of the experiments on the culture of flounder *Platichthys flesus luscus* (Pallas, 1811) and turbot *Psetta maeotica* (Pallas, 1811) at the Romanian Black Sea coast. CIHEAM-Cahiers Options Mediterraneennes. 16: 205-213.