Broodstock Rearing and Spawning of Black Sea Turbot, *Psetta maxima*

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Abstract

Broodstock development and induction of spawning of Black Sea turbot, *Psetta maxima* has been conducted between 1998 and 2001 in the Central Fisheries Research Institute, Trabzon, Turkey. One of the environmental and feeding conditions evident in broodstock management is the optimum water temperature below 17°C which is the present culture condition at the Institute. Likewise, frozen whiting is considered to be a good broodstock diet from the viewpoint of nu-tritional and ecological aspects. Growth of hatchery-bred broodstock fed this diet is shown as the relationship of body weight to age (month) represented as an equation of: $y = 0.072 x + 0.164$ ($R^2 = 0.998$).

Natural spawning was observed for 2-year-old hatchery-bred stock at water temperature of 14°C, however, no spawning was observed by the same broodstock at 3-years of age. On the other hand, induction of spawning for wild-caught broodstock were established through hormonal treatment.

A total of 78 artificial inseminations for 61 wild-caught females were carried out between 1998 and 2001spawning season (April and May). An average yearly production of 1.96 million newly hatched larvae was recorded with an average fertilization rate of 39.5% and a mean hatching rate of 32.5%.

Key Words: turbot, *Psetta maxima*, broodstock, spawning, Black Sea.

Introduction

The turbot, *Psetta maxima*, is an important commercial fish in European, Mediterranean and Black Sea regions. In 1986, commercial farms in Europe produced less than 1,000 tons of Atlantic turbot (Paulsen, 1989) but higher production is expected at present. Accordingly, the biology of Atlantic turbot in relation to its culture has been well studied since 1980's as reviewed by Paulsen (1989) and recently by Fauvel *et al*. (1992) and Imsland *et al*. (1997a and 1997b). In contrast, study on the culture aspect of Black Sea turbot is limited (Spectorova and Doroshev, 1976; Khanaichenko *et al*., 1994).

In Turkey, cultured Black Sea turbot were not sold in the market, because the seeds of turbot were not available. The species found in the market came from the wild and mainly caught by gill nets along the Black Sea coastal waters off Turkey. In order to contribute to the fishery and fish culture industry, the biology of Black Sea turbot had recently been studied including the identification of flatfish in Black Sea and its adjacent waters (Amaoka *et al*., 2001), stock assessment (Zengin, 2000; Suzuki *et al*., 2001) and the larval or juvenile development (Kohno *et al*., 2001; Moteki *et al*., 2001). Induced spawning and cryogenic sperm preservation of this species was first reported by Hara *et al*. (1998a and 1998b). However, there are no detailed informations on broodstock development and sourcing of fertilized eggs.

This paper reports the partial establishment of broodstock, induction of spawning and larval production of Black Sea turbot between 1998 to 2001 conducted by the Fish Culture Development Project in the Black Sea.

Broodstock Development

Environmental conditions

In order to investigate the environmental conditions for rearing hatchery-bred broodstock, 45 -3 year old hatchery-bred Black Sea turbot were stocked in 12m³ tank in November 2000, and reared until September 2001. Feeding activity of the fish, followed by changes in water temperature under natural rearing environmental conditions, was observed from July to September (Figure 1).

Frozen cod in combination with vitamin mix was fed to the fish 3 times a week. In order to control the physical condition of broodstock, cold water from the depth of 40 m was introduced into the tank when the required rearing temperature is more than 20°C.

Broodstock fed poorly at a temperature of 18.6°C (coefficient of variance, CV, of 0.14) in July. Fish fed well (2.5-3.5 kg/day) in August at temperature of 18.9°C (CV: 0.09) and in September at temperature of 16.4°C (CV: 0.1).

The poor food intake in July could probably be due to temperature stress caused by large and irregular fluctuations of water temperature ranging from 15.3 to 23.7°C (CV: 0.14). In August, good food intake was recorded when the water temperature was stable at 17.5°C with just a slight fluctuation (3°C;

CV: 0.06) that continued for 14 days. It is therefore assumed that a small fluctuation of water temperature does not affect fish conditions.

From these findings on water temperature changes in relation to the feeding activities of broodstock in 2001, it was concluded that the control of water temperature is important for broodstock management during summer and that the water temperature should be below 17°C with limited fluctuations for rearing 4 years old hatchery-bred and captive wild broodstock.

Frozen whiting is presently the best feed from the ecological viewpoint (Zengin, 2000) and nutritional aspect (Nezaki and Erteken, in preparation). The former contention was based on the analysis of stomach contents of wild Black Sea turbot wherein whiting was the dominant food. The latter was based on the growth analysis wherein fish fed frozen whiting showed the highest growth rate compared to goby, house mackerel and anchovy.

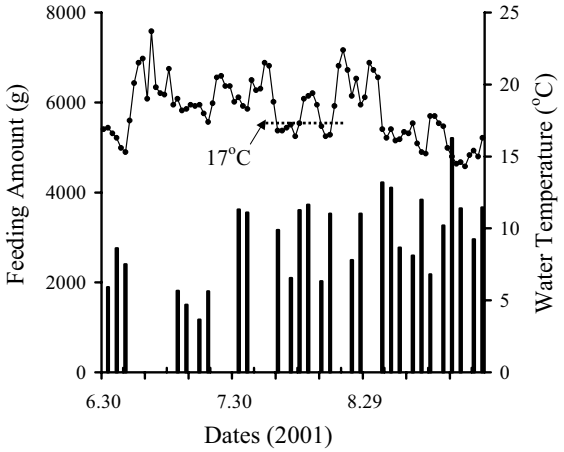

Figure 1. Relationship between changes of water temperature and feeding amount of 4 year-old hatchery-bred Black Sea turbot, *Psetta maxima* stocked in a 12°C tank. Broken line hows upper limit of optimum water temperature for broodstock management.

Growth and survival

On 12th July 1999, 158 hatchery-bred fish, 187g, were initially stocked in four-4 m³ tanks and reared for 149 days. The fish grew to 2.056 g with a final survival rate of 84.2% on harvest by the 27th of September 2001. The food conversion rates with frozen whiting as feed ranged from 3.2 to 6.8. The relationship of body weight to age (month) for hatchery-bred broodstock is represented as an equation: $y = 0.072 x + 0.164$ (R^2=0.998) (Figure 2). Thereafter, several rearing trials were con-ducted using different numbers of tanks (2 to 7 units) of different sizes (3 to 20m³) and at different stocking densities (1.4 to 6.6 kg/m²). Water temperature ranged from 6.1 to 24.1°C. Salinity ranged from 15 to 18 practical salinity unit (psu).

In addition, a rearing trial of wild-caught broodstock, 1.4 -1.7 kg, was conducted from 3rd Nov. 2000 to 7th Sep. 2001 and initially stocked in a 20 m³ concrete tank. From an average weight of 1.7 kg, they grew to 2.2 kg when fed frozen whiting and with a survival rate of 98.5%.

From these findings, year-round rearing technique for both the hatchery-bred and captive wild broodstock is almost established under the above-mentioned culture conditions.

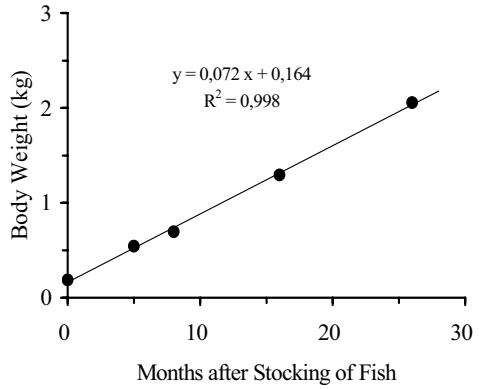

Figure 2. Growth of hatchery-bred Black Sea turbot, *Psetta maxima* reared in the tank.

Spawning

A total of 75.000 unfertilized eggs were collected from 2-year-old hatchery-bred broodstock on 9th and 11th May 2000. Both spawning events were under natural conditions and remarkable changes of water temperature were noted: the water temperature increased abruptly from 12 to 14°C in a few days, and this temperature was maintained for 10 days (Figure 3). However, the 3-year-old broodstock did not spawn even with environmental manipulation.

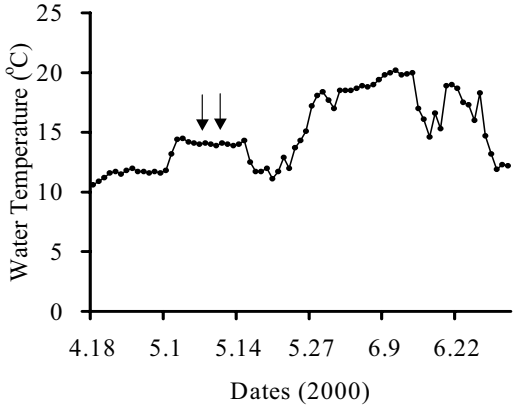

Figure 3. Relationship between changes of water temperature and partial spawning activities of 2-year-old hatchery-bred Black Sea turbot, *Psetta maxima*, reared in a 12°C tank. Arrows show the occurrence of natural spawning on 9th and 11th May at water temperature of 14°C.

To obtain stable egg production, to produce high quality gametes and to control spawning time, it is necessary to develop nutritional, environmental and hormonal manipulation techniques for the induction of spawning of hatchery-bred spawners in the near future.

Spawning of Captive Wild Broodstock

Spawning season

In order to pinpoint the spawning season of wild Black Sea turbot, stages of gonadal development of wild broodstock were investigated from 1998 to 2000. Most of the fish samples were purchased from the market in Trabzon and body length, body and gonadal weights were recorded.

Based on the gonosomatic index (GSI) for two consecutive years monitoring, it was estimated that the peak of the spawning season is from April to May. However, males showed a GSI peak 1-2 months earlier than the females (Figure 4).

Capture of wild caught broodstock from 1998 to 2001

A total of 455 fish consisting of 46 maturing and 113 mature females and 197 mature males were captured by gill nets and/or trawl nets during the spawning seasons of 1998 to 2001 (Table 1). Maturing stages are judged as follows: Gonads of maturing females possess oocytes more than 0.4 mm in diameter but fish did not ovulate. Mature female readily ovulates. On the other hand, mature male exuded milt from the urogenital pore, but immature or spent male did not.

The distribution of sexually mature wild-caught broodstock during the spawning season indicated that maturing females were caught between middle of March and end of April with a peak between April 1 and 15, while mature females were caught between the beginning to mid April and with a peak from May 1 to May 15. Male fish were caught throughout the spawning season from April to May (Table 1). Of the total captive wild fish caught using different gears, 64% of maturing females were captured by a trawl net, while 83% of mature females were caught by gill nets (Table 2).

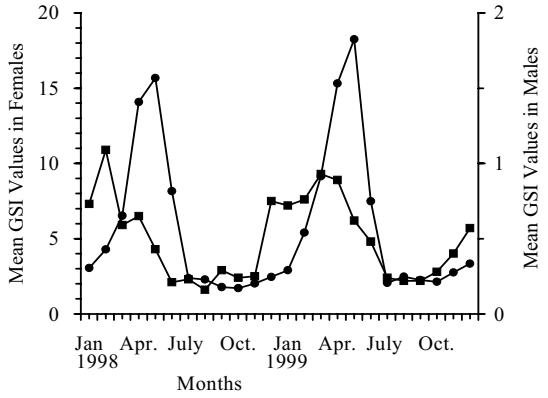

Figure 4. Monthly changes in GSI for male and female.Black Sea turbot, *Psetta maxima*, in the wild. ●, GSI values for females; ■, GSI values for males.

Table 1. Total catch and distribution of wild-caught broodstock of Black Sea turbot, *Psetta maxima* during the spawning seasons of 1998 to 2001.

Grade of maturity and sex	No. of total fish catch for 4 years	Fish distribution in each period (%)				
		March 15-31	April 1-15	April 16-30	May 1-15	May 16-30
Maturing F*	47	17.0	61.7	21.3	0	0
Mature F*	113	0	15.9	36.3	47.8	0
Mature M**	197	5.6	42.6	24.9	24.9	2.0

*Female; **Male

Table 2. Total catch and distribution of maturing and mature females of wild-caught Black Sea turbot, *Psetta maxima* using different fishing gears.

Grade of maturity	Total fish catch	% of catch	
		by gill net	by trawl net
Maturing	47	36.2	63.8
Mature	113	83.2	16.8

Table 3. Number of spawners used, average fertilization and hatching rates, and total larval production from 1998 to 2001.

Year	No. of insemination	No. of fish used	Mean fertilization rate (%)	Mean hatching rate (%) from whole eggs	No. of newly hatched larvae produced
1998	23	19	34.4	28.0	1.880.000
1999	15	13	46.3	36.0	1.650.000
2000	20	17	34.5	28.6	1.970.000
2001	20	12	42.4	37.3	2.260.000
Yearly mean	19.5	15.3	39.5	32.5	1.940.000

Artificial insemination

In order to constantly produce Black Sea turbot seeds, artificial insemination of captive turbot was conducted from 1998 to 2001 during the spawning season (April to May).

A pellet form of luteinizing hormone-releasing hormone analogue was used to induce ovulation of maturing females while a mixture of human chorionic gonadotropin and white salmon pituitary gland was used to increase milt volume (Hara *et al*. 1998a). No hormonal treatments were needed for mature females.

A dry method of artificial insemination was employed. Milts were principally obtained from 2 male fish for fertilization purposes. The water temperature in the maturation tank (2x1x0.5 m) was maintained by a heater at around 15°C.

A total of 78 artificial inseminations were carried out for 61 females, and a total of 24.246 million eggs were obtained with an average fertilization rate of 39.5% (34.4-46.3%) and a hatching rate of 32.5% (28.0-37.3%). Finally, a total of 7.760 million newly hatched larvae were produced with an average yearly production of 1.960 million (Table 3).

Acknowledgements

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