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RESEARCH PAPER

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The Age-Dependent Reproductive Performance of 4th Generation Black Sea Trout (*Salmo labrax Pallas*, 1814) Females

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Abstract

In this study, the age-dependent reproductive performance of 4th generation of hatchery-origin Black Sea trout (*Salmo labrax* Pallas, 1814) females was determined with the aim of understanding breeding characteristics of ideal broodstock management plan for Black Sea trout. The 2^+, 3^+, 4^+ and 5^+ years old females (n=131, 104, 63 and 28, respectively) with weights ranging from 919.45±36.22g to 2439.21±139.28g were used. Reproductive rates of females (2^+, 3^+, 4^+ and 5^+ years old) were determined as 22.14%, 60.30% and 17.56% for 2^+, 17.47%, 72.82% and 9.71% for 3^+, and 19.05%, 68.25%, and 12.70% for 4^+ years old in November, December and January, respectively. Fertilization rates were obtained 94.31%, 95.02%, 96.03% and 93.39%, respectively. The total fecundity was positively correlated with the age and weight (r=0.84) but negatively with the relative fecundity (r=-0.24). The difference between 2^+ and 3^+ age groups' total fecundities was insignificant, and the 4^+ and 5^+ age groups were different from all groups. In addition, there was no significant difference in fertilization rates among all age groups' rates but the 5^+ age group had slightly lower value ($P<0.05$).
For ideal broodstock management in Black Sea trout production, based on the results of this study, it is recommended to use 3^+ and 4^+ years old females with high breeding efficiency and remove 5^+ years and older females from the broodstock.

Introduction

Black Sea trout (*Salmo labrax* Pallas, 1814), distributed along the eastern part of the Black Sea, is an endemic anadromous species belonging to the Salmonidae. It is known that there are three ecotypes of this species: stream, sea, lake (Tabak *et al*., 2001). Black Sea trout feeds and grows in the Black Sea, when it attains sexual maturity, migrates upstream where it was born, to spawn there. Upon spawning they return to the Black Sea (Slastenenko, 1956; Svetovidov, 1984; Geldiay & Balik, 1996; Solomon, 2000). In a previous study, Tabak *et al*. (2001) found that this fish species reached sexual maturity between 2 and 4 years old with the first sexual maturity length of 44.76 cm in their natural environment in eastern parts of the Black Sea, they started to spawn in November and continued until mid-December.

Bio-ecological and cultivation characteristics of Black Sea trout has been studied by Central Fisheries Research Institute (CFRI) researchers since 1998. According to these studies; Black Sea trout eggs were hatch approximately in 38-40 days at 10°C in fresh water (Firidin, Cakmak, & Aksungur, 2012). Subsequent to hatching, larvae consume their yolk sac and constitute pedestal digestive structures within 30 days (Cankiriligil, Cakmak, & Ozcan Akpinar, 2016). Besides, another research carried out with fifth generation of

this species shown that *Salmo labrax* have similarities with one of the most cultured fish species Rainbow trout in terms of culture characteristics (Cakmak, Cankiriligil, & Ozel, 2018). The results of these studies have shown that this species may be an alternative to the commonly cultured rainbow trout in the region. A breeding program is being implemented to this species in order to improve the cultivation characteristics such as, feed conversion ratio, survival ratio, fertilization rate, fecundity, rapid growth until smolt size. The species is consumed popularly by the people of its natural spreading area and has a high economic value. By providing the fish (F_3 ve F_4) which is raised under the applied selectivity program, to the producers, aquaculture of the Black Sea trout has been spread along the eastern parts of the Black Sea. According to Turkish Statistical Institute (TSI, 2016) data, 1440 tons/year Black Sea trout has been produced in Turkey. The Black Sea trout and rainbow trout are produced together in the same facilities by commercial enterprises due to the similarity of their environmental requirements. Since consumer demands to this species have increased the producer interests to produce it, the necessity of ensuring sustainable production of this species together with three ecotypes and determining the cultural characteristics has emerged.

The most important issue for sustainable aquaculture is a good broodstock management. Thus, selection and maintenance of broodstock also play an important role in quality of eggs and juveniles used in production with optimum environmental conditions. For this reason, reproductive performance of 4^{th} generation of (F_4) Black Sea trout broodstock of different ages (2^+, 3^+, 4^+ and 5^+) were examined in this study with the aim of evaluating breeding characteristics of Black Sea trout to constitute a more accurate broodstock management plan.

Materials and Methods

Maintenance of Fish

This study was conducted between 2012 and 2016 in both marine cage research unit of Central Fisheries Research Institute's (CFRI) in Yomra/Trabzon (salinity: 17‰ and temperature: 6.5-29°C) and a commercial fish farm named Altindere fish farm (spring and stream water, 0.5-18.5°C) in Macka/Trabzon (Figure 1).

Fish

The hatchery-origin F_4 Black Sea trout females were used in this study. The fish were grown in freshwater recirculating aquaculture system (RAS) unit up to the smolt size (11.5 cm and 17 g) and then they were transported to the marine cage unit for quick breeding. When the reproducing season is closer, also considering high warmth of seawater in summer, broodstocks were transported to fresh water in June. The female broodstock was composed of 650 fish in the first spawning period (2^+ years old). For each generation, male fish was kept at a rate of 1/1 of the female broodstock. All broodfish were individually marked with electronic tags (Biomark, U.S.A.). The water temperatures of the marine and the freshwater units where the broodstock was kept were measured daily.

A total of 325 of the 650 females were spawned:

Figure 1. Marine cages (Yomra) and Altindere freshwater fish farm (Macka).

131 (919.45±36.22 g) in the first (2^+ years old), 103 (1154.04±62.25 g) in the second (3^+ years old), 63 (1825.333±92.07 g) in the third (4^+ years old) and 28 (2439.21±139.28 g) in the fourth spawning period (5^+ year old). Brood fish were fed with commercial trout feed (Sibal, Turkey) twice a day at a rate 2% body weight per day (Table 1).

Spawning Control and Stripping

Spawning controls of broodstock began in the first week of October in every year and were made weekly during the spawning season. Individuals with ripened eggs and sperm were taken to separate pools. The fish were anaesthetized in a solution of benzocaine of 50 ppm (Oswald, 1978). The total length of the fish (TL) was measured with a ruler with a precision of 1 mm and the weight (W) of the fish with a digital scale with a precision of ±0.01 g. Dry fertilization method was applied (Billard, 1992). Three male individuals were stripped to obtain stock sperm which was used to fertilize at most 3 different female's eggs.

Measurements and Calculations

Eggs were drained and weighed on a scale with a precision of 0.01 g to determine the total egg weight. To determine averages of egg diameter and weight, 20 eggs from each female were measured by using Von Bayer trough (Von Bayer, 1910), weighed on a scale with a precision of 0.01 g. and the measured values divided by the number of eggs. Fecundity is determined by using method of Vladykov (2011) and total fecundity (number of eggs/female) and relative fecundity (number of eggs/1 kg body weight) values were calculated. The eggs were placed in trays in vertical incubators fed with spring water and each tray included one female's eggs only. One day after fertilization, white and opaque eggs that were considered unfertilized were counted and removed from the trays. The fertilization rate was calculated by proportioning the remaining (fertilized) eggs to the total number of eggs. Condition factor was calculated with the condition factor (CF) equation (Ricker, 1975):

$$CF = \frac{W}{L^3} x100$$

where CF is Condition factor, W is Weight (g) and L is Length (cm)

Statistical Analysis

The body weight and length, reproductive rate, total fecundity, relative fecundity, egg diameter and fertilization rate data obtained in this study were subjected to analysis of variance (ANOVA) with Statistica v.10.0 (Statsoft Inc, Tulsa, USA). Significant differences were tested by Tukey test with P=0.05 significance level. Weight-related total fecundity and relative fecundity were analyzed with lineer regression with Statistica v.10.0 (Statsoft Inc, Tulsa, USA)

Results

The water temperature of the freshwater unit where the maintenance and stripping of the brood fish were done was between 0.5°C and 18.5°C for the period from June to February including the gonad maturation period, In the marine unit where the maintenance was performed after the stripping of the fish between February and June, the water temperature ranged from 6.5°C to 29°C (Figure 2).

The F_4 broodstock were studied during four spawning periods starting in 2012 between the ages of 2^+ (34 months) and 5^+ years old. The spawning period of the broodstock began in mid-November each year and continued until the end of January of the following year in freshwater unit. The 22.14% of 2^+ years old broodstock had completed the ovulation and were stripped in November, 60.30% in December and 17.56% in January. The 17.48% of 3^+ years old broodstock were stripped in November, 72.82% in December and 9.71% in January. 19.05% of 4^+ years old broodstock were stripped in November, 68.25% in December and 13.70% in January. The 32.14% of 5^+ years old broodstock were stripped in November and the rest were in December (Figure 3).

The average fecundity for all age groups was calculated as 3664±221 (129-9492) eggs/ female. Total fecundity was found to increase in relation to the fish age (P<0.05) (Table 2, Figure 4a). Mean relative fecundity was found to be 2417±87 (327-5058) eggs/kg (Table 2). The relative fecundity decreased as fish age increased (P<0.05) (Table 2, Figure 4b). Mean egg diameter was measured as 5.5±0.5 mm for all groups. It was found that the difference between egg diameters

Table 1. Features of Commercial Trout Feed

Basic Food Ingredients	Broodstock feed (10mm)
Crude Protein % (min)	45.0
Raw oil % (min)	20.0
Raw ash % (max)	10.0
Humidity % (max)	10.0

of 2^+ and 5^+ year old females was insignificant and in other age groups the difference was significant ($P<0.05$). Body weight, total fecundity and egg size of the females increased due to increase in age, while in 5^+ years individuals despite increasing of the body weight and total fecundity, the egg size decreased (Table 2). The fertilization rate was calculated as 94.32±0.63% for the 2^+ age group which is the first reproductive age group. The fertilization rate increased in relation to the age of the females until the age of 4^+ but the lowest fertilization rate was calculated in the 5^+ age group (93.39±1.39%) (Table 2, Figure 5).

Discussion

It was reported that the brown trout breed between October and December in the northern hemisphere (Needham, Moffett, & Slater, 1945; Horton, 1961; Thomas, 1964; Moyle, 1976) and between the end of May and July in the southern hemisphere (Hobbs, 1937; Hopkins, 1970; MacDowall, 1978). It was found that the Black Sea trout spawn mainly in November and rarely until the middle of December in their natural environment and the gonadal maturity rate of November (77.8%) is higher than the other months (Tabak *et al*., 2001). It was reported that spawning of brown trout begins in the second half of June, peaks in July and lasts until September in Chile (Estay, Noriega, Ureta, Martin, & Colihueque, 2004). According to result of the study conducted in the Eastern Black Sea region of Turkey, rainbow trout (*Oncorhynchus mykiss*) spawn between

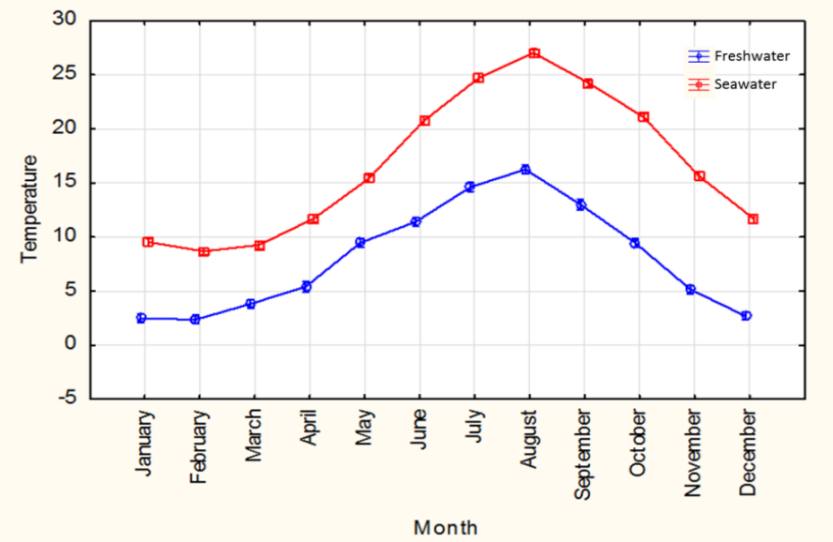

Figure 2. Water temperature (°C) at different months in Marine cages and Altindere freshwater fish farm units.

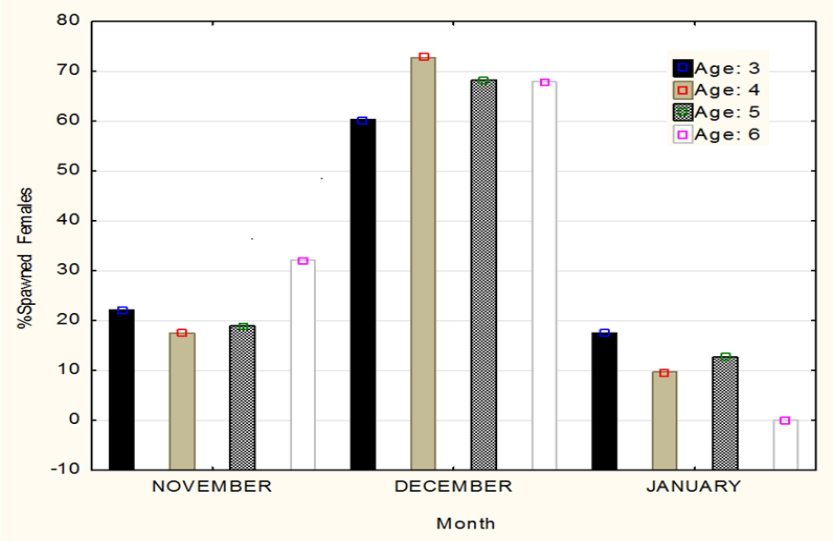

Figure 3. Age-related reproduction rate in reproductive season (%).

Table 2. Reproductive data of the Black Sea trout females at different ages

	2+ year old Mean±SE (n=131)	3+ year old Mean±SE (n=103)	4+ year old Mean±SE (n=63)	5+ year old Mean±SE (n=28)
Weight (g)	919.45±36.22^{d}	1154.04±62.25^{c}	1825.33±92.07^{b}	2439.21±139.28^{a}
Length (cm)	44.51±0.63^{d}	47.40±0.71^{c}	55.61±0.90^{b}	62.4±1.35^{a}
Total fecundity*	2425±117^{c}	2775±127^{c}	4152±243^{b}	5302±396^{a}
Relative fecundity*	2629±73^{a}	2566±75^{ab}	2314±84^{cb}	2159±115^{c}
Egg diameter (mm)	5.23±0.05^{c}	5.54±0.03^{b}	5.73±0.03^{a}	5.28±0.05^{c}
Unit egg weight (g)	0.078±0.001^{c}	0.087±0.001^{b}	0.096±0.002^{a}	0.095±0.003^{a}
Fertilization rate (%)	94.32±0.63^{ab}	95.02±0.63^{ab}	96.03±0.65^{a}	93.39±1.39^{b}
Condition factor	0.980±0.013^{a}	1.002±0.014^{a}	1.006±0.018^{a}	0.979±0.028^{a}

Different letters in the same row indicate significant difference (P<0.05).

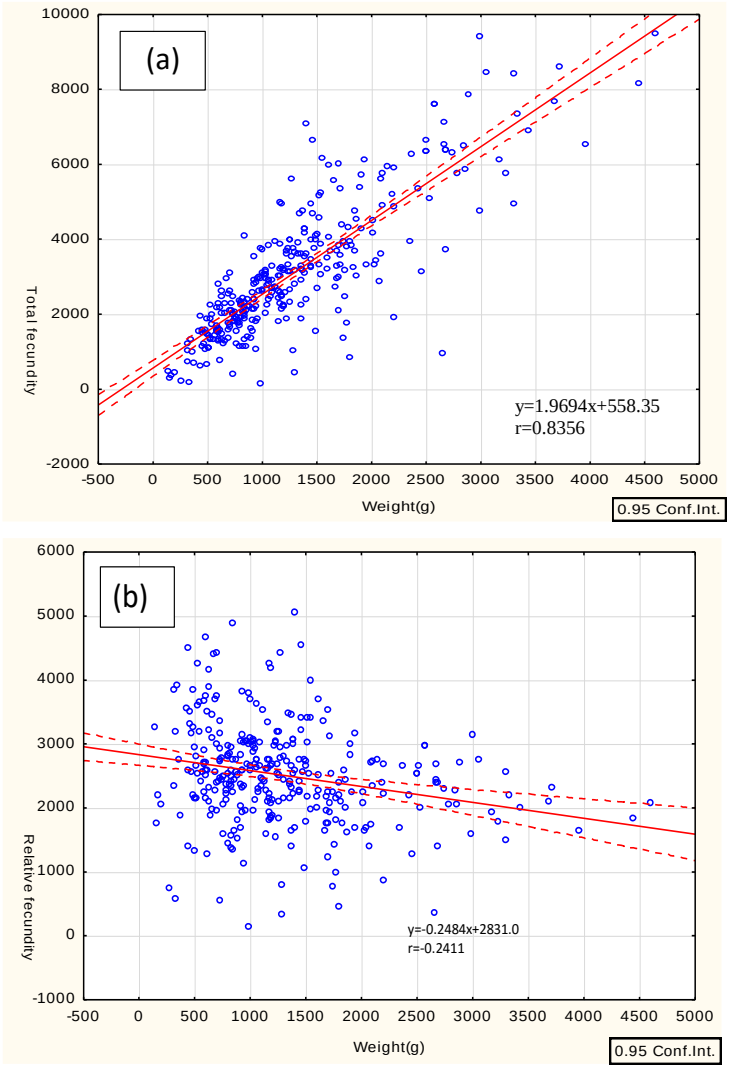

Figure 4. Weight-related total fecundity (a) and relative fecundity (b).

23 December and 26 February (Salihoglu *et al*., 2013). In this study, 19.56% of the 2^{+}, 3^{+} and 4^{+} years old females spawned in November, 67.12% in December and 13.32% in January and 32.14% of the 5^{+} years old females spawned in November and the rest (67.86%) in January. These results were found to be similar to the results of the studies done in the northern hemisphere.

Hatchery origin of Black Sea trout females (F_4) spawn in November, December and January in cultural conditions while Black Sea trout females spawn in November and December in their natural environment (Cakmak *et al*., 2018). One month longer breeding season than the natural individuals is an advantage for the aquaculture production planning. Breeding periods

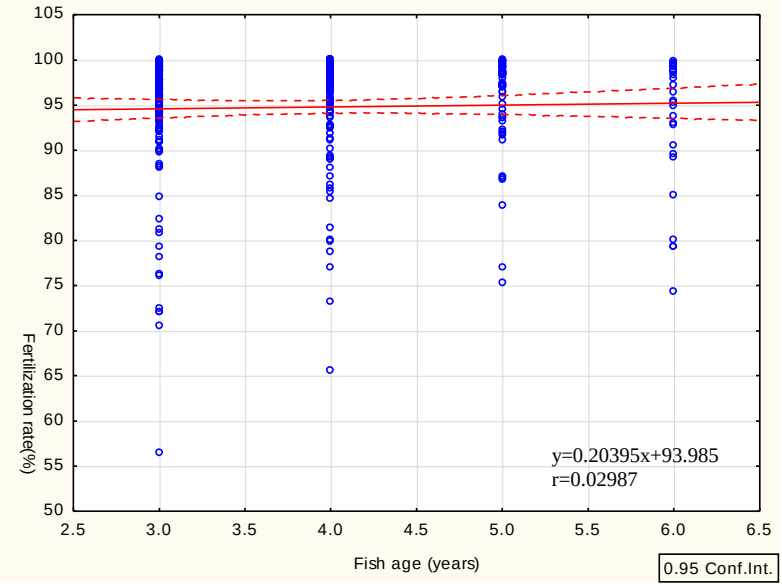

Figure 5. Age-related fertilization rates (%).

of Black Sea trout and rainbow trout do not coincide with each other in culture conditions, an advantage in using the hatchery efficiently for the private companies that produce both species.

Zama & Cardenas (1983) reported that brown trout (*Salmo trutta* L.) reach sexual maturity between the age of 2 and 5 (generally 3 or 4 years old). A study in Chile with brown trout (*Salmo trutta* L.) supplied from Germany showed that the fish reach sexual maturity at 3 years old (Estay *et al*., 2004). Tabak *et al*. (2001) found that the Black Sea trout reach sexual maturity at ages between 2 and 4 in the natural environment. In this study, it was determined that hatchery origin F_4 Black Sea trout females reach sexual maturity at 2^+ (34 months) years old similar to other studies. Due to the selectivity program carried out and culture conditions, the minimum spawning age of F_4 females was 34 months old. In aquaculture, reaching the sexual maturity late is an advantage because before the maturity fish use the energy from feed for somatic growth not for the gonadal development, an advantage that F_4 Black Sea trout also has.

Brown and Kamp (1941) worked on mature brown trout individuals in the Madison River in the United States, and found that the average weight of the 3 years old mature females is 601.2 g. Elliot (1975) developed an equation describing the growth of brown trout and theoretical growth curves for different water temperatures. According to this curve, 3 years old females are expected to have an average weight of 500 g at 10±1.1°C. Estay *et al*. (2004) reported that first breeding weight of cultured form of brown trout females (3 years old) is 470.8±102.5 g. The length of the first sexual maturation of natural Black Sea trout is 44.76 cm (33.83-55.68cm) (Tabak *et al*., 2001). In this study, 2^+ years old hatchery origin Black Sea trout females' first sexual maturation length and weight are 44.51±0.63 cm and 919.45±36.22 g, respectively. Although the first breeding lengths of hatchery-origin (F_4) and natural Black Sea trout females are similar, both of them are considerably longer than the other subspecies of brown trout studied (Brown & Kamp, 1941; Elliot, 1975; Estay *et al*., 2004). This situation can be explained by the facts that the Black Sea trout is an anadromous species and until maturity (2^+ years old) they are located on the upper level of the food chain of the Black Sea. Heavier first breeding weight (919.45±36.22 g) is an advantage in using energy from food for somatic growth up to this weight.

The egg diameter was 4.64mm (n=37) for the female which has the fecundity of 1285 eggs/female (Brown & Kamp, 1941) and 4.67mm (n=24) for the female which has the fecundity of 1176 eggs/female (Toledo, Lemaire, Bagliniere, & Brana, 1993). Estay *et al*. (2004) found that the total fecundity, relative fecundity and egg diameters of cultured form brown trout females were respectively 1182±344 eggs/female, 3577±471 eggs/kg, 4.64±0.11 mm for 3 years old; 1904±595 eggs/female, 2591±900 eggs/kg, 4.77±0.27 mm for 4 years old and 2744±605 eggs/female, 2181±360 eggs/kg, 5.24±0.12 mm for 5 years old. Estay *et al*. (2004) also determined that the most successful breeders were 4 and 5 years old. Tabak *et al*. (2001) reported that the total fecundity, relative fecundity and egg diameter of the natural Black Sea trout females were 3226±320 eggs/female, 1747±70 eggs/kg and 5.48±1.10 mm, respectively. Fecundity and egg diameter of fish are affected by various factors. The most important ones are size, age, genotypic structure and feeding conditions of the female (Healey & Heard, 1984; Bromage, Hardiman, Jones, Springate, & Bye, 1990; Bromage *et al*., 1992). It is accepted

worldwide that there is an increasing in female body weight, total fecundity and egg diameter especially at the second and third spawning (Springate & Bromage, 1984; Bromage & Cumaranatunge, 1988; Bromage *et al*., 1992; Estay, Díaz, Neira, & Fernandez, 1994). Different results obtained in abovementioned studies on total fecundity and egg diameter of brown trout can be explained by the difference in size, age, genotypic structure, environmental conditions and nutritional sources of females used in the studies. Relative fecundity and egg diameter values of cultured form of Black Sea trout determined in our study were found to be similar to those of natural forms from other Black Sea trout studies but not similar to the values revealed from the studies performed with different subspecies of brown trout ($P<0.05$).

Tabak *et al*. (2001) calculated the fertilization rate of the Black Sea trout eggs obtained from natural females as 99.76%. Estay *et al*. (2004) reported that the fertilization rate of the 3, 4 and 5 years old brown trout were 92.0±13.7%, 98.5±4.01% and 95.8±8.33%, respectively. Salihoglu *et al*. (2013) found the average fertilization rate of rainbow trout eggs as 98.7%. In this study, the best fertilization rate (96.03±0.65%) was obtained for the eggs of 4^+ years old females. The fertilization rate of the eggs of 2^+ (94.32±0.63%) and 3^+ (95.02±0.63%) years olds were relatively similar. The lowest fertilization rate was obtained for eggs of 5 years old females ($P<0.05$). Although the fertilization rate of the hatchery-origin F_4 Black Sea trout eggs is lower than that of the rainbow trout and natural Black Sea trout, it is similar to the cultured form of brown trout species.

The Black Sea trout is consumed widely by the people of the region and has a high marketing advantage. However natural stocks have decreased because of the overfishing, pollution and some human mediated disturbances in river beds, which led researchers to work on the aquaculture of Black Sea trout. Exhibiting reproductive performance similar to the brown trout and rainbow trout can be considered as an indication that the F_4 Black Sea trout can be used in aquaculture. Similar breeding values obtained for both rainbow trout and brown trout were also obtained from the F_4 Black Sea trout, which indicates that F_4 can be used in aquaculture.

In conclusion, it was found that the fish at 3^+ and 4^+ years old have most suitable reproductive performance for broodstock management practices in commercial production. Besides, as reproductive performance begins to decline after 5^+ years old, it is necessary to remove 5^+ years old and older females from the broodstock for a good broodstock management. It is necessary to repeat similar studies for the next generations and develop new strains in accordance with the needs of the industry.

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