Acta Aquatica Turcica

E-ISSN: 2651-5474

Home Page: https://dergipark.org.tr/actaquatr 19(4): 289-297, 2023 DOI: 10.22392/actaquatr.1232320

Research Article **Araştırma Makalesi**

Reproductive Performance of Hatchery-Originated Black Sea Salmon Broodstocks' (*Salmo labrax* PALLAS, 1814) F5 and F6 Filial Generations

Kuluçkahane Kökenli F5 ve F6 Nesil Karadeniz Somonu Anaçlarının (*Salmo labrax* PALLAS, 1814) Üreme Performansları

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Received: 11.01.2023 **Accepted:** 24.05.2023 **Published:** 01.12.2023

How to Cite: Özel, O. T., Çakmak, E., Çankırılıgil, E. C., Çimagil, R., & Düzgüneş, Z. D. (2023). Reproductive performance of Hatchery-Originated Black Sea Salmon Broodstocks' (*Salmo labrax* PALLAS, 1814) F5 and F6 filial generations. *Acta Aquatica Turcica, 19*(4), 289-297. https://doi.org/10.22392/actaquatr.1232320

Abstract: In this study, reproductive performances of F5 and F6 generation hatchery-originated female Black Sea salmon (*Salmo labrax*) were evaluated to determine the effects of the active selective breeding program. The study was conducted in freshwater ponds and marine cage systems between 2018 and 2021. For the study, 53 specimens from F5 generation females with an average length of 52.71±7.08 cm and weight of 1561.29±639.76 gr, and also 56 broodstocks specimens from F6 generation females with an average length of 51.92±5.35 cm and weight of 1506.12±417.28 gr were used. Gonad maturity controls and spawning studies were carried out in the 2018-2019, 2019-2020, and 2020-2021 breeding periods along the entire season, lasting from November to January. The number of ovulating females, their fecundity, egg diameters, and fertilization rates of eggs were recorded. In all seasons, 36.17% of F5 females ovulated in November, and the remaining females (63.83%) ovulated in December. Ovulation time in F6 females also showed a similar pattern of 1/3 of females (37.40%) ovulating in November and the remaining majority (62.60%) ovulating in December. According to the results, total egg yields as 4439.31±1675.69 and 4112.08±1041.16 egg/broodstock, relative egg yields as 2313.44±278.48 and 2345.76±516.30 egg/kg, egg diameters as 5.08±0.19 and 5.21±0.17 mm, and fertilization rates as 96.78±3.24% and 96.63±3.73% were determined for F5 and F6 generations, respectively. In both generations, as the size of the broodstock increases, the total egg production increases (F5: r=0.9169, F6: r=0.74254), while the relative egg production (F5: r=-0.2526, F6: r=-0.3486) decreases. When the total egg yields and fertilization rates were compared, the difference between the F5 and F6 groups was insignificant ($p \leq 0.05$). The condition factors of the two generations were found to be statistically similar ($p \leq 0.05$). As a result, it is understood that the maximum broodstock efficiency of the breed has been achieved in the broodstock management as a result of the selective breeding studies applied for the broodstock management.

Keywords
- Black Sea salmon
- Selective breeding
- Reproductive characteristics
- Breeding season

Özet: Bu çalışmada, yürütülen seçici ıslah programının üreme performansına etkisini belirlemek amacıyla, F5 ve F6 nesil kuluçkahane kökenli Karadeniz somonu (*Salmo labrax*) anaçlarının üreme bulguları irdelenmiştir. Çalışma, 2018-2021 tarihleri arasında tatlısu havuzlarında ve deniz ağ kafes sisteminde yürütülmüştür. Çalışmada, ortalama boyları 52,71±7,08 cm ve ortalama ağırlıkları 1561,29±639,76 g olan F5 nesil anaçlardan 53 birey, ortalama boyları 51,92±5,35 cm ve ortalama ağırlıkları 1506,12±417,28 g olan F6 nesil anaçlardan 56 birey kullanılmıştır. Farklı nesil anaçların üreme periyodunda aylık üreme oranları, toplam yumurta verimi, nispi yumurta verimi, yumurta çapı ve döllenme oranları belirlenmiştir. 2018-2019, 2019-2020 ve 2020-2021 üreme periyotlarında (Kasım-Ocak ayları) gonad olgunluk kontrolleri ve sağım çalışmaları yapılmıştır. Sağılan F5 nesil anaçların oranı Kasım ayında % 36,17 iken Aralık ayında % 63,83, F6 nesil anaçlarda ise bu durum Kasım ayında % 37,40 Aralık ayında % 62,60 olarak tespit edilmiştir. Bu sonuçlara göre, sağımı gerçekleştirilen F5 ve F6 nesil

Anahtar kelimeler
- Karadeniz somonu
- Seçici ıslah
- Üreme özellikleri
- Üreme sezonu

anaçların, toplam yumurta verimi, nispi yumurta verimi, yumurta çapları ve döllenme oranları sırasıyla 4439,31±1675,69 ve 4112,08±1041,16 adet/anaç, 2313,44±278,48 adet/kg ve 2345,76±516,30 adet/kg, 5,08±0,19 mm ve 5,21±0,17 mm % 96,78±3,24 ve % 96,63±3,73 olarak belirlenmiştir. Her iki nesil anaçların büyüklüğü arttıkça toplam yumurta veriminde de arttığı (F5: r=0,9169, F6: r=0.74254) fakat nispi yumurta veriminde ise (F5: r=-0,2526, F6: r=-0,3486) azalma olduğu görülmüştür. Toplam yumurta verimleri ve döllenme oranları karşılaştırıldığında F5 ve F6 grupları arasındaki fark önemsizdir. ($p \leq 0.05$). F5 ve F6 nesil anaçların kondisyon faktörlerinin benzer olduğu ($p \leq 0.05$) tespit edilmiştir. Sonuç olarak, damızlık yönetimine yönelik uygulanan seçici ıslah çalışmaları sonucunda türün damızlık yönetiminde maksimum üreme verimini ulaşıldığını anlaşılmaktadır.

1. INTRODUCTION

Black sea salmon, which was only a subject of sportive fishing initially, has become quite common in Türkiye, especially on the eastern shores of the Black Sea, due to high consumer preference. Trabzon Central Fisheries Research Institute (SUMAE) determined the species' basic bioecological and culture characteristics with the scientific studies it started in 1998, thereby revealing the species' aquaculture potential. Within the scope of these studies, both broodstock individuals and eggs were provided to several private enterprises and contributed to the expansion of the species breeding in the Eastern Black Sea Region (Tabak et al., 2001; Çakmak et al., 2007, 2022; Kasapoglu et al., 2020; Özel et al., 2021, Çankırılıgil et al., 2022). Today, 25 private enterprises, which have a total production capacity of 23,256,000 juvenile fish per year, are breeding species, and the production amount for 2021 has been reported as 1.603 tons/year (BSGM, 2022; TÜİK, 2022). In parallel with the projects, selective breeding programs were also implemented to improve the species' culture characteristics. The characteristics for creating breeding stock with easy adaptation to culture conditions and high reproductive efficiency were followed in the applied selective breeding programs.

A good start is essential for sustainable farming. This can only be possible by managing well-known and known breeders with high fertility. Broodstock management practices are essential in business management and the economy to obtain quality and healthy offspring in Black Sea salmon farming, whose culture production is increasing daily. Effective broodstock management practices can improve reproductive performance, resulting in higher-quality offspring and increased sustainability and efficiency in Black Sea salmon farming. Therefore, evaluating the effects of selective breeding programs on the reproductive performance of hatchery-originated female Black Sea salmon can provide valuable insights for future breeding programs and contribute to developing a more sustainable aquaculture industry. This study aims to evaluate the reproductive performance of F5 and F6 generation hatchery-originated female Black Sea salmon, and to determine the effects of the ongoing selective breeding program on their reproductive efficiency. By evaluating the gonad maturity controls, egg yields, fertilization rates, and egg diameters, we aim to assess the success of the selective breeding program in improving the culture characteristics of Black Sea salmon. The results of this study can help inform future breeding programs and improve the sustainability and efficiency of Black Sea salmon farming.

2. MATERIAL AND METHODS

2.1. Broodstock care

The care and feeding of broodstock were carried out in the freshwater unit in the Of province of Trabzon and the marine cages research unit of SUMAE in the Yomra province (Figure 1). Water temperatures were measured daily. The water exchange in ponds is set as 18-20 times/day. The broodstocks were transferred to the freshwater unit in June when the Black Sea water started to warm (18°C), and they were transported to the marine unit in February after spawning. The stock density in cages and ponds was set as 12 kg/m³ in initial, and it reached to 15 kg/m³ in spawning till February.

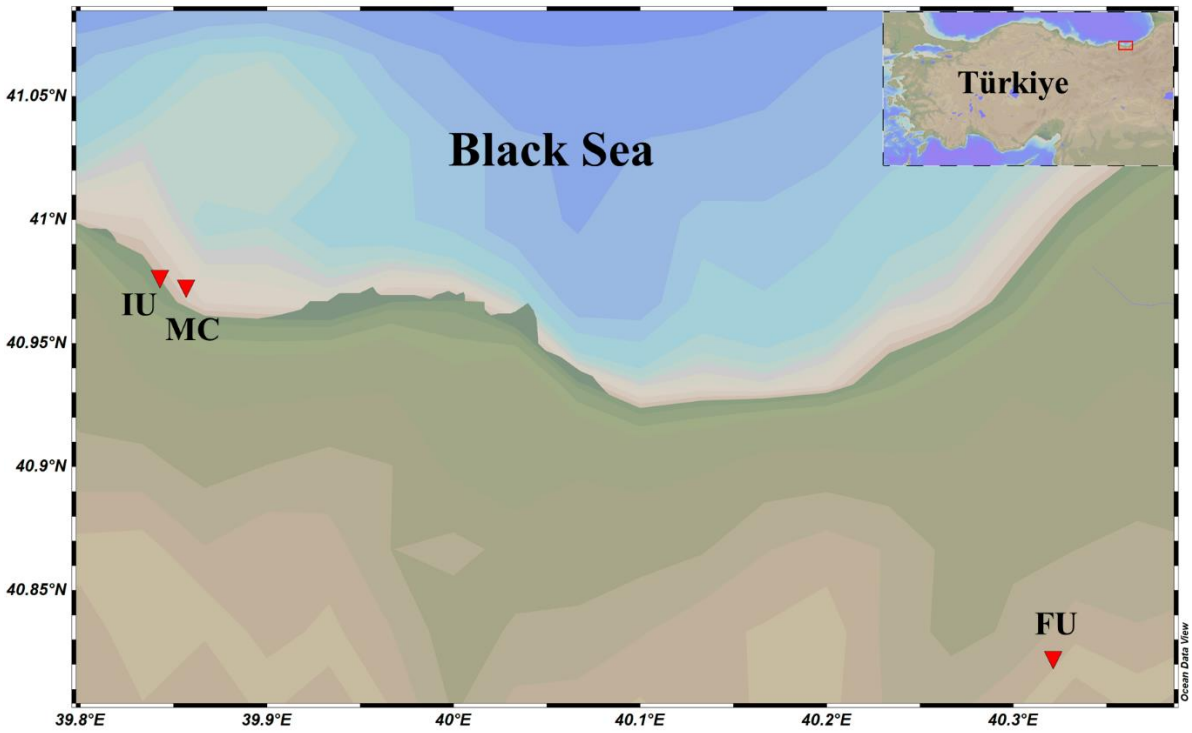


Figure 1. Areas where broodstock individuals are kept. IU: Central Fisheries Research Institute Units, MC: Marine cage research unit, FU: Freshwater unit, altitude: 644 m. The map was created with Ocean Data View (ODV) software (Schlitzer, 2023).

2.2. Broodstock Selection

The study used hatchery-origin F5 and F6 broodstock fish of different ages as the primary material. Among the F5 generation fish stock, there were 53 female fish with an average length of 52.71±7.08 cm, an average weight of 1561.29±639.76 g, and 31 male fish with an average length of 50.22±7.63 cm and an average weight of 1711.98±646.48 g were selected. Moreover, in F6 generations, 56 female fish with an average length of 51.92±5.35 cm, an average weight of 1506.12±417.28 g, and 32 male fish with an average length of 49.12±8.78 cm and an average weight of 1645.18±836.35 were selected (Table 1, 2). Commercial trout feed with 7% moisture, 45% protein, 20% lipid, 10% crude ash, 3% crude fiber, and 4801 Kcal/kg energy content was used in breeding stock feeding. All individuals were marked with electronic markers (12 mm, 134 KHz) for individual monitoring of reproductive efficiency and management of breeding stock (Figure 2).

Figure 2. Marker process of broodstocks (a: Reader, b: injector and markers, c: application of the marker to muscle tissue)

2.3. Spawning Studies

Maturity controls of the breeders were started in the second week of October, 15 days before the breeding period, considering the milking dates of the previous years, and milking was carried out at two-week intervals until the end of the breeding period. Egg and sperm-maturing individuals were taken into separate tanks in the hatchery. Broodstock individuals were anesthetized before spawning by exposure to a 50 ppm solution of benzocaine-acetone (Oswald, 1978). The dry spawning method

was applied. Firstly, male individuals were milked, and sperm stock was formed by sperms of at least three male individuals. The stock sperm fertilized the spawned eggs of 5 female fish. Twenty-five minutes after fertilization, the eggs were washed with the water used in the hatchery to remove the residues and prepared for incubation.

2.4. Determinations of Reproductive Characteristics

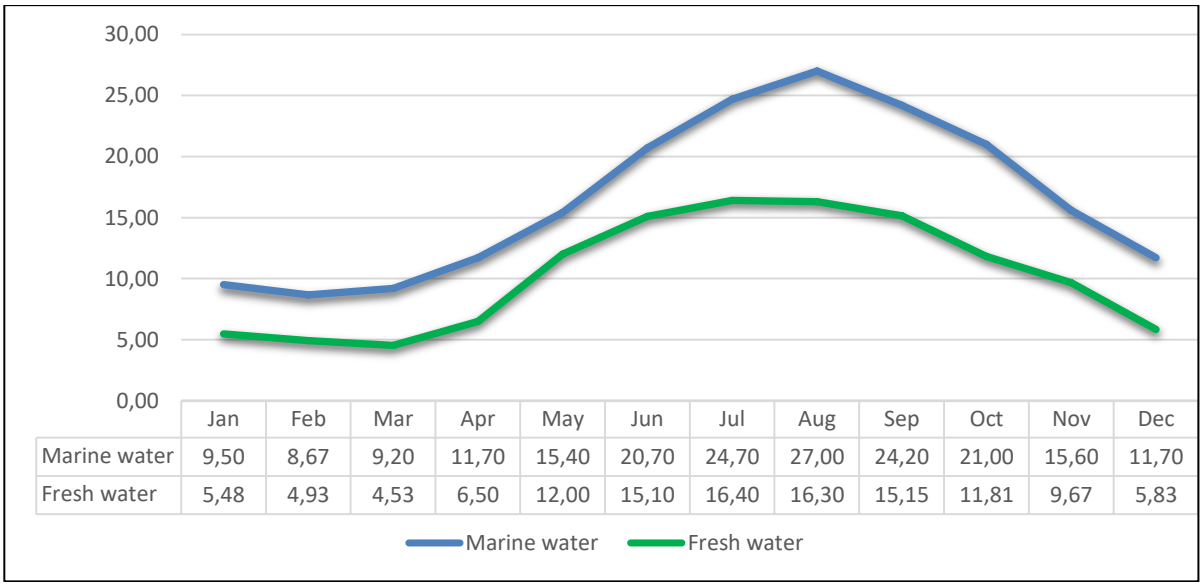
A ruler with a precision of ±1 mm was used for the length measurements of the broodstocks, and a scale with a precision of ±0.01g was used for the measurements of body weight and total egg weight. Total egg weight was determined by weighing the eggs with an accuracy of 0.01 g. Average egg diameters were measured with Von Bayer ruler (Von Bayer, 1910) with 20 eggs for each broodstock, and the number was calculated by dividing the number of eggs. The same 20 eggs were weighed with a scale with a precision of 0.001 g, and this weight was divided by the number of eggs. Egg production (fecundity) was determined by the gravimetric method (MacGregor, 1957), and total egg production (number of eggs per broodstock) and relative egg production (number of eggs per kg body weight) were calculated. Eggs were placed in vertical flow brood cabinets fed with spring water using separate pans for each rootstock. One day after fertilization, the white and opaque eggs were considered unfertilized, counted, and removed. The fertilization rate was calculated by proportioning the remaining eggs (fertilized eggs) to the total number of eggs.

2.5. Statistical Analyses

The data of the study were analyzed by t-test. Differences were evaluated at the 5% significance level ($P<0.05$). Relationships between parameters were calculated in correlation analysis. Minitab statistical program was used in analyses.

3. RESULTS

The average water temperature of the marine net cage unit was determined as 11.27±3.06 °C (min: 8.6 °C, max: 20.7 °C), and the salinity rate was determined as 17 ‰. The average water temperature of the freshwater unit was measured as 11.91±4.28 °C (min: 4.0 °C, max: 18.5 °C) (Figure 3).



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Marine water	9,50	8,67	9,20	11,70	15,40	20,70	24,70	27,00	24,20	21,00	15,60	11,70
Fresh water	5,48	4,93	4,53	6,50	12,00	15,10	16,40	16,30	15,15	11,81	9,67	5,83

Figure 3. Water temperatures of the marine cage and freshwater units

Breeding studies started in November and were completed at the end of December for each year 36.17% of F5 broodstocks and 37.19 % of F6 broodstocks were spawned in November, and all remaining broodstocks were spawned in December (Figure 4).

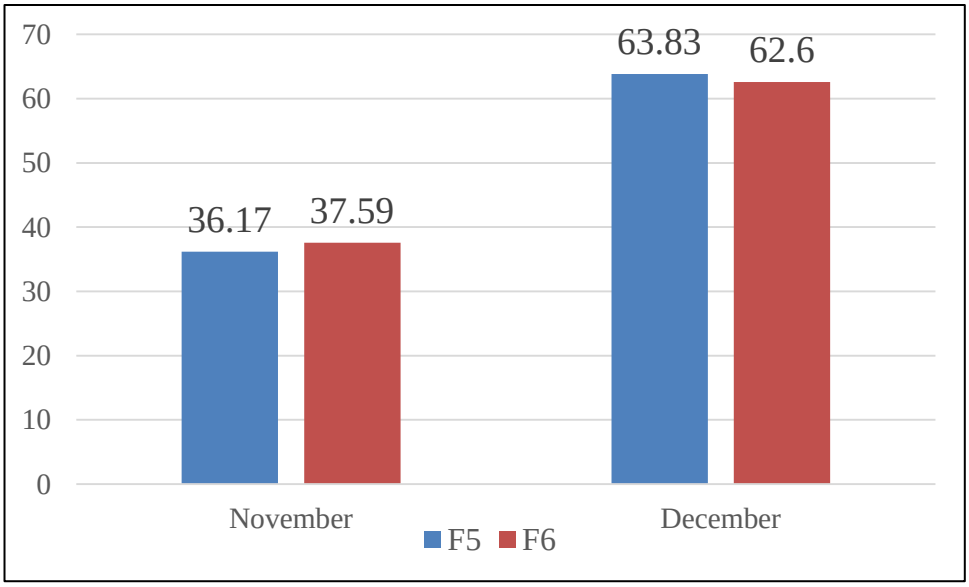


Figure 4. Reproduction rates of generations during the breeding period (%)

According to the results, total egg yields were 4439.31±1675.69 egg/broodstocks and 4112.08±1041.16 egg/broodstocks, and relative egg yields were 2313.44±278.48 egg/kg and 2345.76±516.30 egg/kg for F5 and F6 broodstock individuals, respectively. In broodstock groups, It was determined that there was a positive relationship between broodstock weight and total fecundity and a negative relationship between broodstock weight and relative fecundity (Table 1; Figures 5, 6). It was observed that the difference between egg yields of F5 and F6 generations was statistically insignificant (Table 1) (P<0.05). Mean egg diameters were found as 5.08±0.19 mm and 5.21±0.17 mm, and mean egg weights were found as 0.083±0.01 g and 0.088±0.01 g for F5 and F6 broodstocks, respectively. Mean fertilization rates were calculated as 96.78±3.24 % for the F5 generation and 96.63±3.73 % for the F6 generation. It was observed that the difference between fertilization rates and the egg's diameter and weight was found insignificant for both groups (Table 1) (P<0.05).

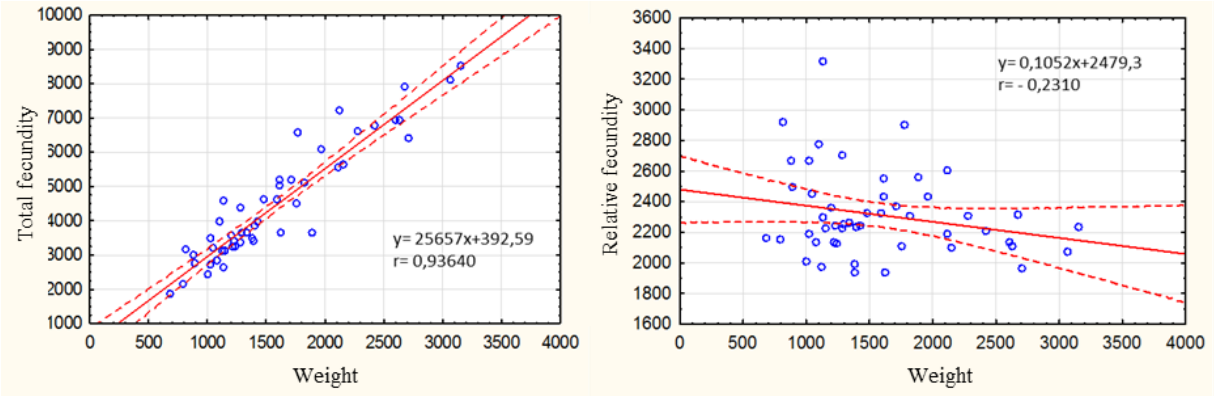


Figure 5. Total and relative fecundity weight relationship of F5 broodstocks

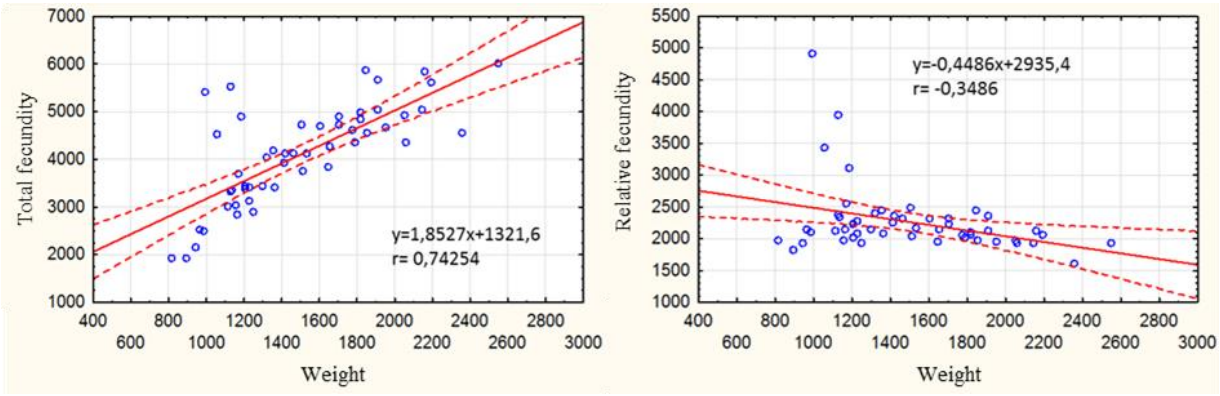


Figure 6. Total and relative fecundity weight relationship of F6 broodstocks

Table 1. Reproductive efficiency of F5 and F6 generation broodstocks

Parameters	Filial generation	
	F_5 (n=53)	**F_6 (n=56)**
Length (cm)	52.71±7.08[a]	51.92±5.35[a]
Weight (g)	1561.29±639.76[a]	1506.12±417.28[a]
Total egg weight (g)	376.03±165.73[a]	365.47±109.05[a]
Egg weight (g)	0.083±0.01[b]	0.088±0.01[a]
Egg diameter (mm)	5.08±0.19[a]	5.21±0.17[a]
Total fecundity (egg count/broodstock)	4439.31±1675.69[a]	4112.08±1041.16[a]
Relative fecundity (egg count/broodstock kg)	2313.44±278.48[a]	2345.76±516.30[a]
Fertilisation rate (%)	96.78±3.24[a]	96.63±3.73[a]
Condition factor	1.07±0.15[a]	1.05±0.16[a]

*Different letters in a line indicate a statistical difference.

Table 2. Some characteristics of the male individuals

	Length (cm)	Weight (g)	Condition factor
F5 (n=31)	50.22±7.63[a]	1711.98±646.48[a]	1.29±0.18[a]
F6 (n=32)	49.12±8.78[a]	1645.18±836.35[a]	1.26±0.10[a]

*Different letters in a column indicate a statistical difference.

4. DISCUSSION

Although studies on *Salmo labrax* are limited, significant studies have been conducted on brown trout (*Salmo trutta*) belonging to the same genus. Several studies have been conducted about brown trout spawning season, fertility, natural behavior, and culture characteristics. The reproduction season of the brown trout, which takes three months, occurs between October and December in the northern hemisphere (Needham et al., 1945; Horton, 1961; Thomas, 1964; Moyle, 1976), while it was between late May and July in the southern hemisphere (Hobbs, 1937; Hopkins, 1970; MacDowal, 1978). It is reported that brown trout give offspring in Chile in June, and reproduction reaches the maximum in July and continues until September (Estay et al., 1994). It is known that the maturation and spawning times of Black Sea salmon individuals in fish production facilities and their natural habitats in the Northwest Caucasus vary depending on environmental conditions, especially temperature (Makhrov et al., 2011). According to previous studies, on the Turkish coasts, Black Sea salmon mainly spawns in November and rarely until mid-December in the natural environment (Tabak et al., 2001), whereas, under culture conditions, it has been reported that the spawning period begins in November, peaks in December, and continues until February (Çakmak et al., 2022). A study conducted in a private enterprise operating in the Eastern Black Sea Region of Türkiye determined that rainbow trout (*Oncorhynchus mykiss*) gave offspring between the last quarter of December and the last quarter of February (Salihoğlu et al., 2013). Our study determined that F5 and F6 breeding stocks gave offspring in November and December, and the breeding season was completed in 2 months. It is seen that selective breeding studies applied for the creation of breeding stock from marine (anadromous) individuals are effective in completing the milking period in 2 months. This reproduction pattern in the

F5 and F6 stocks is similar to the findings of Needham et al. (1945), Horton (1961), Thomas (1964), Moyle (1976), and Tabak et al. (2001). The number of enterprises culturing Black Sea salmon in Türkiye is increasing daily. Especially for private enterprises that breed Black Sea salmon together with Rainbow trout, breeding Black Sea salmon in the early period and rainbow trout in the late period will provide an advantage in hatchery usage.

Brown & Kamp (1941) measured the egg diameter of 4.64 mm (n=37) for brown trout (*Salmo trutta*) with 1285 eggs per brood in their study in the USA. Toledo et al. (1993) measured the egg diameter of 4.67 mm (n=24) for Brown trout (*Salmo trutta*) with 1176 eggs/brood yield in their study in Spain. Estay et al. (1994) found that the total egg production of brown trout in culture form at different ages was 1182±344 - 2744±605 eggs/broodstock, the relative egg production was 3577±471 - 2181±360 eggs/kg, and the egg diameters were 4.64±0.11 - 5.24±0.12 mm. Tabak et al. (2001) reported that the total egg yield of natural Black Sea trout broodstocks was 3226±320 eggs/broodstock, the relative egg yield was 1747±70 eggs/kg, the egg diameter was 5.48±1.10 mm. Çakmak et al. (2022), within the scope of selective breeding of Black Sea salmon, it was determined that the total egg yield, the relative egg yield, and the egg diameter of F2, F3, and F4 generations varied between 3202±1665- 3664±1220 eggs/broodstock, 2428±709-2417±586 eggs/kg varied between, and 5.52±0.34-5.45±0.21 mm under culture conditions. Egg production and egg size of fishes are affected by several factors. The most important are broodstock size, age, genotype, and feeding conditions (Haeley & Heard, 1984; Bromage et al., 1990, 1992). The reproductive yields and egg sizes of F5 and F6 individuals obtained in this study were similar to studies by Estay et al. (1994), Tabak et al. (2001), Çakmak et al. (2022), while these were found to be higher than the findings of other researchers. It is seen that this difference is caused by the fish size used in the studies, the anadromous feature, and the difference in environmental conditions. The findings obtained in this study overlap with those of the F3 and F4 generations, and it is understood that the breeding data remained stable in the breeding study with these generations.

Estay et al. (1994) reported that the fertilization rate in eggs of different ages ranged from 92.0±13.7 % to 98.5±4.01 % in their study with brown trout of culture origin. Çakmak et al. (2022) calculated the fertilization rate of eggs obtained from natural broodstocks (F0) adapted to the culture conditions within the scope of the selective breeding program they carried out in Black Sea salmon 93.46±5.35 %. They reported that the fertilization rate of eggs from F1, F2, F3, and F4 generations ranged from 95.28±6.29 % to 98.25±1.81 %. The fertilization rate findings obtained in this study are similar to those conducted with cultured brown trout.

5. CONCLUSION

The Black Sea salmon is among the new species introduced into production in the aquaculture sector. To improve the culture characteristics of the fish, selective breeding programs were applied to create an F6 generation having high adaptability to culture conditions. The results of the study showed that selective breeding was an effective tool for improving the culture characteristics of the Black Sea salmon. Some parameters related to reproduction, such as fertilization and hatching rates, were found to be significantly increased in the F6 generation compared to the wild population. Moreover, the reproduction data in the last three generations were similar and stable, indicating the success of the breeding program. These findings suggest that selective breeding can be used to improve the culture characteristics of Black Sea salmon in the culture environment. Future studies should focus on improving the species' survival rate, growth, and adaptation to different environmental conditions.

ACKNOWLEDGEMENT

We want to thank the all crew members our Institute for their support on technical issues and infrastructure preparation and use during the study.

FUNDING

This work has been prepared using the data obtained from the "Investigation of possibilities of using some phytobiotic added feeds in feeding of Black Sea salmon (*Salmo labrax*)" project supported by the Ministry of Agriculture and Forestry.

CONFLICT OF INTERESTS

There is no conflict of interest between authors.

AUTHOR CONTRIBUTIONS

Methodology: EÇ, OTÖ, ECÇ; Experimenting: OTÖ, EÇ, ECÇ, RÇ, ZDB; Data analysis: OTÖ, EÇ; Manuscript writing: EÇ, ECÇ, OTÖ; Supervision: EÇ. All authors approved the final draft.

ETHICAL STATEMENTS

All experiments were carried out considering the ethical rules of the authorities, with the approval coded as ETIK-2017/1 by the Ethical Committee of Animal Experiments of Central Fisheries Research Institute.

DATA AVAILABILITY STATEMENT

All relevant data were given in the manuscript.

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