Comparison of Reproductive Performance of Black Sea Salmon Broodstock (Salmo labrax PALLAS, 1814) Reaching First Sexual Maturity at Different Ages

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Abstract

In this study, it was aimed to determine the individual first maturation age diversity in hatchery originated F6 generation Black Sea salmon (*Salmo labrax*) broodstock created by applying a selective breeding program. The study was carried out in freshwater ponds and marine net cage systems between 2018–2021. In the study, 136 broodstock reaching the first sexual maturity at the age of 22 months, 87 of the broodstock reaching the age of 34 months and 3 of those reaching the age of 46 months were evaluated. Total egg production, relative egg production, egg diameter and fertilization rates were determined at the first stripping of broodstock that reached sexual maturity at different ages. The first gonadal development measurements and stripping studies were carried out for broodstock aged 22 months in 2018–2019, 34 months in 2019–2020 and 46 months in 2020–2021. Total egg yields of 22, 34 and 46 months old broodstock, which were stripped, were calculated 1108.98 ± 40.73, 3869.02 ± 138.43 and 5899.52 ± 1143.78 egg/broodstock, relative egg yields were calculated 3024.87 ± 87, 2291.90 ± 89.52 and 1816.00 ± 284.51 egg/broodstock, egg diameters were measured 4.40 ± 0.00, 5.07 ± 0.00 and 5.35 ± 0.00 mm, fertilization rate were determined %92.21 ± 0.87, %95.69 ± 1.65 and 89.83 ± 2.77 respectively. In the broodstock, individuals with a first maturation age of 22 months have predominantly red spots with a white halo around the perimeter, individuals with a first maturation age of 34 months have predominantly silvery coloration with black spots, individuals with a first maturation age of 46 months have completely silvery coloration with black spots.

1. Introduction

The aquaculture sector has been in constant search for the introduction of highly consumer-like species into culture production. Brown trout and salmon species, which were focused on farmed production for conservation and sportive fishing in the early days, are among the species with high commercial potential and consumer appreciation. One of these species is the Black Sea salmon.

Although the Black Sea salmon, which is one of the endemic species of the Black Sea, was named differently by some researchers, *Salmo labrax* is still valid today. Slastenenko (1956) and later scientists (Svetovidov, 1984; Lelek, 1980; Solomon, 2000) named the individuals were sampled from the rivers in the Caucasia and the Eastern Black Sea, as Black Sea trout and Black Sea salmon to indicate their origin and transition features to the sea. It is known that the species continues to exist in the Black Sea as three ecotypes: sea, river and lake. Svetovidov (1984), Geldiay and Balik (1996) reported that marine ecotype individuals spend most of their lives in the sea, especially the time that includes the feeding period, and they lay their eggs by entering the rivers flowing into the Black Sea during the reproduction period. Slastenenko (1956), Çelikkale (1994), Geldiay and Balik (1996) reported that lake ecotype individuals spend their lives in the lake, they do not migrate for breeding and feeding. Slastenenko (1956), Geldiay and Balik (1996) reported that river ecotype individuals are resident in the stream and live there all their lives, do not migrate between the sea and streams, but migrate for short distances in the stream environment, between the source, the tributaries and the mouth of the stream. Large variations in color are seen in all three ecotypes of *Salmo labrax* (Slastenenko 1956; Svetovidov,1984). Although marine
ecotype juveniles have scattered black and red spots on the sides of their bodies in freshwater, when they return to the sea, they gradually lose this color and pattern and turn into a silvery white color. However, it has been reported that there is no significant color and pattern difference between juveniles and adults of lake and river ecotype individuals (Çelikkale, 1994). It is possible to encounter individuals with different appearances according to color, form, mottling and smoltification in the same basin (Svetovidov, 1984). Fish belonging to the salmon genus can easily adapt to aquatic areas with suitable environmental conditions, more than one interspecies form can coexist in the same aquatic area. Some of these can be defined as separate species (Gunther, 1866). Several studies have confirmed the coexistence of Atlantic salmon \((Salmo salar)\), brown trout \((Salmo trutta)\), as well as endemic species \((S. marmoratus, S. letnica, S. ischchan, S. carpio, and S. platycephalis)\) (Frost et al., 1967; Behnke, 1968; Dorofeeva, 1998).

Nikandrov and Shindavina (2007) reported that Black Sea salmon is one of the species with the largest size among brown trout. In the literature review, different findings related to the size that this species can reach have been found. Kocabaş (2009) reported that Black Sea salmon spend most of its life in the sea and can grow up to 100 cm in length and 26 kg in weight. Barach (1962) reported that the female Black Sea salmon caught by him in the Kodori river was 16.7 kg and 116 cm. Similarly, Solomon (2000) reported that Black Sea salmon, which came from fishing in Batumi in different months, was sold in the markets as the largest size 16 kg. Kottelat and Freyhof (2007) reported the size of the largest Black Sea salmon as 80 cm. Çakmak et al. (2022) reported that the largest Black Sea salmon caught to date in Turkey was 98 cm long, weighed 16.5 kg, and had an obvious marine ecotype (anadromic) character. Adaptation of the Black Sea salmon to the cultural conditions in Turkey was achieved with the studies started in 1998, after 2007 its cultivation started to become widespread, and today it is increasing in the whole country, especially in the Eastern Black Sea Region (Çakmak et al. 2011). Mainly anadromic F6 broodstock with high adaptation to culture conditions was created with the selective breeding studies carried out.

In this study, 6th generation farmed broodstock of Black Sea salmon, which is included in the brown trout family, were examined, individuals who were found to reach their first sexual maturity at the age of 22, 34 and 46 months were evaluated.

2. Materials And Methods

2.1. Broodstock

In the study, hatchery origin F6 generation Black Sea salmon broodstock was used. The reproductive data of 136 broodstock with an average height of 32.53±0.28 cm and an average weight of 364.67±8.34 g reaching the first sexual maturity at the age of 22 months, 87 broodstock with an average height of 50.45±0.42 and average weight of 1425,68±37,80 g reaching the first sexual maturity at the age of 34 months, 3 broodstock with an average height of 62,6±1,10 cm and average weight of 2650,35±18,45 g reaching the first sexual maturity at the age of 46 months evaluated (Table 1). The incubation period of
Black Sea salmon is 60 days on average (600 days/degrees) until the first feed intake, and the first feed intake date is accepted as zero in the age calculation.

Commercial trout feed with 10% moisture, 45% protein, 20% lipid, 10% ash, 3% crude fiber and 4801 Kcal/kg energy content was used in feeding the broodstock. All broodstock were marked with electronic marks (12 mm, 134 KHz) for individual monitoring of reproductive efficiency (Figure 1). In the study, 83 male broodstock with an average length of 49.67±18.20 cm and a weight of 1478.58±947.32 g were used.

2.2. Broodstock Rearing

The rearing of the broodstock was carried out at the Gürpınar freshwater plant in Of District of Trabzon Province and at the Yomra sea cages research unit of the Central Fisheries Research Institute (SUMAE) (Figure 2). Water temperatures were measured daily in the marine net research unit and the freshwater unit. The broodstock were transferred to the freshwater unit in June, when the Black Sea water started to warm (18°C), and again to the marine unit in February after stripping. In the broodstock ponds fed with freshwater, the water change is adjusted to be 18-20 times/day. Broodstock are stocked at 15 kg/m³ in marine net cages and freshwater ponds.

2.3. Reproductive Controls and Stripping

Controls for gonadal development of the broodstock were made 15 days in advance, taking into account the time of the previous stripping start. In all breeding seasons, this time corresponded to after the second week of October. These controls performed every two weeks were continued until the stripping of all broodstock was completed. Male and female individuals, who were found to have completed the gonadal development during stripping controls, were placed in the tanks kept in the hatchery unit separately according to their gender. Broodstock to be stripped were anesthetized using benzocaine (Oswald, 1978) solution at an application dose of 50 ppm. Dry stripping method was applied. First, at least 3 male individuals were stripped and sperm stock was created. The sperm stock created was used to fertilize the eggs of 5 female fish. 5 minutes after fertilization, water was added until 3 cm above the eggs and until the stripping container was full after 15 minutes. In order to prevent the eggs from being without oxygen, water was added and the eggs were mixed. This treatment continued until the eggs hardened. After about 25 minutes, the hardened eggs were washed with hatchery water to remove the residues and were prepared for incubation. An incubation pan was used for each broodstock’s egg and the pans were labeled with the tag number of broodstock.

2.4. Measurements and Calculations

A height scale with ±1cm precision was used for the total height measurements of the broodstock, and ±0.01 g precision scales were used for the body weight and total egg weight measurements. Average egg diameter, 20 eggs for each broodstock were measured in a Von Bayer vessel (Von Bayer, 1910) and the number was calculated by dividing the number of eggs. The same eggs were weighed with a balance.
with a precision of 0.001 g, and the total weight of 20 eggs was found, and the weight of one egg was calculated by dividing the number by the number of eggs. Egg yield (fecundity) was determined by gravimetric method (MacGregor, 1957) and total egg yield (number of eggs per broodstock) and relative egg yield (number of eggs per kg body weight) were calculated. Eggs were placed in cabinet incubators with vertical flow fed with spring water using separate pans for each broodstock. One day after fertilization, the white and opaque eggs were considered unfertilized, counted and removed. Fertilization rate was determined by calculating the ratio of the number of unfertilized eggs to the total number of eggs.

2.5. Statistical Analysis

The results obtained in the study were obtained by performing a one-way ANOVA test with the help of SPSS 14 statistical analysis program. Differences were evaluated at the 5% significance level (P<0.05).

3. Results

The maintenance of the broodstock was carried out in the net cage unit with Black Sea salinity (0.17%) and freshwater unit during the study. The average water temperature was measured at 11.27±3.06°C (min: 8.6°C, max: 20.7°C) during the maintenance period (November-June) of the marine net cage unit. The average water temperature was recorded as 11.91±4.28°C (min: 4.0°C, max: 18.5°C) during the usage period of the freshwater unit (June-January) (Figure 3).

The mean height-weights of 22, 34 and 46 months old broodstock used in the study were measured as 32.53±0.28-364.67±8.34, 50.45±0.42-1425.68±37.80 and 62.6±1.10 cm- 2650.35±18.45 g, respectively. The directly proportional increase in the average length and weight of broodstock depending on the increase in first maturation age was found to be statistically significant (Table 1) (P<0.05).

The stripping period recorded between 2018-2021 started in the second week of November and continued until the end of December. 31.62% of the broodstock reaching the first sexual maturity at the age of 22 months were stripped in November and 68.38% of them were stripped in December. Likewise, 63.22% of the broodstock reaching the first sexual maturity at the age of 34 months were stripped in November and 36.78% of those were stripped in December. However, all of the broodstock that reached the first sexual maturity at the age of 46 months were stripped in December. While the stripping of broodstock that reached sexual maturity at 22 and 34 months spread to November and December, stripping period of the group that reached sexual maturity at 46 months was conducted only in December (Figure 4).

The total egg yield of broodstock that reached the first sexual maturity at 22, 34 and 46 months, were 1108.98±40.73, 3869.02±138.43 and 5899.52±1143.78 eggs/broodstock, respectively. As for that, their relative egg yields were 3024. 87±87.52, 2291.90±89.52 and 1816.00±284.51 eggs/kg were calculated. It was observed that the increase in total egg yield in direct proportion to the age of the fish was statistically significant. In the evaluation of the relative egg yield between stocks, it was found that 34 months old stock was similar to 22 and 46 months old stock, while 22 month old stock and 46 month old stock were
4. Discussion

Brown trout is at the forefront of species with high nutritional value and consumer appreciation on a global scale. Many studies have been carried out on subjects such as their natural behavior, cultural characteristics and breeding due to these features. Zama and Cardenas (1983) reported that the age of sexual maturity of brown trout (*Salmo trutta*) is 2-5, and maturation occurs mainly at 3 and 4 years old. Gjerde (1984) obtained a production stock of wild Atlantic salmon from Norway by fertilization of females reaching sexual maturity at 4 and 5 years of age with males reaching first sexual maturity at 1, 4 and 5 years of age. In this production stock, all fish reaching sexual maturity at the age of 3 are male, the majority of the 4-year-olds are male, while the sex ratio is equal in the 5-year-old group. Tabak et al. (2001) determined that Black Sea salmon reached their first sexual maturity between the ages of 2-4 in the natural environment. Estay et al. (2004) determined the first maturation age of all fish as 3 years old in the study they carried out with the German origin culture form Brown trout (*Salmo trutta*) in Chile. In this study, reproductive age and reproductive yields of 6th generation cultured broodstock of Black Sea salmon, which is included in the brown trout family, were evaluated. It was seen that individuals reaching the first sexual maturity at the age of 22, 34 and 46 months continued to exist in the broodstock. It was also observed that this result was similar to the studies on the first maturation age of brown trout, and individuals reaching late sexual maturity had similar characteristics to Atlantic salmon. It is important to create a broodstock according to preference from individuals who reach the first sexual maturity at

<table>
<thead>
<tr>
<th>Parameters</th>
<th>22 Months Old (n:136)</th>
<th>34 Months Old (n:87)</th>
<th>46 Months Old (n:3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>32,53±0,28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50,45±0,42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62,6±1,10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight after stripping (g)</td>
<td>364,67±8,34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1425,68±37,80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2650,35±18,45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Condition factor</td>
<td>1,05±0,02</td>
<td>1,09±0,01</td>
<td>1,08±0,03</td>
</tr>
<tr>
<td>Total egg weight (g)</td>
<td>58,13±1,96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>321,13±12,38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>571,35±127,39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg weight (g)</td>
<td>0,05±0,01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0,08±0,02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,09±0,09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg diameter (mm)</td>
<td>4,40±0,00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5,07±0,00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5,35±0,00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg number (adet/anaç)</td>
<td>1108,98±40,73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3869,02±138,43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5899,52±1143,78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fecundity (adet/kg)</td>
<td>3024,87±87,52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2291,90±89,52&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1816,00±284,51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>92,21±0,87</td>
<td>95,69±1,65</td>
<td>89,83±2,77</td>
</tr>
</tbody>
</table>

Table 1. Reproductive data of broodstock reaching first sexual maturity at 22, 34 and 46 months
different ages, taking into account the consumer appreciation of the species whose culture production is becoming widespread day by day. Especially the enterprises that produce in the terrestrial area and have restaurants, considering the customer demand, prefer individuals with red spots (who reach sexual maturity at an early age) despite their higher production costs. On the other hand, enterprises that produce large sizes prefer individuals who reach sexual maturity late and therefore have lower production costs.

Many studies have been conducted on issues such as spawning season, fertility, natural behavior and cultural characteristics of brown trout. It has been reported that brown trout give offspring in the 3-month period between October and December in the northern hemisphere (Needham, 1945; Horton, 1961; Thomas, 1964; Moyle, 1976), and in the 3-month period between late May and July in the southern hemisphere (Hopkins, 1970; MacDowall, 1978). It has been reported that brown trout start to give offspring in Chile in June, reach the highest point in July, and reproduction continues until September (Estay et al., 2004). It has been reported that the maturation and spawning times of Black Sea salmon living in fish production facilities and natural waterways of the Northwest Caucasus show significant differences depending on environmental conditions, especially temperature (Makhrov et al., 2011). It has been reported that Black Sea salmon broodstock continue to reproduce mainly in November and rarely until mid-December in the natural environment on the Turkish coasts (Tabak et al., 2001). It has been reported that the cultured Black Sea salmon started to breed in November under cultured conditions, peaking in December and continuing until February (Çakmak et al., 2022). It was determined that it gave offspring between the last quarter of December and the last quarter of February (Salihoğlu et al., 2013). In this study; the broodstock that reached the first sexual maturity at 22 and 34 months of age were stripped in November and December, and the broodstock that reached the first sexual maturity at the age of 46 months were stripped in December. It is seen that selective breeding studies applied for the creation of broodstock from marine ecotype (anadromous) individuals are effective in completing the stripping period of the broodstock in a 2-month period. The data obtained in the studies to determine the stripping season are similar to the reproduction season of brown trout distributed in the Northern hemisphere and different from the reproduction period of brown trout distributed in the Southern hemisphere and rainbow trout produced in the Eastern Black Sea region of Turkey. In the northern hemisphere, reproduction of Black Sea salmon in the early period and rainbow trout in the late period will provide advantages in hatchery management for private enterprises that produce Black Sea salmon together with rainbow trout.

Brown and Kamp (1941) calculated the total egg yield of brown trout (Salmo trutta) in the USA as 1285 eggs/broodstock and measured the egg diameter as 4.64 mm (n=37). Toledo et al. (1993) calculated the total egg yield of brown trout (Salmo trutta) in Spain as 1176 eggs/broodstock and the egg diameter was 4.67 mm (n=24). Gaudemar and Beall (1998) calculated the egg yield of 630 Atlantic salmon with an average weight of 3985 g as 7771 eggs/broodstock in their study of Atlantic salmon females spawning in a controlled flow channel. Estay et al. (2004) reported that the total egg yield of brown trout of cultured forms of different ages was between 1182±344 - 2744±605 eggs/broodstock, the relative egg yield was between 3577±471 - 2181±360 eggs/kg and the egg diameters were between 4.64±0.11 - 5.24±0.12 mm. in Chile. Tabak et al. (2001) calculated the total egg yield of Black Sea salmon broodstock in the natural
environment as 3226±320 eggs/broodstock, the relative egg yield of 1747±70 units/kg and measured the egg diameter as 5.48±1.10 mm. Çakmak et al. (2022) reported that the total egg yield of F2, F3, F4 generation broodstock was 3202±1665-3664±1220 eggs/broodstock, the relative egg yield was 2428±709-2417±586 eggs/kg and egg diameters were 5.52±0.34-5.45±0.21 mm in their studies on selective breeding of Black Sea salmon. In general, egg yield and size in fish are affected by various factors such as broodstock size, age, genotypic structure and feeding conditions (Haeley and Heard 1984; Bromage et al., 1990, 1992).

In this study, the total egg yield of the broodstock reaching the first sexual maturity at 22, 34 and 46 months of age were calculated as 1108.98±40.73, 3869.02±138.43 and 5899.52±1143.78 eggs/broodstock, relative egg yields were 3024.87±87.52, 2291.90±89.52 and 1816.00±284.51 eggs/kg and mean egg diameters were measured as 4.40±0.00 cm, 5.07±0.00 cm and 5.35±0.00 cm, respectively. It is seen that the total egg yield and egg diameters of the broodstock that reach the first sexual maturity at the age of 22 months are similar to the results of Brown and Kamp and Toledo et al., but they are different from other researchers’ who work on farmed broodstock results. While total egg yield, relative egg yield and egg diameter of broodstock reaching the first sexual maturity at 34 and 46 months of age were found similar to studies of Estay et al., Gaudemar and Beall, Tabak et al., Çakmak et al. who studied brown trout and Atlantic salmon broodstock, it was found to be different from the results of other researchers working on wild broodstock. It is seen that the differences in total egg yield, relative egg yield and egg diameters are due to the size of the broodstock depending on the first maturation age. According to the ecotypes to be preferred in the production of Black Sea salmon, it will be important for the profitability of the enterprise to take into account the breeding data in broodstock management and breeding studies.

Estay et al. (2004) calculated that the fertilization rate ranged from 92.0±13.7% to 98.5±4.01% in the study they carried out with brown trout, a cultured form in Chile. Çakmak et al. (2022) calculated the fertilization rate as 93.46±5.35% in the wild Black Sea salmon production studies adapted to the culture conditions in Turkey, and reported that the fertilization rate of eggs stripped from F1, F2, F3 and F4 generation broodstock varied between 95.28±6.29 - 98.25±1.81%. It is seen that the fertilization rate findings obtained in this study are similar to the results of the study conducted with cultured brown trout.

Black Sea salmon, which is among the species with high socio-economic value related to human consumption and sportive fishing, is similar to brown trout (Salmo trutta) and Atlantic salmon (Salmo salar) in terms of first maturation age and some characteristics. Individuals with the first reproductive age of 22 months have the typical characteristics of brown trout with early sexual maturity, red colored white halo mottling, slow growth and being settled, while individuals with first reproductive age of 34 and 46 months have the characteristics of Atlantic salmon like late sexual maturity, black mottling, silvery coloration, rapid growth in especially marine water and being anadromous. Different ecotypes of the species, which are still in the domestication stage, are preferred by commercial enterprises with different marketing networks. The general characteristics of ecotypes should be taken into account, considering...
producer and consumer preferences in future studies to be carried out on genetically supported breeding of Black Sea salmon.

Declarations

Acknowledgments

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Ethical Statement

The experimental protocols were conducted in accordance with the approval of the experimental animals ethics committee of the Trabzon Central Fisheries Research Institute (protocol No.: ETIK-2017/1).

Competing Interest

The authors declared that there is no conflict of interest.

References


Figures

Figure 1

Individual tags applied to broodstock (a: Tag reader, b: tag injector and tags, c: application of the tag to the muscle tissue, d: the appearance of the tag in the muscle tissue)

Figure 2

The sites where the broodstock kept (1: SUMAE, 2: Net cage research unit (40°57’35.05”N, 40°19’17.44”E, Altitude:0m), 3: Freshwater unit (40°49’20.59”N, 40°19’17.44”E, Altitude:644 m)
Figure 3

Water temperatures of marine net cage research unit and freshwater unit
Figure 4

Number of broodstock reaching sexual maturity at different ages during the reproductive period

Figure 5
Relationship between total and relative fecundity-weight of stock reaching first sexual maturity at 22 months of age

**Figure 6**

Relationship between total and relative fecundity-weight of stock reaching first sexual maturity at 34 months of age

**Figure 7**

Relationship between total and relative fecundity-weight of stock reaching first sexual maturity at 46 months of age