

Wild *Salmo trutta labrax* from the Bıçkı Stream in the Marmara Region: Gamete Quality and First Reproduction Under Aquaculture Conditions

Devrim Memiş¹ , Gökhan Tunçelli¹ , Güneş Yamaner¹ 

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ABSTRACT

In this study, the quality and reproductive success of wild Black Sea Trout (*Salmo trutta labrax*) were investigated. In total 32 wild trout (27.3±13.6 g and 13.9±2.3 cm) from the Bıçkı Stream in Kocaeli Province in the Marmara Region were kept in circular fiberglass outdoor flow-through tanks (1.5m ø) at the mean water temperature of 11.8±4.5 °C. In November 2016, when the water temperature was 8.4 °C, the first spawning of wild trout occurred. Sperm volume (ml), Motility (%) and VCL (µm/sec) values were determined to be 7.5±2.5, 70.42±0.83 and 70.35±3.98, respectively. The weight (g), total fecundity and egg diameter (mm) values of female fish were listed to be 513.75±78, 1.171±171, and 3.84±0.02, respectively. In the experiment, 5,859 wild Black sea trout eggs were fertilized. The eyed-egg stage occurred 24 days (288 day-degree) post fertilization and the survival rate from fertilization to the eyed-stage was 28%. Wild Black Sea trout larvae from P1 (Paternal Generation) hatched 48 days after fertilization with the hatching rate of 7%.

Keywords: Wild trout, sperm quality, egg quality, fertilization, first spawning

ORCID IDs of the author:

D.M. 0000-0001-7378-0165;
G.T. 0000-0003-1708-7272;
G.Y. 0000-0003-1886-4985

¹Istanbul University, Faculty of Aquatic Sciences, Department of Aquaculture and Fish Diseases, Istanbul, Turkey

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Correspondence:
Güneş Yamaner
E-mail:
gyamaner@istanbul.edu.tr;
gunesyamaner@gmail.com

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INTRODUCTION

Domesticating and cultivating wild fish are the first stages of aquaculture. In these stages, huge losses can occur in the fish stock due to stress. Aquaculture success of a species depends on the losses at this stage and the reproductive ability of individuals adapted to culture conditions (Bromage and Roberts, 1995; Teletchea et al., 2014; Özgür et al., 2016).

The Salmonidae family has many species of commercial, cultural and environmental value. Thirteen species in the family have been listed as either "endangered," "critically endangered" or "vulnerable," according to The International Union of Conservation of Nature - Red List of Threatened Species (IUCN, 2020). For decades, the pollution of water, habitat destruction, hydro-electric power stations, sand-mines, weirs, dams and the lack of control overfishing have caused a decrease in the Salmonid species

population of Turkey and the world. The rearing of endangered salmonid species for both commercial and ecological purposes has received considerable attention. From an ecological perspective, the rearing of wild fish in culture conditions may provide potential solutions for the rehabilitation of damaged populations. Aquaculture is one of the most widely used methods to increase the natural populations after adaptation to the culture conditions of endangered species (Cabrita et al., 2009).

According to the environment or geographical region where they are adapted, the genus *Salmo* is present in almost all cold streams and rivers of Anatolia with high diversity, such as *Salmo abanticus*, *S. caspius*, *S. platycephalus*, *S. rizeensis*, *S. coruhensis*, *S. tigridis*, *S. labecula*, *S. opimus*, *S. chilo*, *S. kottelati*, *S. okumusi*, *S. euphrataeus* and *S. munzuricus*. Black Sea Trout (*Salmo trutta labrax*) are distributed along the eastern part of the Black Sea, and are endemic

anadromous species of Salmonidae family. This species lives in the Black Sea until sexual maturity and migrates upstream in order to spawn (Turan et al., 2009).

Reproduction and bio-ecological characteristics, first feeding problems, and growth properties of Black Sea Trout have been studied systematically and the majority of studies have been conducted regarding the development of aquaculture practices in order to restock their natural habitat (Çakmak et al., 2004; Aksungur et al., 2005).

Collecting gametes from wild fish under captive conditions can only be achieved by creating conditions similar to natural conditions. A successful adaptation process is possible by the "no mortality" and "reproduction" of the existing species. After the adaptation periods are completed, aquaculture success depends on obtaining gametes and the quality and quantity of gametes obtained. The monitoring of gamete quality is a key issue for efficient artificial reproduction strategy. The quality of gametes allows us to make certain predictions to the gamete's capacity for successful fertilization or survival possibility (Migaud et al., 2013).

In Black Sea trout, there have been certain spawning techniques and protocols developed (Çakmak et al., 2004). In addition, the determination of gamete quality has become a main step to optimize breeding techniques. Quality and quantity of gametes can be influenced by factors including fish origin, fish health and age, time of stripping during the spawning season, nutrition and pre-spawning broodstock management (Bobe and Labbe 2010).

The aim of the present study is to describe for the first time, the reproduction specialty and gamete quality of both genders of wild Salmonid species (*Salmo trutta labrax*) from the Bıçkı Stream and provide information for the fertilization rate of newly adapted fish.

MATERIALS AND METHODS

Broodstock and rearing system

Fish were captured two years before the fertilization experiment by an electroshock device in the Bıçkı Stream (40°34'42.99"N; 29°55'57.60"E) in Kocaeli Province on 28 January 2014 and transported by aerated transfer tank to Istanbul University Faculty of Aquatic Science, Sapanca Inland Fish Culture Research and Application Unit.

In total 32 wild trout (27.34±13.66 g, and 13.91±2.33 cm) were placed in circular fiberglass outdoor flow-through tanks (1.5 m diameter) at a mean water temperature of 11.79±4.55°C and they were kept under culture conditions until ready for the reproduction. The first feeding of wild trout was maintained by conducting social interaction with domesticated rainbow trout, which already had experienced the feeding process. Wild trout and rainbow trout of the same length were fed commercial trout feed (2-3 mm depending on the fish size; 48% protein; 17% oil; 11% ash) until their first spawning (Başçınar et al., 2010b).

Reproductive performance

In order to investigate wild Black Sea trout spawning adaptability in culture conditions, gamete maturation was checked between September and January in 2015 and 2016. The gender and stage

of the sexual maturity of each fish were determined via investigation of eggs or sperm. The first spawning activity occurred two years post-capture. In total five female (mean weight of 476.32±107 g) and two male (mean weight of 529±51 g) broodstocks spawned for the first time at 8.4°C in November 2016. Gentle hand pressure was applied to the abdomen to obtain eggs and sperm samples. Contamination of blood, feces, and urine during stripping were avoided.

Determination of gamete quality

1. Egg quality

The total number of eggs for each individual were estimated gravimetrically. The subsamples of about 1 g of eggs were counted for each fish and the total fecundity was calculated to be $F=nG/g$ where F is fecundity, n is the number of eggs in the subsample, G is the total weight of the fish and g is the weight of subsample (Hunter et al., 1985). Fifty eggs from each female were taken at random, placed under the stereomicroscope and photographed, measured to the nearest 0.01 mm using a stereomicroscope. An average egg size for each female fish was then calculated.

2. Sperm quality

The sperm volume (ml) was calculated using labelled glass beakers. The sperm cell motility parameters (Motility, VCL) were determined with the Computer Assisted Sperm Analysis (CASA, CEROS, Hamilton Thorne, Beverly, MA, USA) system connected to a CX41 microscope (Olympus Japan). Recordings were made with a digital camera (U-TV1X-2 Tokyo, Japan) at 60 images per second using Salmonid variables pre-determined in the Hamilton configuration. Sperm were considered immotile at velocities $<20 \mu\text{m s}^{-1}$, and motile at velocities $>20 \mu\text{m s}^{-1}$. Hatchery water was used as a sperm activation solution.

3. Fertilization experiment

The fertilization was performed by the dry method. The eggs of each female were fertilized with two male's pooling sperm. The sperm and eggs were gently stirred for 15-20 seconds, then 250 ml water from broodstock holding tanks was added. The eggs were left for 30 minutes until completion of swelling. The eggs were placed on the vertical incubation system which has constant well water (12.0°C) (2 L/min) in the hatchery (Okumuş et al., 2007). The fertilization rates were measured at the eyed stage and calculated as the number of eyed eggs \times initial egg number⁻¹ \times 100 and hatching stages calculated as several hatching larvae \times eyed eggs⁻¹ \times 100 (Hunter et al., 1985).

Statistical analysis

The data obtained from the analysis are given as mean value and standard deviation (\pm SD). An independent T-test was performed using STATISTICA v.8.

RESULTS AND DISCUSSION

Physicochemical parameters of water

Water temperature was measured weekly and found to be 11.79±4.55°C in outdoor tanks, and 12 °C in the incubation unit. The dissolved oxygen level varied between a minimum of 8.2 and a maximum of 9.4 mg/l. The water pH level ranged between a minimum of 7.4 and a maximum of 8.2 during the study.

Reproductive performance of female individuals

The first spawning activity took place two years later, while the wild *S. trutta labrax* were kept under aquaculture conditions. Spawning occurred in November 2016 when the water temperature was 8.4°C. Five female individuals spawned and a total number of 5,859 eggs were collected (Table 1). The eggs ($M=3.84$, $SD=0.02$) were yellow, spherical, non-adhesive and demersal.

Table 1. Weight, total fecundity and the mean egg diameters of wild *S. trutta labrax* females.

Individuals	Weight of female (g)	Total fecundity (egg / individual)	Egg diameter (mm) (n=50)
1	327	1247	3.82±0.08
2	470	1044	3.82±0.08
3	624	1300	3.85±0.06
4	514	1330	3.84±0.07
5	447	938	3.88±0.07
Mean±SD	476.32±107	1171±171	3.84±0.02

*Values with different superscript letters and numbers are significantly different ($p<0.05$, Independent T test).

Salmonid fish reach sexual maturity at the age of 2-5 years (Bromage and Roberts 1995). In this study, we observed the first ovulation two years from capture. This is consistent with the observations in this study where the individuals who were given gametes but did not mature. It has been well documented in the literature that spawning among Salmonids generally occurs during the autumn-winter period: the beginning of October to the end of January. It is indicated that additional genetic conditions, the origin of broodstock, feeding regime, size and age of individuals were the main factors controlling the spawning, and consequently, fertilization success. Geldiay and Balik (1996) reported that the spawning age of salmonid species is between 2-4 years in Turkey. The present study shows that after two years in captivity in trout farm conditions, wild Black Sea trout were able to naturally spawn in November.

The amount and size of the egg are the two main parameters for the determination of the quality of female broodstock individuals in aquaculture (Bromage and Roberts 1995). Several studies (Okumuş et al., 2007; Woynarovich et al., 2011) have stated that the fecundity of Salmonid species is about 300-2.000 eggs × broodstock⁻¹. The fecundity of *Salmo trutta macrostigma* was found as 1.322±233 eggs × individual⁻¹ (Alp et al., 2010). Reported as a new Salmonid species of Alakır trout (*Salmo kottelat*), the fecundity was changed to between 1.035-1.632 eggs × individual⁻¹ (Kanyılmaz, 2016). For some *Salmo trutta* species, fecundity was reported as a maximum of 1.250 eggs × individual⁻¹ (Arslan and Aras 2007). Aksungur et al. (2011) found a total fecundity of 3.226±320 numbers of eggs per individual in the broodstock of marine ecotype Black Sea trout. Some researchers, however, have reported a higher number of eggs produced by brown trout, such as 2.000-5.000 eggs × kg⁻¹ (Geldiay and Balik 1996); 3.099 eggs × kg⁻¹ (Alp et al., 2010). In our study, the number of

spawned eggs per female (n=5) was found as 1.172±171. Fecundity values achieved in our study showed similarities with other Salmonid fish reports.

Egg size is another key parameter for measuring the egg quality. The egg size per diameter may vary among fish species and within the same species (Bromage and Roberts 1995). In rainbow trout egg diameter ranges from 4.9 to 7.2 mm, while egg diameter in brown trout ranges from 5 to 5.33 mm (Gjedrem and Gunnes 1978). Egg diameter in Black Sea trout was found as 3.0-5.3 mm (Sonay 2008), 4.5-4.7 mm (Başçınar et al., 2010a), and 4.5-5.1 mm (Erbaş and Başçınar 2013). In our study, egg diameter was recorded as 3.8±0.02 mm and it was in agreement and comparable with earlier reports.

Reproductive performance of male individuals

Spermiation occurred in three males before the spawning of females and in two males at the same time the females spawned. Sperm volume and the kinematic parameters of sperm (motility (%), and VCL (µm/sec) values gained in the experiment) are given in Table 2. Sperm volume ranged from 5 ml to 10 ml per individual. Sperm cell motility was higher than 70% in each individual, and the mean motility percentage was 70.42±0.83% for five males.

Table 2. Sperm (volume, ml) and Spermatological parameters (motility (%), VCL (µm/sec) of the wild *S. trutta labrax* (mean±SE).

Sperm and Spermatological parameters	Values (Mean±SE) (n=5)
Sperm volume (ml)	7.5±2.5
Motility (%)	70.42±0.83
VCL (µm/sec)	93.79±6.51

Sperm volume in fish is directly related to water temperature, stripping frequency, age of the individual, presence of females in the pool and rearing conditions (Büyükhatoğlu and Holtz 1984). In salmonids, the total sperm volume stripped from an individual varies between 5-30 ml during a complete breeding season. While a total sperm amount of 4.5-18 ml was reported in brown trout, 19.6 ml sperm volume was reported in rainbow trout (Rainis et al., 2005). Total sperm volume was reported as 8.4±0.4 ml in *Salmo trutta macrostigma* (Yavaş et al., 2011), 8.45±1.32 ml (Erbaş and Başçınar 2013), and 1.6±0.04 ml (Şahin et al., 2013) in Black Sea trout. In the present study, total sperm volume was detected as 7.5±2.5 ml in males. Our findings for sperm counts in the present study show parallel findings with earlier reports on trout.

High sperm cell motility is required for successful fertilization and it is the most important parameter for determining the fertilization ability of sperm cells. Sperm cell motility can change between 0% and 100% under aquaculture conditions, where any manipulation processes are applied. Ruling out the details and variables in sperm cell freezing or sperm cell activation studies, more than 70% of the cells in a fresh sperm sample is a reason for preference (Cabrita et al., 2009). In fresh sperm collected from salmonid fishes without any manipulation, the spermatozoa mo-

tility was determined between 20-100% in brown trout (Dziewulska, Rzemieniecki, & Domagała, 2008); 50-89% in rainbow trout (Dziewulska, Rzemieniecki, & Domagała, 2008; Ekici et al., 2012; Şahin et al., 2013), and 60-90% in Black Sea trout (Erbaş and Başçınar 2013). In our study, sperm motility was determined as $70.42 \pm 0.83\%$. These motility values determined in Black Sea trout are similar to earlier reports on Black Sea trout as well as other trout species mentioned above.

The VCL value, the curvilinear velocity related to the sperm motility, also varies among species and within the same species in salmonids. The sperm cell with higher speed is more successful in fertilizing (Lahnsteiner et al., 2011). In salmonid fishes, various VCL values were determined as $38.1-149.5 \mu\text{m}/\text{sec}$ in Chinook salmon (*Oncorhynchus tshawytscha*) (Rosengrave et al., 2009); $96-127 \mu\text{m}/\text{s}$ in Atlantic salmon (*Salmo salar*) (Rosengrave et al., 2009); $191.8 \pm 56.9 \mu\text{m}/\text{s}$ (Dziewulska, Rzemieniecki, & Domagała, 2008); $102.50 \pm 18,39 \mu\text{m}/\text{s}$ (Tunçelli and Memiş 2020) $59.42 \pm 24.63 \mu\text{m}/\text{s}$ in *Salmo trutta labrax*, and $141.2-240 \mu\text{m}/\text{s}$ in *Oncorhynchus mykiss*. In our study, VCL values were determined as $93.79 \pm 6.51 \mu\text{m}/\text{s}$. VCL values achieved in our study showed similarities with the previous reports.

Fertilization and survival rate of larvae

In the experiment, some of the 5,859 Black Sea trout eggs were fertilized. The eyed egg stage was determined at 24 days (288 day-degree) post-fertilization and the survival rate from fertilization to the eyed stage was 28%. Black Sea trout larvae hatched 48 days after fertilization with a hatching rate of 7% (Figure 1).

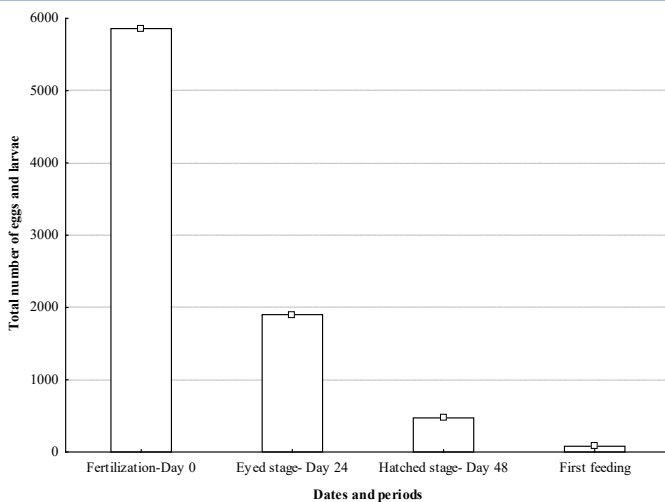


Figure 1. Total number of fertilized, eyed and hatched larvae of wild trout (*S.trutta labrax*).

The temperature of the incubation period greatly impacts the survival of fish embryos (Cabrita et al., 2009; Simcic et al., 2015). Reference for the optimum temperature for incubation and hatching is between 8-14°C for Salmonid (Pennell and Barton 1996), however, incubation time, development of embryonic and larval stages, hatching and swim-up larvae as day-degree reveal differences in Salmonids (Başçınar et al., 2010a). These studies

showed that the reproduction period, gamete quality and fertilization rate in different conditions can be different between interspecies and between species. It was reported that in brown trout, the incubation temperature varied between 10.5-10.9°C and the rate of hatching was determined to be between 32 and 57% (Demir et al., 2010). In the other study, *S. trutta labrax* eggs hatched after 38 days under 10°C (Firdin, Çakmak, & Aksungur, 2012). According to Marten (1992), in order to gain a high survival rate in brook trout, the incubation temperature should be maintained between 3-8°C during the incubation period. Some species of salmonids, such as Brook trout embryos, grew much faster in the warm water treatment than their siblings in the cold-water treatment. This was consistent with previous studies of other species, showing individuals in warmer environments had earlier hatch dates (Cook et al., 2018). In the present study, the eggs of wild Black Sea trout incubated at 12.0°C during the incubation period, the water temperature changed between 8.4 and 12.3°C (11.3 ± 1.2). Although the water temperature was considered suitable for Black Sea trout eggs in the incubation unit, the low hatching rate is thought to be related to the low quality of gametes. As is well known, the quality of eggs can be determined by healthy larvae in the hatchery after fertilization. While the number and size of the eggs are important criteria for determination of gamete quality of the female broodstock, the rate of fertilization and eyed egg ratio are also important for the quality. During the incubation, survival rates of Salmonid eggs may vary 0-100% (Bromage et al., 1992). The survival of eggs during the incubation period and hatching larvae recorded in the present study was lower than previously reported for some salmonids species (Pennell and Barton 1996). Neither the egg number nor egg size proved to be useful indicators of egg quality and egg viability of wild oradapted broodstock of *S. trutta labrax*. The hatching rate of larvae of wild broodstock individuals was calculated as >10% despite fertilization. The majority of eggs were lost due to mortality during embryogenesis which could indicate that there were several factors causing the egg loss (Cabrita et al., 2009). Lower survival rate during the incubation conditions could possibly be explained by the lower adaptation to culture conditions of broodstock individuals, even though these individuals were given the gametes. Gamete growth occurred in wild broodstock, but gamete maturation was not enough for the embryonic development for first spawning under culture conditions (Bobe and Labbe 2010). Also, the egg quality should be affected by broodstock nutrition, genetics, environmental conditions and any stress factors such as handling and spawning induction (Migaud et al., 2013).

Sperm quality has been described as "the ability of sperm to successfully fertilize an egg" (Rurangwa et al., 2004). This definition reflects the former consensus that sperm quality only affected fertilization rates, while paternal effects on offspring viability are limited to genetic information carried by the sperm quality of the father rather than his sperm. However, embryonic survival and development are affected by sperm quality (Labbe et al., 2017). Because of oxidative stress during spermatogenesis, if there is some damage to the chromatin or RNA in the spermatozoa, the sperm still have the ability to fertilize an egg. However, embryonic development will be disturbed due to damage to these mole-

cules (Johnson et al., 2011). The present study determined acceptable sperm quality with regard to the motility and kinematic parameters of motility. All parameters were found to be similar to the salmonid sperm quality, which is described in previous studies (Dziewulska et al., 2008; Ekici et al., 2012; Şahin et al., 2013) but together with information on fertilization and embryonic survival rates of present study, these data showed that there should be a paternal effect on reproduction performance.

CONCLUSION

In conclusion, *Salmo trutta labrax* individuals, which were caught from their natural habitat (Bıçkı stream) could be adapted to culture conditions. As was observed, all individuals could have finished the oogenesis and spermatogenesis cycle under culture conditions in consideration of first spawning. However, the first gametes of these individuals showed bad quality for both genders, which led to low and unacceptable rates for the aquaculture conditions. The first spawning from the wild parental generation results can be considered as ordinary results and the success will increase in the next generations. With regard to the sustainable culture conditions of this species in farms, the ways of increasing the "breeding success" and "gamete quality" of this wild trout species should be investigated in future studies.

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Ethics committee approval: Ethical approval was not required for this study.

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Disclosure: -

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