

## Bir Alabalık İşletmesindeki Gökkuşluğu Alabalığı Damızlıklarının Üretim Performansı Üzerine Bir Çalışma

Mustafa Erkan ÖZGÜR<sup>1</sup>

İsmail BAYIR<sup>2</sup>

<sup>1</sup> Inonu University, Surgu Vocational High School, Fishery Program, Malatya, Turkey

<sup>2</sup> Erzincan University, Kemaliye Hacı Ali Akin Vocational High School, Fishery Program, Erzincan, Turkey

\*Corresponding author:  
e-mail: mustafa.ozgur@inonu.edu.tr

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### Özet

Bu çalışma bir alabalık işletmesinin damızlıklarının performans analizi üzerine yapılmıştır. Çalışmada, gökkuşluğu alabalığı (*Oncorhynchus mykiss*) dişi damızlıklarından elde edilen yumurtaların, yumurta çapı  $5.58 \pm 0.18$  mm, nispi yumurta üretimi  $1607.25 \pm 84.55$  adet, toplam yumurta üretimi  $6024.66 \pm 357.97$  adet olarak belirlenmiştir. Kuluçka periyodunda dölleme oranı, gözlenme oranı ve çıkış oranları sırasıyla,  $98.84 \pm 2.04$  %,  $97.14 \pm 5.08$  ve  $79.36 \pm 12.43$  olarak hesaplanmıştır. Besin keseli larva, besin kesesini 23 gün sonra tüketmiş ve aktif yüzmeye başlamış olup, canlılık oranları besin keseli larva için  $99.49 \pm 0.26$  ve yavru dönemi için  $85.51 \pm 22.31$  olarak tespit edilmiştir. Erkek damızlıklardan elde edilen sperm kalite parametreleri, sırasıyla pH ( $7.35 \pm 0.21$ ), sperm hacmi ( $40.41 \pm 21.60$  ml sperm), spermatozoa yoğunluğu ( $10.43 \pm 2.06 \times 10^9$  spermatozoa/ml) ve spermatozo motilitesi  $86.50 \pm 5.45$  olarak bulunmuştur.

Çalışmada, dişi damızlık ağırlıkları ile toplam yumurta üretimi, nispi yumurta üretimi ve yumurta çapı arasında önemli pozitif ilişkiler tespit edilmiştir ( $R^2=0.99$ ;  $P<0.05$ ). Sonuç olarak, elde edilen yumurta kalite parametreleri, bu çalışmanın amacı doğrultusunda temel kalite parametrelerini içeren sınırlar arasında olduğu ve performansının iyi olduğu sonucuna varılmıştır.

**Anahtar Kelimeler:** *Oncorhynchus mykiss*, üretim performansı, yumurta ve sperm kalite parametreleri, Turkey.

## A Research on Reproduction Performance for Broodstocks of Rainbow Trout in A Fish Farm

### Abstract

This study carried out reproduction performance about broodstocks of rainbow trout in a fish farm. In the study, egg diameter  $5.58 \pm 0.18$  mm, relative eggs production per kg  $1607.25 \pm 84.55$ , absolute egg production per individual  $6024.66 \pm 357.97$  of broodstocks of Rainbow trout (*Oncorhynchus mykiss*) was determined. After eggs collection, the ratios of fertilization, eye formation and hatching during incubation period were  $98.84 \pm 2.04$  %,  $97.14 \pm 5.08$  % and  $79.36 \pm 12.43$  %, respectively. Twenty-three days after yolk-sac larvae stage, yolk-sac sack is exhausted and free swimming begins, leading to feeding stage. Yolk-sac larvae term survival ratio at this stage and fry survival ratio were determined to be  $99.49 \pm 0.26$  %,  $85.51 \pm 22.31$ , respectively. Quality of sperm examined about pH ( $7.35 \pm 0.21$ ) and volume ( $40.41 \pm 21.60$  ml of sperm, density ( $10.43 \pm 2.06 \times 10^9$  spermatozoa/ml) and motility ( $86.50 \pm 5.45$  %) of spermatozoa.

There was significant positive relationships between broodstocks weight and absolute eggs production, relative eggs production and egg diameter (Adjusted  $R^2=0.99$ ;  $P<0.05$ ). From egg quality parameters point of view, it was determined that, the values obtained were within the limits of the purpose in which this study was built.

**Keywords:** *Oncorhynchus mykiss*, reproduction performance, egg and sperm quality parameters, Turkey

## INTRODUCTION

High quality gametes are necessary for successful aquaculture development. In salmonid and other finfish industries, the proportion of gametes surviving the embryonic period is highly variable [9, 18]. One species that is grown worldwide due to its marketability and adaptability is the rainbow trout (*Oncorhynchus mykiss*) [15]. Although this species has been cultured for many years, this industry still experiences some embryonic mortality and/or poor quality eggs from their broodstock [27]. Accumulating evidence suggests that most of the problems with rainbow trout embryo viability can be attributed to the female [21].

In *Oncorhynchus mykiss* farming, fertilization, eye formation and hatching ratios, form the main criteria used in determining egg quality. Several owners of *Oncorhynchus mykiss* farms have chosen eye formation ratio as eggs quality determinant, and the high ratio of eye formation value, shows high quality of eggs [6].

Long incubatory life with hatching ratios, hatching of healthy and regularly developing individuals; determine the quality of good eggs. Fecundity, post-spawn per fish (absolute fecundity) or kg is known as the number of eggs per fish live weight (relative fecundity). Egg size, diameter or unite volume of number of eggs or expressed as the weight of eggs spawned, is known as an important factor in defining the fecundity of broodstocks [7]. For this reason, egg size and its quantity are evaluated as the production characteristics of broodstock. Fecundity and egg size of *O. mykiss* can be measured easily on the calcifying (hardening) egg after spawning. Even though there is no clear-cut opinion on the determinant of egg quality, factors such as fertilization, eye formation, and larva survival ratios are all taken into consideration when discussing egg quality. This is because; it is known that broodstocks under the same conditions can exhibit very different egg qualities. Several factors are known to have effects on egg production and on the quality of larva in fish-farming conditions [7, 8, 10, 16, 22, 24]. Different researchers in Turkey and many other countries have carried out studies on the production performance (egg production, egg quality and the factors affecting these qualities) in *Oncorhynchus mykiss* under culture conditions. In fact, there is no sufficient information on the production properties of

*Oncorhynchus mykiss* broodstocks found in our country. In order to work towards increasing productivity in the future, it is therefore, necessary to know the prevailing conditions.

## MATERIALS AND METHODS

### Experiment Location and Water Supply

This study was done during the production season (2010) in Fish Hatchery Center of Kemaliye Hacı Ali Akın Vocational High School Aquaculture Department of Erzincan University in Kemaliye district, Erzincan province at Turkey. Spring water got from a source known locally as 'Egin-zo' is used in the center and runs directly along a single course to the center. It flows through covered canals. Throughout the study, water temperature was measured thrice/day, while water flow was measured once/week.

### Broodstocks Care and Spawning

Until production season, broodstocks comprising 150 females (four-years old) and 120 males (three-years old) were kept in 4 different pools measuring 8x4x1 m with males at the water entrance and the females kept behind the males. The broodstocks were fed 1<sup>-day</sup> with 52 % raw protein, 12 % raw oil, 2.200 kcal<sup>-kg</sup> energy of commercial Ecobio extruder and 10 mm diameter pellet feed until they were satisfied. During weekly controls, 18 broodstocks of spawning stages represented by all the broodstocks were chosen at random. In the production season, the broodstocks were controlled once a week. Those ready to spawn were collected in a different pool and kept without feeds for two days until spawning occurred between 4–10 days of maturity. Before measuring the weights, lengths and prior to spawning preparations, 100 mg of benzokain<sup>L</sup> (anesthesia) was applied on the fishes [19]. During the experiment, live weight of the broodstocks was measured with a digital balance of 1 g sensitivity and total lengths determined with 1 mm measurement wooden stick. At the beginning of the study, some of the physical and chemical parameters in the research pool and incubator boats water were determined. Also, throughout the study, both pools' water temperature and that of the incubator vessels were measured daily with 1°C thermometer, the pH measured with a portative pH meter while dissolved oxygen (DO) (mg<sup>-l</sup>) was measured using a portative oxygen meter [1].

### Sperm Quality

We used anesthesia (100 mg of benzocain/L) before we spawn, weight and measure their length to mature fish. Spawn was became by massage from the front to back of fish abdomen [19].

While Sperm volume of mature fish was measured by a 50 mL of glass, pH of sperm was measured by insided papers and looked for color scale with pH indicator papers which is Merck-Neutralit mark (spaced 5.5-9.0).

The duration of sperm motility in each male was assessed microscopically in order to support the findings which were obtained during the fertilization experiment. Spermatozoa were activated by mixing a minute volume of milt by means of the tip of a toothpick into a drop of the water ( $10 \pm 1^\circ\text{C}$ ). Motility was then evaluated at 400 X magnification. The sperm motility duration was taken as the time at which 50% of the activated spermatozoa ceased forward movement. This was obtained using a stopwatch. This procedure was repeated five times for each of ten males and only good quality milt, i.e.  $\geq 80\%$ ;  $5^+$  motility was employed [26].

When we determined density of spermatozoa and to make easy counting, we firstly prepared a dilution that took 10 ml of 0.07 % NaCl solution, dropped inside 1-2 bead from 3 % of eusin. Later sperm was diluted with this prepared dilution by  $1000^{-1}$  proportional and density of spermatozoa was determined by hemocytometric method with thoma slide [20].

### Fertilization

Spawning was done according to dry fertilization method. Eggs of each female were spawned into a dry dish and on it; sperm of two males was added. A disinfected feather was used in the egg and sperm mixture. Water was poured over it; mixture again; and was left for 30 minutes for fertilization and hardening. For the total quantity of eggs per fish (absolute egg production), 1 g was taken from the total number of eggs weight (with 0.0001 g sensitive balance) and calculated according to weight method.

### Egg Collection and Yolk-sac Larvae

Eggs diameter measuring were arranged side by side in Von Bayer boat (30 cm) and measured with the help of 1 mm sensitive compass. All counted eggs were stocked in incubators with vertical water flow and were watched until the exhaustion of the nutrients sacks. The incubation vessel was provided with 1 L/minute source of

water. Yolk-sac larvae (from hatching to complete absorption of yolk-sac) were observed until exhaustion of yolk-sac sack. After the larval period, the feeding period was observed for 30 days. According to fry development periods, 0.2-2 mm size of 45-50 % raw protein, 7-15 % raw oil, powdered feed with 2 % raw cellulose contents and granule feeds were given 4-6 times daily at different intervals until weights increased by 4 %. As from the 30th day, fry weights with nutrient sacks were measured at an interval of 15 days and development of the fry continuously observed. The fry weights were measured with a 0.001 g sensitive balance [20].

### Investigated Parameters

In the study, weight and total lengths of male and female broodstocks, absolute and relative eggs production, egg diameter, rate of fertilization during incubation period, eyed-egg formation ratio, hatching ratio, larvae survival ratio duration of consumption of yolk-sac, survival ratio, increase live weight and specific growth ratio of fry, pH and volume of sperm, density and motility of spermatozoa were determined.

The eggs quality parameters during the incubation period were done according to the formula below [23]; The fry growth parameters during feeding period were done according to the formula below [10];

Fertilization ratio (%)  

$$= (\text{Fertilized egg number} / \text{Total number of eggs}) \times 100$$

Eyed egg ratio (%)  

$$= [(\text{Eyed egg number} - \text{Number of spoiled eggs}) / \text{Number of fertilized eggs}] \times 100$$

Egg hatching ratio (%)  

$$= (\text{Number of live larvae} / \text{Number of fertilized eggs}) \times 100$$

Ratio of number of survived larva (%)  

$$= (\text{Number of free swim fry} / \text{Number of larvae}) \times 100$$

Live weight increase (CA%)  

$$= [(CA_s - CA_b) / CA_b] \times 100$$

CA % = Percentage of live weight increase;

$CA_b$  = Initial group weight of fishes (g);

$CA_s$  = Final weight of fish group (g).

Specific growth rate (SGR %)  
 $= [(\ln CA_s - \ln CA_b)/T] \times 100$

SGR = Specific growth rate:

$CA_s$  = Live fish weight at end of experiment (g);

$CA_b$  = Fish weight at start of experiment (g);

T=Duration of experiment (days);

In = logarithm according to base).

### Statistical Analysis

Results are expressed as mean values  $\pm$  standard deviation and used regression and other statistical analysis by SPSS 11 packet program for Windows software packet [28].

## RESULTS AND DISCUSSION

In the study, water temperature, dissolved oxygen (DO) and pH values were measured daily. Throughout the research period, water temperature was  $10 \pm 0.1^\circ\text{C}$ , pH  $7.4 \pm 0.2$  and DO recorded as  $9.6 \pm 0.32$  mg/L. During the experimental period, water temperature, pH and DO values did not show much variations and this difference was identified statistically ( $p > 0.05$ ) as insignificant.

Egg diameter, fertilization ratio, eyed-egg ratio, hatching ratio and free-swimming ratios are accepted as criteria for egg quality. It was estimated, the number of white eggs and their fertilization ratio within the first 36 hours after fertilization, the number of eggs with eyes and eyes ratio after 15-20 days, hatched eggs and ratio after 30-31 days. According to this, the average of fertilized eggs ( $98.84 \pm 2.04\%$ ), eye formation ratio ( $97.14 \pm 5.08\%$ ), hatching ratio ( $79.36 \pm 12.43\%$ ), larvae survival ratio ( $99.49 \pm 0.26\%$ ) and fry survival ratio ( $85.51 \pm 22.31\%$ ) were identified, respectively (Table 1).

According to the results of Linear Regression Analysis, there was forceful positive relationships between broodstocks weight and absolute eggs production, relative eggs production and egg diameter (Adjusted  $R^2 = 0.99$ ;  $P < 0.05$ ) Table 2. It was observed that during the consumption of nutrients sacks by the fries, the average fry weight was  $0.28 \pm 0.13$  g. But after the start of normal feeding, in 15 days  $0.37 \pm 0.07$  g, and in 30 days, it was  $0.57 \pm 0.16$  g. It was found that in 30 days, average live weight was  $1.16 \pm 0.51$  % and specific growth ratio  $2.46 \pm 0.91$  % (Table 3).

pH and volume of sperm, density and motility of spermatozoa were investigated for sperm quality parameters (Table 1). Our results of sperm quality in this study were included to the normal standards for the sperm quality parameters which were reported by authors [5, 12, 13, 20].

Reproduction of *Oncorhynchus mykiss* under farming conditions is known to occur between late autumn and winter months. Spawning periods of different types of genotypes in *Oncorhynchus mykiss* span between 6–8 weeks. Among the most important factors that affect egg production in *Oncorhynchus mykiss* are; close family relations among broodstocks, water quality, quantity and quality of feeds, fish age and weight [4, 9]. Fecundity (number of eggs) and egg diameter are the most used criteria in egg production. Increase in broodstock weight is followed by increase in number and diameter of eggs [7].

There was forceful positive relationships between broodstocks weight and absolute eggs production, relative eggs production and egg diameter (Adjusted  $R^2 = 0.99$ ;  $P < 0.05$ ). But some researchers had reported a negative and weak relation between quantity eggs and egg diameter [16, 17]. According to Bromage et. al., (1992) [9], despite the positive relations between quantity of eggs and its diameter, it is reported that, there is a more powerful relationship between the broodstocks weight and egg diameter. However, no relationship is seen between egg diameter and fish weight especially in 2 years old spawning fish. It is reported that, apart from broodstocks weight and feeding, other factors also determine egg diameter. There are different results that arise from the relationship between broodstock weight and egg diameter. Positive relation ( $R^2 = 0.43$ ) [9], strong relations ( $R^2 = 0.66$ ) [7, 25] and a weak relation ( $R^2 = 0.16$ ) [17] had been found. Since egg diameter and its size are to a great extent, linked with the weight of the broodstocks, there is therefore, a direct positive relation between them.

Obtaining different results in different media would be attributed to environmental conditions, broodstock genotype and differences in feeding mode, size and age. In *Oncorhynchus mykiss*, egg quality is determined by fertilization, eye formation, hatching and 4 months survival ratios [7].

In this research,  $98.84 \pm 0.48\%$  was found as the fertilization ratio. In other studies done on

the fertilization ratio of *Oncorhynchus mykiss*: 90.88 % [22], 90 % [7] and 91.87% [14] were found. When those values were compared with the results of this study, it was rather found to be low. In addition to this, from fertilization ratio point of view, values reported by 98.50 % [3] and 82.91-98.9 % [2] showed a parallel correlation.

According to Springate et. al. (1984) [24], ovulation and spawning period are some of the most important factors that affect fertilization ratio. These researchers have also stated that, the ratio of fertilization 4-6 days after ovulation is almost 100 % but later decreases sharply below 70 %. This condition could be accepted as a reason for obtaining different fertilization ratios in this study contrary to other researchers.

Besides this, spawning period, fertilization medium, egg and sperm qualities, too, are the other factors that affect fertilization ratio of fish eggs. The high fertilization ratios in this study could be attributed to a proper choice of good spawning time. Fertilization ratio of eggs is a criterion used in estimating the survival ratios in the latter cycles [9].

The rate of eye formation of the incubated eggs in the study was identified as  $97.14 \pm 1.20$

%. Other studies done for eye formation ratio on *Oncorhynchus mykiss*: 93.90 % [3] and 86.02 % [14], were reported, and are less and nearly for eyed-egg ratio in this study.

In this study,  $79.36 \pm 2.93\%$  was reported as the hatching ratio of the incubated eggs. *Oncorhynchus mykiss* eggs: Springate and Bromage (1985) [25]; 47.71-58.67 % found lower than the results found in this study, Aydın and Çelebi (2000) [3]; 83.8 %, Atamanalp et. al. (1996) [2]; 91 %, and Kurtoğlu et. al. (1998) [17]; 96 %, higher than the values found in the study.

There are very few data on the farming of *Oncorhynchus mykiss* broodstock. In fact, incubators can produce quality fry that would have superior properties over their broodstocks through the identification of the characteristics of the broodstock before production because; production of fry by *Oncorhynchus mykiss* is affected by several factors.

As a conclusion, good suggestions on obtaining quality fry from quality broodstocks have been offered. It is hoped that this research would contribute to more production of this important species of fish in our country.

**Table 1.** Quality parameters of egg and sperm, weight and total length of broodstocks

Female Broodstocks	n=18	Range	X±SD
Weight of female broodstocks (g)		3679.00-3810.00	3747.33±39.18
Total length of female broodstock (cm)		61.00-63.50	62.29±0.47
Egg diameter (mm)		5.40-5.80	5.58±0.18
Absolute egg production (number of egg/individual)		5597.59-7024.39	6024.66±357.97
Relative egg production (number of egg/kg)		1511.75-1848.52	1607.25±84.55
Fertilization ratio (%)		92.80-100.00	98.84±2.04
Eye formation ratio (%)		77.91-99.84	97.14±5.08
Hatching ratio (%)		56.54-96.56	79.36±12.43
Larvae survival ratio (%)		98.94-99.86	99.49±0.26
Fry survival ratio (%)		20.12-99.14	85.51±22.31
<b>Male Broodstocks</b>			
Weight of male broodstocks (g)			2690.50±62.07
Total Length of male broodstocks (cm)			55.69±0.47
Sperm pH			7.35 ±0.21
Sperm Volume (ml)			40.41±21.60
Spermatozoa Density (x10 <sup>9</sup> /ml)			10.43±2.06
Spermatozoa Motility (%)			86.50±5.45

**Table 2.** Normal P-P of Regression Standardized Residual

Model	R	R Square	Adjusted R Square	S.E. of the Estimate
1	0.999 <sup>a</sup>	0.999	0.999	1.44

a Predictors: (Constant), Egg diameter, Absolute egg number, Relative egg number

b Dependent Variable: Weight

**Table 3.** Weight of fry for 30 days after exhaustion of nutrients sacks

Parameters	Range	X±SD
Weight of fry (g)	0.17 – 0.70	0.28±0.13
Weight of 15-day fry (g)	0.28 – 0.50	0.37±0.07
Weight of 30-day fry (g)	0.36 – 0.91	0.57±0.16
Increase in live weight (%)		1.16±0.51
Specific growth ratio (%)		2,46±0,91

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