A STUDY ON INCREASING THE FERTILIZATION YIELD OF TROUT EGGS

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Abstract

Studies providing trout eggs and enhancing efficiency of fertilization have increased in Turkish trout aquaculture industry recent years. Fertilization methods are important for obtaining better quality offspring and rising living creatures as much as broodstock management and feeding regimes. In this study, four different fertilization methods were tested in order to increase the fertilization ratio and determine the most suitable fertilization method for trout eggs. The experiment was carried out in 4 different treatment groups with 3 replicates for each. In related treatment groups, the fertilization of eggs were performed with actifish solution "AS" (15 ml actifish, 1 L of water), isotonic solution "IS" (7 gr NaCl, 1 L of water), $NaCO_3$ solution (8 gr $NaCO_3$, 1 L of water) and Classical method (CM). At the end of the experiment, the fertilization rates between gropus, eyeing rates and the effects of the solutions on sperm quality were analysed; it was aimed to determeine the most efficient artificial fertilization method for trout eggs. The highest fertilization rates were seen in the group "AS" %95.01±0.47 ($p<0,05$) while the lowest rates were seen in the group "$NaHCO_3$" %90.72±2.54 ($p<0,05$). The eyeing rates were resulted in the groups "AS" %87.71±0.36, "CM" %69.94±2.48, "IS" %81.57±1.75 and "$NaHCO_3$" %58.53±5.74 respectively. According to these results, the fertilization method performed with "$NaHCO_3$" was found deficient for the artificial fertilization and eyeing rates of trout eggs; while the method "AS" was found as the most influential method to increase the fertilization of trout eggs.

Key words: *Onchorynchus mykiss*, trout, artificial insemination, egg, fertilization media

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) became the most important species, with 108,038 ton farming, among the cold-water fish which are farmed in Turkey [1]. Therefore, an increasing demand emerged for research on providing quality eggs and fingerlings for fish farms. When an average of 40% loss from stripping to marketing size in commercial enterprises is taken into account, there is a need for about 720 million eggs in our country according to 2015 data under normal circumstances.

Most of the trout farming enterprises in our country try to produce their own fingerlings. However, they cannot produce the fingerlings they need because of the lack of a special care and nutrition for the fish which they spare for breeding. Therefore, many enterprises satisfy their egg and fingerling needs with other enterprises. Despite this great need, there are few enterprises who only produce and sell eggs and fingerlings. Under the circumstances, the enterprises cannot produce at full capacity or alternatively, the importation of eyed eggs comes up. Therefore, the most important priority of the enterprises which operate as hatcheries is to produce large numbers of eggs and fingerlings of good quality. To achieve this goal, development and employment of productive methods is quite important.

In this study, the aim is to increase the fertilization rates in comparison with the classic method by applying the prepared different fertilization solutions on the eggs taken from the rainbow trout broodstock which have an important role in the water products sector in our country and to determine the rate of eyed eggs. In the said studies, the research was on which method would be useful in increasing the

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productivity of trout eggs. It is also aimed to reduce the number of male broodstock kept in the enterprises besides increasing the fertilization rate, by having the producers use this study. In this way, prevention of waste of labour force, time and material source is among the expected outcomes.

MATERIAL AND METHOD

This study was conducted in Çobanlar trout enterprise in the Aegean (Muğla) region. The enterprise is one which produces fingerlings and especially serving size fish. The broodstock fish which are 3-4 years old male and female fish were kept together in a pool of 15x4x3m size until the production season. These broodstock were fed with pellet feed once a day which has 45% crude protein, 15% crude fat and 3000 kcal/kg energy. The 20 female and 10 male broodstock fish (3-4 years old) were randomly chosen in the study. The feeding was stopped before the weighing and stripping day. Clove oil at a rate of 40-60 ppm was anaesthetically applied to the broodstock fish before the stripping. Afterwards, the fish were dried with a dry cloth and weighed on a 1 g sensitivity scale and their total length was measured with 1 cm sensitivity, then they each were stripped into separate plastic containers according to the dry-egg system [2].

The broodstock fish were weighed again after the stripping. In this way, the weights of the broodstock fish before and after the stripping were measured. After each female trout was stripped into a dry container with the dry fertilization method, sperm liquid of 2 male trout were added on the eggs. 1 to 26 ml. sperm liquid was taken from each male broodstock (882.444±130.336 gram). 4 fertilization methods were used in the test. Isotonic solution and $NaCO_3$ solution were added on grouped eggs, each of 30.000 eggs at an amount of 1 l. Then, sperm at an amount of 1.5 ml was added on each of 10.000 eggs. The mixture (egg - solution - sperm) was stirred. This mixture was left to rest for 15 minutes and finally, the eggs were washed with fresh water. The eggs which were washed with fresh water were placed in cartridges after full inflation. In the classic method, the same procedures were followed without adding solution. The Actifish solution was added at a ratio of 1;9 to the sperm and was mixed with the egg. 12 incubation trays at a side length of 27x43x5 cm and 2 incubation troughs at a size of 5x72x2270 were used in the study. Approximately 15.000 to 20.000 eggs were placed in the incubation troughs. Water at a flow rate of 3-4 l/min were fed into the incubation troughs. In the study, water temperature was 8.4 °C during fertilization on average; and 9.1 °C until the eggs were eyed. (8-11°C).

Broodstock weights, totaland relative egg fecundity, egg diameter, fertilization, eyeing rate, total spermatozoa numberand sperm count were determined in the study. The spermatozoa density was determined with haematocrit method. 10 µL sperm was added on 990 µL 0.7% NaCl solution with a straw and it was diluted. A drop of diluted sperm was added on the thoma lami (depth 0.1 mm) and was closed in a way to keep the air out, 3 to 5 minute was given for sperm cells to settle and it was recorded as spermatozoa density x 10^9 spz/mL by counting in the light microscope (x 400) [3]. The diameter of the eggs (mm) were measured by laying them together in a 30 cm Von Bayer trough.

Eyeing rate and fertilization is accepted as the criteria showing the quality of the egg. Fertilization rate is the rate of the remainder of the eggs after the dead eggs are removed by choosing the eggs 1 day after the fertilization. Broodstock weights, total and relative egg fecundity, egg diameter, fertilization and eyeing rate were determined in the study. Regression analyses were conducted among various parameters by using this data [4]. Excel and SPSS 15 were used in evaluating the data.

RESULTS AND DISCUSSIONS

As a result of linear regression analyses, positive relationship between broodstock fish weight and total fecundity (R^2=0.67), positive but weak relationship between broodstock weight and relative fecundity (R^2=0.19), positive high relationship between total fecundity and length (R^2=0.67), positive but weak relationship between relative fecundity and length (R^2=0.074), positive high relationship between broodstock weight and egg diameter (R^2=0.36), positive relationship between length and egg diameter (R^2=0.36),

positive relationship between total spermatozoa number and sperm count (ml) (R^2=0.25) and positive relationship between sperm count-male broodstock weight were found (Figure 1, 2, 3, 4, 5, 6, 7, 8).

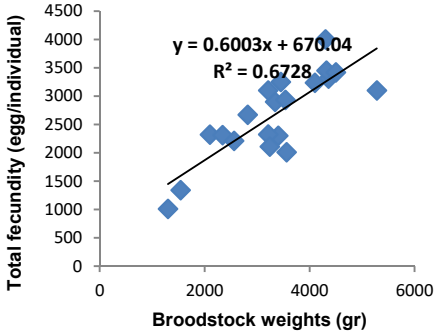

Fig. 1 Total fecundity-Broodstock Weight

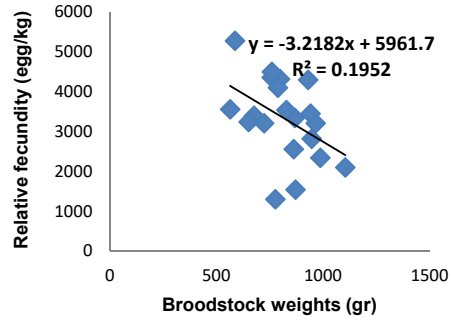

Fig. 2 Relative fecundity-Broodstock Weight

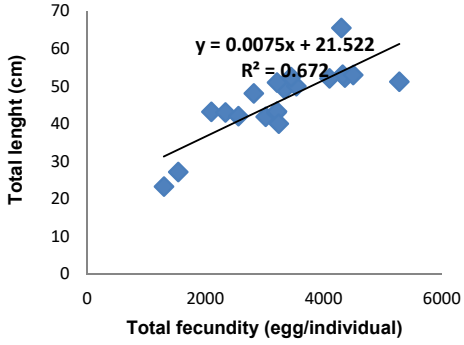

Fig. 3 Total fecundity-total lenght

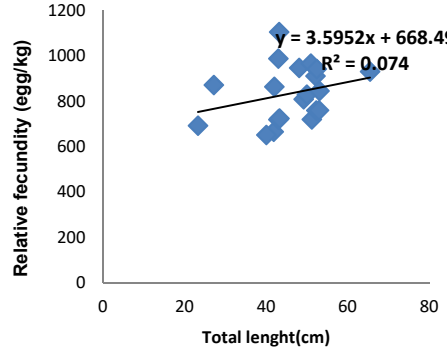

Fig. 4 Relative fecundity-total lenght

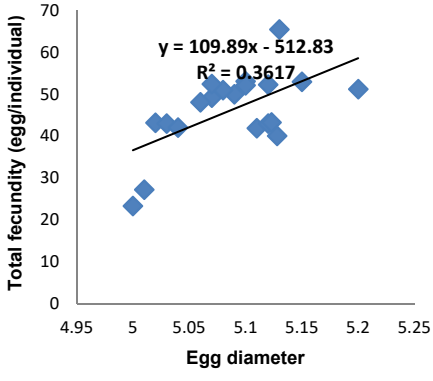

Fig. 5 Total fecundity-Egg diameter

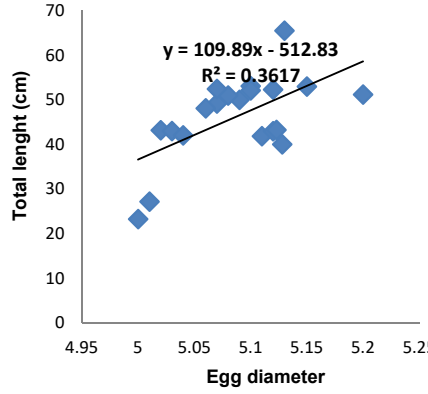

Fig. 6 Total lenght-egg diameter

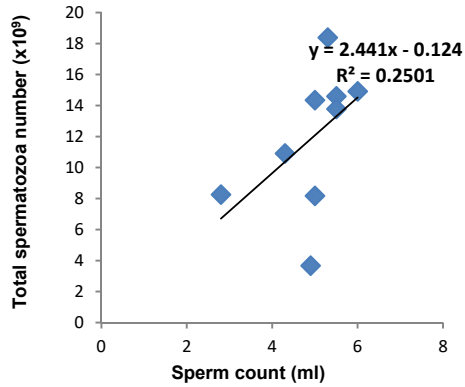

Fig. 7 Total spermatozoa number (x10^9) - Sperm count (ml)

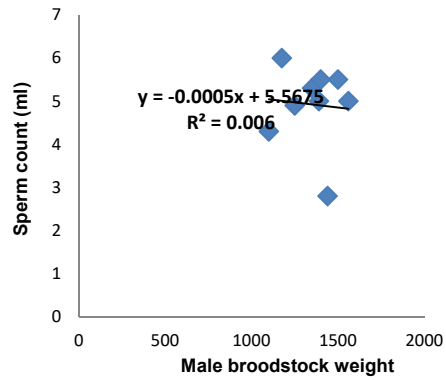

Fig. 8 Sperm count (ml)-Male broodstock weight

The effects of actifish solution, classic method, isotonic solution and $NaHCO_3$ solutions on fertilization and eyeing were compared in the test (Table 1). As a result of the measurements, the fertilization rates were found to be 95.01±0.47% in the actifish solution; 92.55±0.24 % in the classic method; 94.69±0.12 % in the isotonic solution and 90.72±2.53 % in the $NaHCO_3$ solution. It was determined that there was a statistical difference between diluting actifish method and other fertilization methods which were used ($p \leq 0.05$).

Eyeing rates as a result of the measurements conducted after the eggs became eyed are given in Table 1. As a result of the measurements, the eyeing rates were found to be 87.71±0.36% in the actifish solution; 81.57±1.75% in the ısotonic solution; 69.94±0.12 % in the classical method and 58.53±5.74% in the $NaHCO_3$ solution. It was determined that there was a statistical difference between diluting solution and other fertilization methods which were used ($p \leq 0.05$).

Table 1 n= 3 average+standard error. There is a statistically significant difference among groups indicated with different exponentials in the same column (P<0.05)

Egg (count)	Fertilization Methods	Fertilization rates (%)	Group Rates (%)	Eyeing Rates (%)	Group Rates (%)
4442	Actifish solution	94.55	95.01±0.47^a	87.80	87.71±0.36^a
4442		95.49		87.30	
4442		95.00		88.02	
4442	Classical method	92.34	92.55±0.24^{ab}	67.31	69.94±2.48^b
4442		92.81		72.26	
4442		92.50		70.24	
4442	Isotonic solution	94.75	94.69±0.12^a	81.04	81.57±1.75^a
4442		94.55		80.14	
4442		94.77		83.52	
4442	$NaHCO_3$ solution	92.30	90.72±2.53^b	65.06	58.53±5.74^c
4442		92.07		56.28	
4442		87.79		54.25	

Being closely related, the water quality, feed amount and quality, broodstock age and weight are the most important ones among the factors affecting the egg productivity in trout [5,6]. Fecundity and egg diameter are the most used two criteria in egg production. As the weight of the broodstock fish increases, the fecundity and egg diameter increase [7]. In this study, total egg fecundity of 2-year-old rainbow trout average live weights of which are 3286.5±1015.35 was determined as averagely 2719.7±827.711 in

number. Total egg fecundity values were found to be higher than that of [8] and [9] and lower than that of [10]. Relative egg fecundity is 834.757±123 in number per kg. (651- 1105) and is lower than the change limits (1500-2000) of values by [8]. Relative egg amount reduces with the increase of broodstock weight and egg diameter [5]. The egg diameter determined in the study is 5.08±0.05 and is greater than the 4.09-4.35 mm value of [11] and 3.5-5.0 mm value of [12] and is smaller than the 5.2 mm value of [8]. Since egg productivity and diameter are largely depending on the broodstock weight, there is no direct positive relationship between them.

In this study, it was determined that there is a positive relationship between broodstock weight and total egg fecundity (R^2=0.67). Some researchers [7,5,13] found a strong correlation between these two parameters (R^2=0.45-0.71) while [8] found quite a weak relationship due to reasons such as the use of the broodstock for the first time, care and feeding, uncertain time of stripping (R^2=0.24).

Average weight of the male broodstock used in the study is 1322.5±169.46 g. Total amount of sperm (milk) stripped from the male broodstock is 50.3 ml. [14] reported that the fertilization in salmon fish is monospermic and that there is 10–25x10^9 spermatozoa in a millilitre of milk at a volume of 3.5-20 mL taken from a male broodstock at a time under normal circumstances. [15] observed that the sperm density varied between 9.2-14.1x10^9 ml in the tests they carried out on sperm compositions of rainbow trout, brown trout and Atlantic salmons. While [16] found 6.54-11.36x10^9 /ml sperm cell in the first year in tests on rainbow trout, they obtained 6.14-7.72x10^9 /ml spermatozoid in the second year from the same broodstock. While[17] state the spermatozoa density in trout as 9-26x10^9 spermatozoa/ml[18]states it as 3-10 x 10^9. In the study [9] conducted on the effect of sperm and egg quality of rainbow trout (*Oncorhynchus mykiss*) on the number of eggs, they determined the spermatozoid density to be averagely 11.43±0.58x10^9/ml. [19] determined the spermatozoid cell amount to be 8.9±3.8x10^9 in number per millilitre. In the study [20] conducted on sperm cryopreservation of rainbow trout, they determined the spermatozoa density to be 12.71±6.37x10^9 spermatozoa/ml and determined that there is a negative correlation between sperm amount and spermatozoa density ($p<0.05$). [21] reported in his study that this value varies between 1.1 and 4.4x10^9/ml.

In this study, research was conducted in order to find solutions for problems encountered in the fertilization of eggs in trout which has the biggest production rate (57%) in water products farming in Turkey. Although trout has the highest tonnage in terms of the annual production amount, it is not possible for this number to significantly rise due to the unforeseen losses in production. One of the important matters in trout farming is fertilizing the eggs taken from female broodstock at a high rate and putting the eggs to good use. When compared to a carp species which spawns 100.000 to 200.000 eggs per kg of its body weight, a female trout broodstock spawns 500 to 2000 eggs in number per kg of its body weight [7].Researches show that the fecundity of female trout is lower than other fish and therefore the eggs obtained in the hatcheries should be put to good use. The best method in the fertilization of the eggs taken from the female broodstock in hatcheries should be determined having regard to the current fertilization success and a production plan should be prepared in line with the protocol of the fertilization method. Of the fertilization methods used in this study which are classic method (CM), isotonic solution (IS), $NaHCO_3$ solution and actifish solution (AS) methods, the actifish solution (AS) method in which the fertilization rate is highest is determined to be the method in which the eggs are best used ($p<0.05$). When fertilization and eyeing results are analysed, it is seen that there is less loss in practices with actifish solution than with isotonic solution, classic method and $NaHCO_3$ solutions. As a result, there are many advantages of preferring actifish solution (AS) method in artificial fertilization rather than other methods. These advantages have

importance in terms of the used male and female broodstock amounts, egg and sperm amounts, fish stocking areas and used labour force amount. It is expected to popularize the use of actifish solution method in production because of the gains all these advantages will earn both Turkey and a commercial enterprise which produces trout.

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