Effect of age on reproductive efficiency of adult rainbow trout, *Oncorhynchus mykiss* Walbaum, 1972

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Abstract

Rainbow trout, *Oncorhynchus mykiss* is one of the most important cold water fishes in Iran. For successful artificial propagation of rainbow trout, the quality and quantity of brooders and sexual materials are key factors. In the present study, we investigated the age-dependent changes of reproductive efficiency. For this purpose, the brooders were divided into age classes and then three age groups of male and female (i.e. 3, 4 and 5 years old) selected randomly from each age classes. At the time of propagation, total length and weight of brooders before and after stripping, egg diameter, total weight of stripped eggs and sperm quality parameters including sperm density and spermatocrit were measured. Afterward, the brooders were crossed randomly. According to our results, the higher percentage of fertilization (%98), hatching (%96), survival (%94.5) and also the larvae with more active feeding (n= 3070) were found when the 4 year old males were crossed with 5 year old females (p<0.05). Our results confirmed the age-dependent changes of reproductive efficiency in rainbow trout. We conclude that the cross between 4 year old males and 5 year old females of rainbow trout can enhance the reproductive efficiency.

Keywords: Survival, Fertility rate, Fertilization, Rainbow trout

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Introduction

Salmonids are the most important cultured fish species throughout the world and their culture has become commonplace among countries during centuries (Lee and Donaldson, 2001). Among salmonids, the culture of rainbow trout is more widespread due to the meat quality, sport fishing and simple method of culture and propagation. At present, it is well recognized that the use of high quality gametes from fish broodstocks is of great importance for ensuring the production of viable larvae for aquaculture (Kjorsvik *et al*., 1990; Billard *et al*., 1995; Yaron, 1995). Usually, the sperm motility, sperm concentration, egg diameter, fertilization and hatching rate are used as indices of gamete quality. A few studies showed that fish age influences quality of gametes and survival rate of larvae. In rainbow trout, older and weighty females produced eggs with higher size compared to small and younger individuals (Gall, 1974). In captive striped Bass *Morone saxatilis*, the 3 year old fish produced the greatest number of spermatozoa, sperm concentration and spermatocrit compared to the 1 and 12 year old fish. In addition, during short-term storage at 4 ºC, extender-preserved sperm samples of the 3 year old group showed higher percentage of motile spermatozoa and duration of sperm motility, compared to the other two groups (Vuthiphandchai and Zohar, 1999). Büyükhatipoglu and Holtz (1984) observed that second-season spawners of rainbow trout produced milt with better quality than first-season spawners in terms of milt volume and sperm concentration. In captive striped Bass (*M. saxatilis*), 3-year-old fish had higher sperm quality than 1-or 12-year-old fish, based on higher sperm production and increased sperm longevity during short-term storage. However, the fertilizing capacity of virgin and repeat spawners was comparable in Atlantic cod (*Gadus morhua*) (Trippel and Neilson, 1992). Positive correlations were found between milt volume and body size (weight and length) in Atlantic salmon (*Salmo salar*) and rainbow trout (Gjerde, 1984).

Rainbow trout brooders with different ages are held nowadays in Iran that is very costly for fish farmers. Therefore, removal of brooders with low efficiency is necessary. The aim of the present study is determination of age-dependent changes of reproductive efficiency of rainbow trout. For this purpose, the effect of brooder's age on fertilization, egg eyeing and hatching percent and also the survival percent of alevins were evaluated.

Materials and methods

Fish

The experiment was carried out during the spawning season at the Kalardasht Salmonids Reproduction Centre, Iran. Totally, 60 adult males and females of rainbow trout in three age classes including 3, 4 and 5 year old fish were selected randomly from brooder's ponds (each age class composed of 10 females and 10 males). To identify mature fish, the brooders were checked every other days. Before stripping, the fish were anaesthetized using 200 ppm of clove extract (Hajirezaee *et al*., 2010a) and then their total weight and length were measured.

Sperm quality parameters assessment

The collection of semen and egg samples was carried applying massage from the anterior portion of the belly (testis or ovary region) towards the genital papilla. Special care was taken to avoid the contamination of semen and eggs by water, mucus, blood cells, faeces or urine. The semen volume was measured using scaled vials. The sperm density and spermatocrit were measured according to Hajirezaee *et al*. (2010 a, b). For spermatocrit assay, microhaematocrit capillary tubes (75 mm in length and 1.1–1.2 mm in diameter) were filled with semen and one end of each tube was sealed with clay. The capillary tubes were centrifuged at 5000 rpm for 10 min in a centrifuge. The spermatocrit is defined as the ratio of white packed material volume to the total volume of semen × 100. For sperm density assay, semen was diluted 1000 times by pipetting 10 µl semen in 990 µl of 0.7% NaCl. A haemocytometer counting chamber was used to determine the spermatozoa density. A droplet of the diluted milt was placed in a haemocytometer slide (depth 0.1 mm) with a coverslip and sperms were counted using light microscopy. After 3–5 min (to allow sperm sedimentation), the number of spermatozoa was counted in 16 individual cells, then calculated according to Caille *et al*. (2006). After the weighting of stripped eggs and brooders, the eggs and semen samples from each age class were pooled separately in order to minimize variations in gamete quality. The egg diameter was measured by caliper. A little amount of milt was allocated for analysis of sperm density and sperm motility and the remainder was used for fertilization.

Fertilization assay

The pooled eggs from each age class were distributed equally to three trays with three replicates (altogether 9 trays). Afterwards, the pooled semen samples were added equally to trays containing pooled eggs and then mixed for three min. In this regard, nine states (or 9 treatments) were considered as follows: T_1: 3 year old males *Vs.* 3 year old females; $T_{2:}$ 3 year old males *Vs.* 4 year old females; $T_{3:}$ 3 year old males *Vs.* 5 year old females; $T_{4:}$ 4 years old male *V* . 3 years old female; $T_{5:}$ 4 year old males *Vs.* 4 year old females; $T_{6:}$ 4 years old male *Vs.* 5 years old female; $T_{7:}$ 5 year old males *Vs.* 3 year old females; $T_{8:}$ 5 years old male *Vs.* 4 years old female; $T_{9:}$ 5 year old males *Vs.* 5 year old females

Twenty four hours after fertilization, a batch of 50 eggs from each tray was fixed in formaldehyde (%5) + Acetic acid (%4) solution and then fertilization

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percent was calculated according to below formula:

% Fertilization= (total number of fertilized eggs / total number of eggs) × 100.

The eggs were treated with malachitegreen (1 g/L) every other day for a period of 45-60 min to avoid the fungal pollution during incubation period. After 19 days from incubation, the eyeing eggs were discerned from dead eggs by shocking (Aas *et al*., 1991). For this, the eggs were poured into a tray from a height of 20 cm. In such situation, the dead eggs became white but the eyeing eggs did not show any color change. The eyeing percent was calculated as follows:

% Eyeing = (Total number of eyeing eggs/ total number of fertilized eggs) × 100.

Then, 30-35 days after fertilization, the alevins were hatched. The hatching percent was calculated according to the following formula:

% Hatching percent = (Total number of alevins / Total number of eyeing eggs) × 100.

When the alevins absorbed approximately two third of yolk sac (50 days after fertilization), the survival percent was calculated as follows:

Survival percent= (Total number of alevins / Total number of alevins) × 100.

Data analysis

The SPSS software was used for data analysis. As the percentage data (% fertilization, % eyeing, % hatching and % survival) did not have a normal distribution, proportional data were converted by angular transformation (arcsin VP). One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different. Significant level of 0.05 was considered for comparing averages.

Results

The mean of the some properties of rainbow trout brooders has been shown in Tables 1 and 2.

The highest and lowest fertilization percent were observed in males (4 years old) × females (5 years old) and males (3 years old) × females (4 years old), respectively (p<0.05) (Fig.1). Also, the highest and lowest egg survival percent until eyeing stage were observed in males (4 years old) × females (5 years old) and (male 4 × female 3), respectively (p<0.05) (Fig. 2). The highest and lowest hatching percent were found in males (4 years old) × females (5 years old) and males (5 years old) × females (4 years old) (p<0.05) (Fig. 3). Similar to egg survival percent, the highest values of the emerged alevin number was found in males (4 years old) × females (5 years old) while the lowest value was observed in males (4 years old) × females (3 years old) (p<0.05) (Fig. 4). The highest and lowest survival percent of alevins until entire absorption of yolk sac were observed in males (4 years old) × females (5 years old) and males (4 years old) × females (4 years old), respectively (p<0.05) (Fig. 5). The number of larvae with active feeding was maximum in males (4 years old) × females (5 years old) and was minimum in males (4 years old) × females (4 years old) (p<0.05) (Fig. 6).

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Table 1: The mean±SD of some properties of rainbow trout males.

Fish age	Brooder's weight before stripping (g)	Brooder's weight after stripping (g)	Total length (cm)	Semen volume (mL)	Sperm density (10^9 spz/mL)	Spermatocrit (%)
3	896.3±87.37	873.67±84.32	41.67±87.37	15.93±2.81	13.5 ±7.77	60.3±12.069 [a]
4	1260±324.95	1229±315.35	45.6 ±2.76	23.12±8.71	7.43±3.70	39.6 ±12.19 [b]
5	1396±304.69	1355.33±298.78	49.4 ±2.59	31.87±24.35	5.71 ±2.73	36.3±16.39 [b]

The values with different letters in the figure are significantly different (p<0.05).

Table 2: The mean±SD of some properties of rainbow trout females.

Fish age	Brooder's weight before stripping (g)	Brooder's weight after stripping (g)	Total length (cm)	Weight of eggs (g)	Egg diameter (mm)	Number of eggs/g	Absolute fecundity
3	7611±114.6 [a]	624.6±71.4 [a]	40.33 ±1.1 [a]	114. 3±31.5	4.4 ±0.21 [a]	14.4±0.31 [a]	479.7±1737.8 [a]
4	1532±125.1 [b]	1271±112.6 [b]	47.7±0.81 [b]	206.67±27.7	4.9±0.1 [b]	11.9±0.21 [b]	389±2893.3 [b]
5	1764±159.8 [b]	1443.6±120.3[b]	53.03±15.6[b]	251±50.4	4.7 ±0.17 [b]	12.7±0.98 [b]	696±536.6 [c]

The values with different letters in the figure are significantly different (p<0.05).

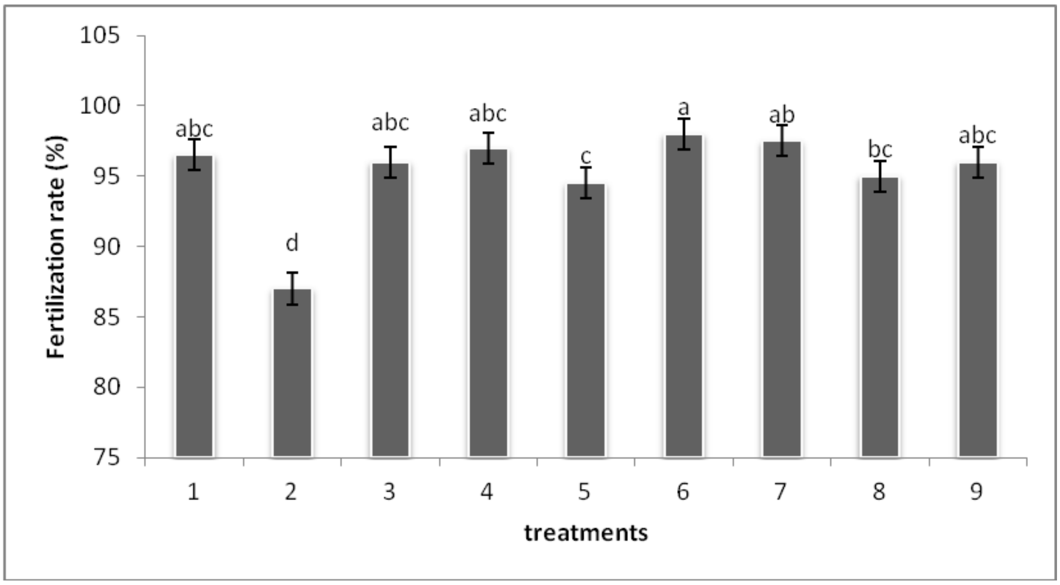

Figure 1: Fertilization percent in experimental treatments (Mean±Sd). Graph1: 3 year old males *Vs.* 3 year old females; 2: 3 year old males *Vs.* 4 year old females; 3: 3 year old males *Vs.* 5 year old females; 4: 4 years old male *V* . 3 years old female; 5: 4 year old males *Vs.* 4 year old females; 6: 4 years old male *Vs.* 5 years old female; 7: 5 year old males *Vs.* 3 year old females; 8: 5 years old male *Vs.* 4 years old female; 9: 5 year old males *Vs.* 5 year old females (Different letters on the graph indicate significant differences within groups (*p*<0.05)).

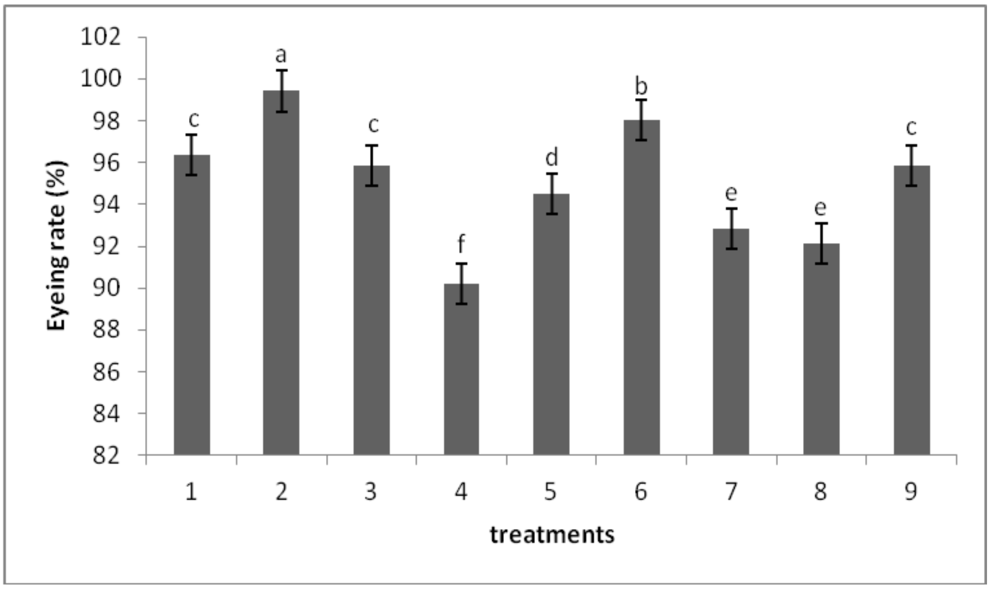

Figure 2: Eyeing percent in experimental treatments (Mean±Sd). (Different letters on the graph indicate significant differences within groups (p<0.05)).

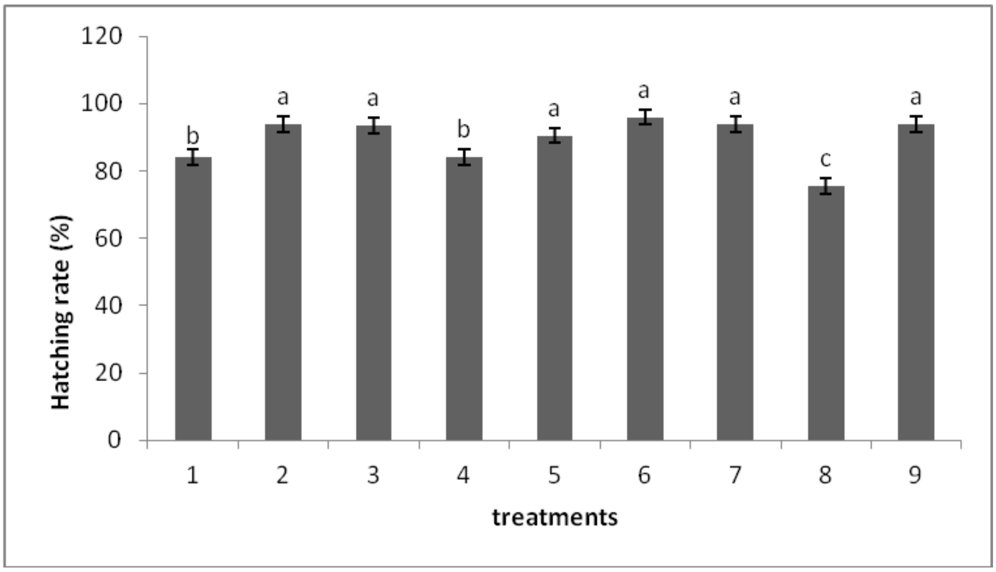

Figure 3: Hatching percent of eggs in experimental treatments (Mean±Sd). (Different letters on the graph indicate significant differences within groups (p<0.05)).

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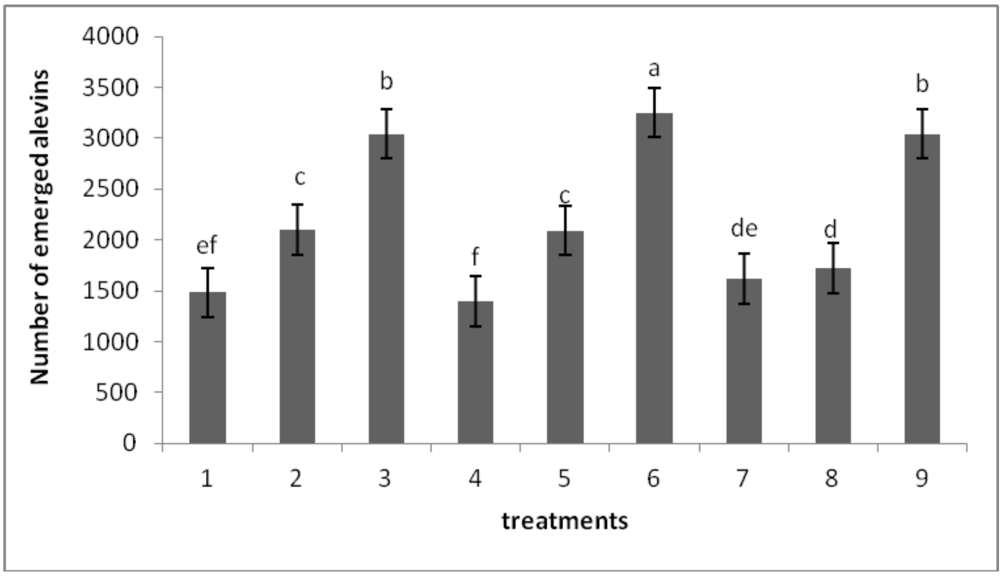

Figure 4: The number of emerged alevins in experimental treatments (Mean±Sd). (Different letters on the graph indicate significant differences within groups (*p*<0.05)).

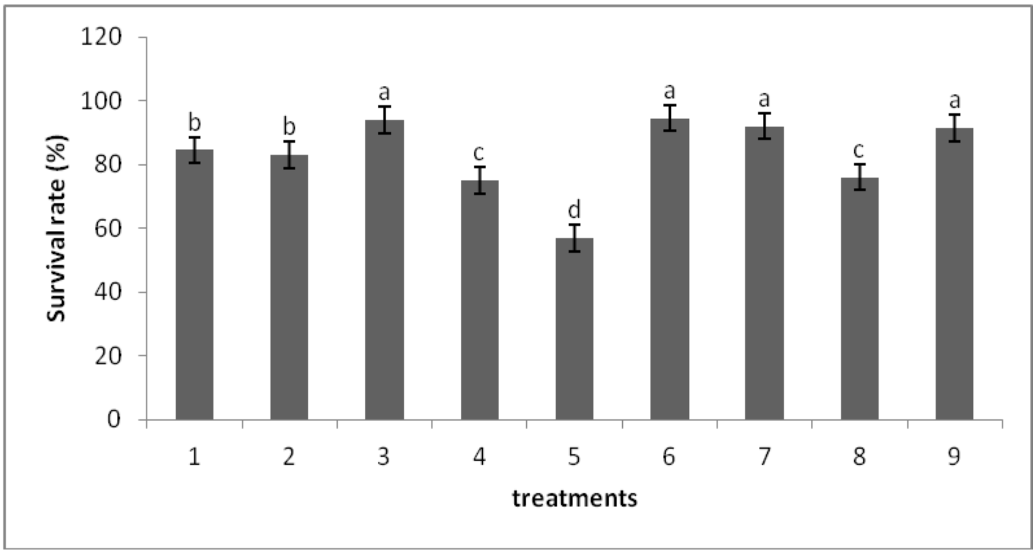

Figure 5: The survival percent of alevins until entire absorption of yolk sac in experimental treatments (Mean±Sd). (Different letters on graph indicate significant differences within groups (*p*<0.05)).

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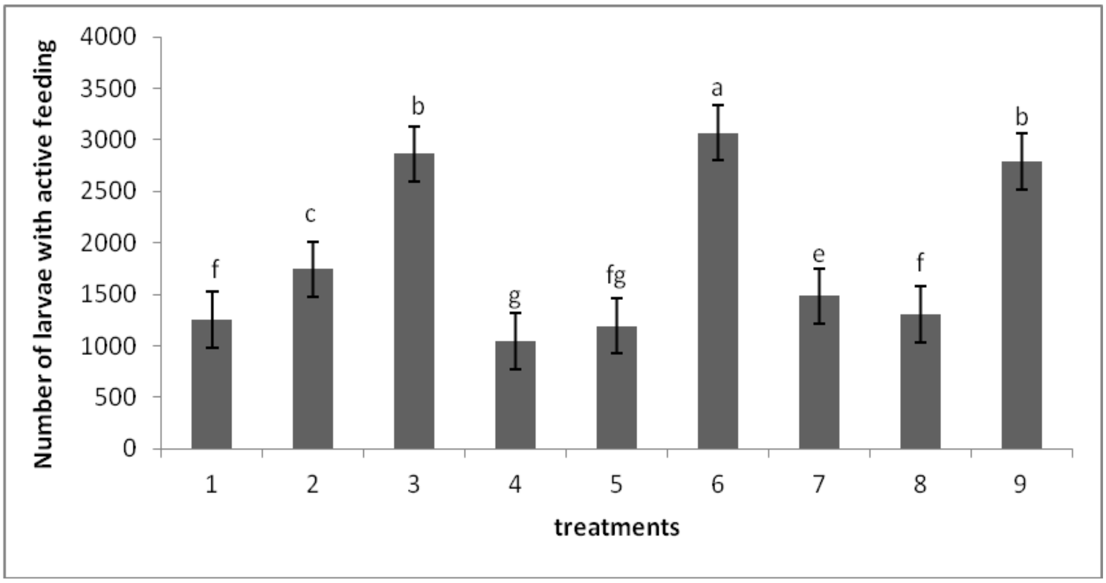

Figure 6: The number of larvae with active feeding in experimental treatments (Mean±Sd). (Different letters on graph indicate significant differences within groups (*p*<0.05)).

Discussion

Since the healthy larvae are obtained from healthy gametes, therefore, any investigation of effect of brooder's age on reproductive efficiency and survival rate of obtained larvae involves consideration of gamete quality (Kjorsvik *et al*., 1990). According to our results, 4 and 5 year old females produced eggs with more diameters compared to 3 year old females. Also, the absolute fecundity was higher in older females than in younger individuals. These results are similar to Pitman (1979) where 5 year old females of rainbow trout produced eggs with more diameter and hatching rate and also larvae with high growth rate and low mortality. The positive relationship between brooder's weight and fecundity has been confirmed previously in wild and cultured stocks of Atlantic salmons, *Salmo salar* (Thorpe *et al*., 1984; Brannas *et al*., 1985; Kallio, 1986; Eskelinen and Ruohonen, 1989; Eskinaro *et al*., 1997). Also, similar results have been found in other salmons (Bagenal, 1969). The egg quality and especially egg diameter and weight have positive impact on fertilization rate and improvement of egg incubation. In the present study, the 5 year old females had more fecundity compared to 3 and 4 year old females. On the other hand, the 5 year old females produced also bigger eggs and more larvae with higher survival rate. Therefore, this result suggests that egg size have a positive relationship with egg and larval quality during incubation period. In Siberian sturgeon, *Acipenser baeri,* a positive relationship was found between egg size and brooder's size and weight and also size of hatched alevins

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(Gisbert *et al*., 1999). Additionally, similar results have been reported for Chum salmon, *Oncorhynchus keta*, Chinook salmon (Flower, 1972, Beacham and Murray, 1985), Caspian brown trout (Rahbar and Khara, 2015), Kutum, *Rutilus frisi*i (Fallah Shamsi and Khara, 2015) and Shemaya (Rahbar *et al*., 2013). In our study, the values of egg numbers per gram of eggs were higher in 3 year old females compared to 4 and 5 year old individuals. This could be due to the smaller size of eggs in these females than in 4 and 5 year old females. In 3 year old males, the values of spermatocrit and sperm density were higher than in 4 and 5 year old individuals. Similar results were observed in sockeye salmon, *O. nerka* and Atlantic salmon where the spermatocrit values were higher in 3 year old males than in other age classes (Daye and Glebe, 1984; Hoysak and Liley, 2001). Nevertheless, the highest and lowest values of semen volume and sperm density were observed in 5 year old males. Tekin *et al*. (2003) reported the positive relationship of semen volume with length, weight and age of brooders, although this relationship was negative between semen volume and sperm density. In the present study, the highest percentage of fertilization, eyeing and hatching was obtained when the 4 year old males were used for insemination as the maximum values of hatching rate was observed previously in 4 year old males of rainbow trout by Lorestani (2004). Also, a positive correlation was found between sperm density and spermatocrit as reported previously by Rakitin *et al*. (1999). Thus, such relationship suggests that the evaluation of sperm quantity by spermatocrit determination is better than spermatozoa counting method in terms of facility and time.

A few studies showed that the sperm motility in rainbow trout decreased as the sperm density increased and vice versa (Liley *et al*., 2002; Tekin *et al*., 2003). It is likely that with increasing of sperm density, more ATP stores are used for motility and thus the motility duration decreases. In other words, when the sperm density is high, the ATP stores are distributed between more numbers of spermatozoa. In such situation, it is obvious that the quota of each sperm from ATP decreases. Our study confirms the effect of age on sperm’s motility duration and spermatocrit. According to previous studies, the duration of 30 seconds has been measured for total duration of sperm motility in rainbow trout (Liley *et al*., 2002; Rurangwa *et al*., 2004).

In conclusion, we crossed different age classes of adult rainbow trout to identify the best age with maximum reproductive efficiency. Our result showed that the 5 year old females *Vs.* 4 year old males have more reproductive efficiency.

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