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Effect of male age in Caspian brown trout, *Salmo trutta* broodstock on reproductive performance

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Abstract

In this study, three different age groups of male broodstocks (4, 5 and 6 years respectively) were used to fertilize eggs obtained from nine females. The results showed that 6 year old males had maximum body weight (1766 g), total length (56.3 cm) and sperm volume (31.83 ml). Results did not show significant difference in spermatocrit and spermatozoa concentration among age groups (p>0.05). Our study showed maximum fertilization rate (98.5 %), survival rate until eyes pigmentation (91.17 %), hatching rate (94.5 %) and survival rate until absorption of yolk sack (97.16 %) for 4 years treatment group. Such positive relationships were detected between sperm production characteristics (spermatozoa concentration, spermatocrit and sperm volume) and fertilization parameters. Based on our results, it can be concluded that 4 year old males have high efficiency leading to fertilization success.

Keywords: Age, Male, Artificial insemination, *Salmo trutta*

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Introduction

The Caspian brown trout, *S. trutta*, is a vulnerable anadromous fish, list as conservation species in the southern part of Caspian Sea (Kiabi *et al*., 1999; Niksirat and Abdoli, 2009). Since 1999, the International Union for Conservation of Nature (IUCN) registered this fish as a species in danger of extinction, so the Iranian Fisheries Organization has conducted artificial reproduction of this species. This organization is also growing juveniles to the smolt level and releasing them into the sea (Mojazi *et al*., 2005). The Caspian brown trout enters the northern parts such as Shirood and Cheshme Kileh Rivers for spawning. The use of high quality gametes from captive fish broodstock is of great importance for ensuring the production of viable larvae (Kjorsvik *et al*., 1990). Semen quality is a key factor in fertilization success (Gage *et al*., 1995; Stockley *et al*., 1997). Techniques used to assess sperm quality in fish include monitoring sperm density and motility and fertilization success (Aas *et al*., 1991; Tekin *et al*., 2003). This success may depend on the timing and position of sperm release, ability of the sperm to compete with other males and the amount of sperm released (Gage *et al*., 1995). The sperm to eggs ratio, fertilization technique (Chereguini *et al*., 1999) and size and age of males (Chechun *et al*., 1994) are important factors that have been evaluated for maximizing fertilization rate. Liley *et al*., (2002) studied effect of male broodstocks age, social experience and spermatozoa concentration and motility on *in vitro* fertilization parameters of rainbow trout (*Oncorhynchus mykiss)*. Also, spermatological traits of rainbow trout were investigated at different age classes (Tekin *et al*., 2003). Another research about influence of different male ages on sperm characteristics and fertilization capacity was carried out by Lorestany *et al*. (2006) in rainbow trout and Alinya *et al*. (2013) in common carp (*Cyprinus carpio*). Other studies were carried out on the reproductive performance, sex ratio, growth and survival rate of rainbow trout in 3 to 5 year-old males, respectively. The aim of the present study was to investigate the effect of broodstock age on gamete quality, fertilization and survival rates of *S.trutta*.

Materials and Methods

Broodfish

The experiment was carried out at the Kalardasht Salmonids Reproduction Center (KSRC), Iran, during 2008-2009 spawning season (December to March). The fish for the broodstock were captured from Cheshme Kileh River during upstream migration and then transferred to KSRC. Altogether 18 matured fish were selected and transferred to hatchery (three mature males for ages 4, 5 and 6 and also 9 females). The average total weight of males were ±57.7g, 1466±46.7g and 2150±86.6g while the total length were 44.6±1.5cm, 50.3±0.5 and 56.3±2.8cm, respectively. The average total weight and total length of females were 1275±46.8 and 53.3±1.9cm, respectively. All brooders were anaesthetized in 100 ppm of MS_{222} (tricaine methane sulfonate) prior to sperm sampling. Scales of Caspian brown trout were used to determine age according to the method suggested by Heinimaa and Heinimaa (2004).

Sperm quality parameters assessment

Sperm and eggs were collected by manual stripping. The sperm was collected in graded tubes for each male. About 1.5 ml of sperm was obtained from each male separately and immediately transported to laboratory under cool condition (Alavi and Cosson, 2005) for measurement of sperm quality parameters (spermatozoa concentration and spermatocrit). The spermatocrit was defined as the ratio of volume of white packed material to the total volume of semen ×100 (Rurangwa *et al*. 2004). Microhaematocrit capillary tubes (75mm length, 1.1–1.2 mm diameter) were filled with semen and one end of each tube was sealed with clay. The capillary tubes were centrifuged at 3000 rpm for 8 min (Sigma, 13 USA). Spermatozoa concentration was measured by counting the number of spermatozoa in a sample diluted with D sodium bicarbonate (5g Na_2HCO_3, 10 cc Formalin, 100 cc water) in a hemocytometer, under X 400 magnification (Suquet *et al*., 1992).

Fertilization assay

After stripping, the pooled eggs from each female (three samples) were distributed equally to plastic dishes. Afterward, semen samples obtained from each age (three samples) were added equally to dishes containing pooled eggs and then mixed. After hardness (for 45 min), the fertilized eggs were delivered to the hatchery. For prevention of disorder, each treatment was divided into three parts and was set in three trays; comprising nine trays. The hatchery trays were covered with a red plastic plate to protect the eggs from sun light. These trays were placed in incubators with cold running water (8°C) until fertilization (6-7 days), eyeing (14-15 days), hatching (30-35 days) and absorption of yolk sack stage (55-60 days). The trials were carried out in three replicates to verify the findings. The fertilization rate was determined according to Bromage and Cumaranatunga (1998) method. About 80 eggs were randomly sampled from each tray and then fixed in formalin solution (5 ml formalin 40% + 45 ml water). Then, eggs were evaluated under a Stereomicroscope (Meiji EMZ-1). The eggs with visible nervous cord in the back of larval body were considered as fertilized eggs and others without such trait were considered as unfertilized eggs. The eyeing stage was determined according to procedure of Aas *et al*. (1991). Eyeing rate defined as the number of eyed eggs divided by the initial number of eggs used for fertilization. The embryo reached to the hatching and absorption of yolk sack stage was recorded as index of fertility (Billard and Gillet, 1981). Hatching rate was defined as the number of hatched larvae divided by the initial number of eyed eggs. When larvae approximately absorbed two third of yolk sack, the amount of larvae survival until absorption of yolk sack was calculated by counting lost larvae. Healthy larvae were poured to trays for manual nutrition (Billard and Gillet, 1981).

Data analysis

Mann Whitney test was used for normality of data distribution and homogeneity of variance. One-way ANOVA was employed to analyze the data and then were compared by Duncan test. The level of significance was considered at $p<0.05$. Then, the linear and non-linear regression models were

investigated using regression fits. Sperm traits were used as independent variables and the parameters of seminal fluid as dependent. All statistical analyses were performed using the statistical program SPSS 13. Data are presented as mean±SD.

Results

Sperm quality parameters of three age groups are shown in Table 1. The body weight, total length and sperm volume (p<0.05) increased significantly with increasing of age. As age of the males increased, so did the sperm volume, although gradually (Table1). The relationships between sperm traits and fertilization parameters are depicted in Figs. 1-5, respectively. There was significant positive relationships between the spermatozoa concentration and spermatocrit with fertilization rate (Figs. 1 and 2). Maximum spermatozoa concentration (17.6±7.77) and spermatocrit (38.0 ± 10.44) were observed for the 4 year old class. However, spermatozoa concentration and spermatocrit did not show significant change among ages. The maximum fertilization rate (98.5 %) was observed for the 6 year old males (p<0.05). Maximum eyeing, hatching and survival rates until absorption of yolk sack was found to be 91.17%, 94.5% and 97.16%, respectively which related to the 4 year-old males (Table 2, p<0.05).

Table 1: The mean biometric and sperm production characteristics of the male broodstocks of Caspian brown trout.

	Age of brooders		
Parameters	**4-year**	**5-years**	**5-years**
weight (g)	866.67 ± 57.74 ^{b}	1100 ± 0.0 ^{b}	1766.67 ± 152.75 ^{a}
Total length (cm)	1.53± 44.67 ^{c}	0.58 ± 50.33 ^{b}	2.08 ± 56.33 ^{a}
sperm volume (ml)	1.71 ± 17.74 ^{b}	2.87 ± 22.31 ^{ab}	6.22 ± 31.83 ^{a}
spermatozoa concentration (10^9 cell/ml)	7.77 ± 17.6	19.6 ± 8.14	27.7 ± 16.16
Spermatocrit (%)	10.44 ± 38.0	10.12 ± 28.67	17.01 ± 35.33

Values marked with a similar letter are not significantly different at p<0.05.

Table 2: Fertilization parameters in different age groups in Caspian brown trout.

	Age of brooders		
Parameters	**4-years**	**5-years**	**5-years**
Fertilization rate (%)	0.58 ±5.98 ^{a}	0.50± 96.66 ^{b}	0.50± 98.5 ^{a}
Eyeing (%)	91.17 ± 1.75 ^{a}	87.33 ± 0.29 ^{b}	91.16 ± 1.80 ^{a}
Hatching (%)	94.5 ± 1.5 ^{a}	86.33 ± 0.76 ^{c}	91.66 ± 0.28 ^{b}
Survival rate until absorption of yolk sack stage (%)	97.16 ± 0.77 ^{a}	92.33 ± 1.52 ^{c}	95.83 ± 0.28 ^{b}

Values marked with a similar letter are not significantly different at p<0.05.

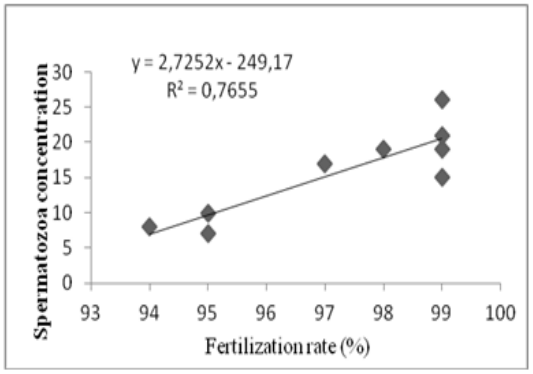

Figure 1: Correlation between spermatozoa concentration (× 10^9/mL) and fertilization rate in Caspian brown trout.

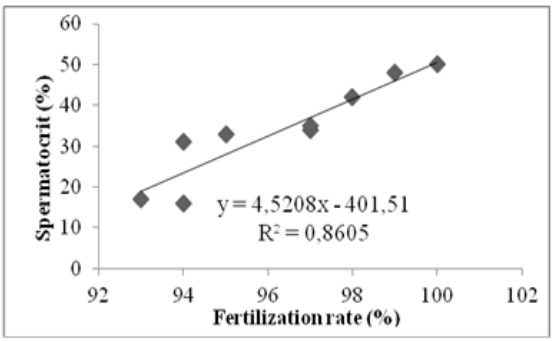

Figure 2: Correlation between spermatocrit and fertilization rate in Caspian brown trout.

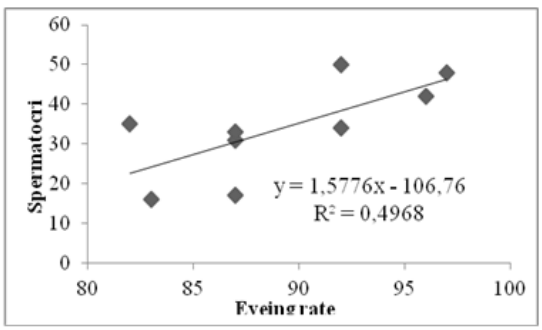

Figure 3: Relationship between spermatocrit and eyeing rate in Caspian brown trout.

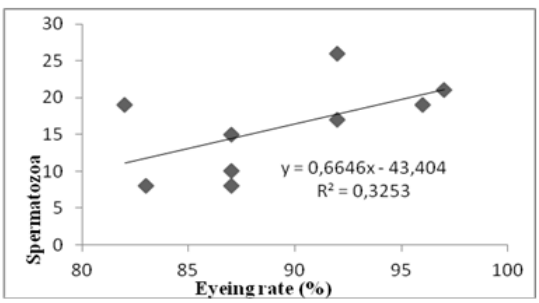

Figure 4: Relationship between spermatozoa concentration and eyeing rate in Caspian brown trout.

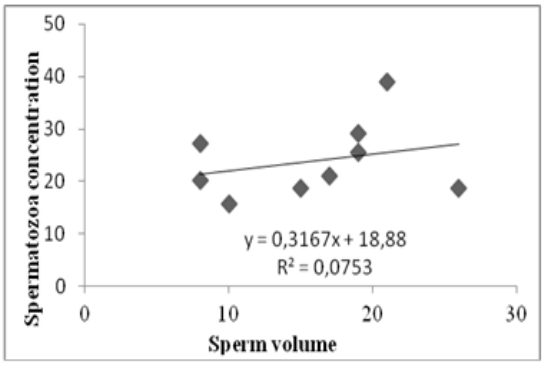

Figure 5: Relationship between spermatozoa concentration and sperm volume in Caspian brown trout.

Discussion

In various fish species, sperm volume is different (Moczarski, 1976), influenced by sequential stripping, age, weigh and strain (Ingermann *et al*., 2002). In the present study, 6 year old males had more sperm volume then 4 and 5 year-old fish. These results are in agreement with reports on rainbow trout (*O. mykiss*) (Shamspour, 2008). Tekin *et al*., (2003) reported such positive relationship between sperm volume and age as well as length and weight of fish. In this study, 4 year old males had higher spermatocrit and spermatozoa concentration than 5 and 6 year-old fish. Some authors confirmed that increase in male ages leads to decrease in spermatocrit value (Liley *et al*., 2002; Tekin *et al*., 2003). Also, in sockeye salmon (*O. nerka*) similar findings were observed between male age and spermatocrit (Hoysak and Liley, 2001). In rainbow trout and sockeye salmon it has been demonstrated that older males have lower spermatozoa concentration (Hoysak and Liley, 2001; Liley *et al*., 2002; Tekin *et al*., 2003; Lorestany *et al*., 2006). Our findings showed a positive relationship between spermatocrit and spermatozoa

concentration (Fig. 5). These results are in agreement with reports on rainbow trout, *O. mykiss* (Lorestany *et al*., 2006; Shamspour, 2008), Atlantic cod, *Gadus morhua* (Rakitin *et al*., 1999) and Atlantic halibut, *Hippoglossus hippoglossus* (Tvedt *et al*., 2001). Some authors confirmed that spermatocrit decreased with increasing male broodstocks age (Liley *et al*., 2002; Tekin *et al*., 2003). In our experiment, spermatozoa concentration decreased with age. An explanation for this is that sperm volume increased in larger fish and as the relationship between sperm volume and sperm density is reverse, therefore with increasing age, sperm volume will increase, but its concentration will decrease (Tekin *et al*., 2003). The maximum spermatocrit was observed in 4 year old and also maximum fertilization rate, eyeing, hatching rates were observed in this age group. When the spermatocrit decreased, fertilization rate, eyeing, and hatching rates were decreased. Several published data revealed positive correlation between fertilization success and sperm quality parameters (spermatocrit, spermatozoa concentration and sperm volume). For example, significant positive relationship was observed between spermatocrit and fertilization rate in Atlantic salmon (*S. salar*) and rainbow trout, respectively (Aas *et al*., 1991; Lorestany *et al*., 2006). Similarly, in sockeye salmon and rainbow trout significant positive correlation was reported between spermatozoa concentration and fertilization parameters (Hoysak and Liley, 2001; Liley *et al*., 2002) which is in agreement with our results. The effect of mating different age classes of broodstocks on reproductive performance, sex ratio, and growth and survival rate of rainbow trout was investigated by Kayam (2004) and Shamspour (2008). These authors noted that young male broods have greater reproductive potential than older male broods. The present study indicates that 4 year old male broodstocks have high potential in terms of sperm quality parameters for fertilization success. The results of this study can be used in artificial breeding programs to produce suitable larvae for breeding and reproduction.

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