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[Short Communication]

Effect of age on reproductive performance in female Caspian brown trout (*Salmo trutta caspious*, Kessler 1877)

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ABSTRACT

Caspian brown trout (*Salmo trutta caspius*) is one of the economically valuable species in the Caspian Sea. Artificial propagation and production of larvae are the main problems in the early culture of this species. The purpose of this paper is to study the effect of reproductive performance of female broods on opposition reproduction efficiency in Caspian brown trout in the breeding season of 2009. Three groups of female broods (4, 5 and 6 years old) were fertilized with 9 male fish individually. The results showed that, 6 year old females have maximum body weight (2150.0 ± 86.6 g), total length (59±2 cm), eggs weight (255.0 ± 30.51 g), egg size (5.37 ± 0.058 mm) and absolute fecundity rate (3060 ± 366.15), while highest average number of ovules in each gram of body weight (16.33 ± 0.58) and relative fecundity (2.08 ± 0.12) belonged to 4 years old females. There were significant differences in mean fertilization rate and survival rate until absorption of yolk sack stage ($p<0.05$) among the treatments studied. The present study showed that the eggs produced from fertilization of 6 year old female eggs and male mixed milt showed maximum average fertilization percentage (97.5 %), survival rate until eyed stage (92%), hatching percentage (93%), and survival rate until absorption of yolk sack (94.5%).

Keywords: *Age, Artificial propagation, Caspian brown trout, Female broods, Iran.*

INTRODUCTION

The Caspian brown trout (*Salmo trutta caspius*) is a Salmonid indigenous to Iranian Waters and its population in the Caspian Sea is decreasing strikingly. In 1999 the IUCN registered this fish as a species in danger of extinction, so the Iranian Fisheries Organization has been conducting artificial propagation of the brood stocks that enter rivers south of the Caspian Sea for spawning. This organization also has been growing fries to the smolt stage for the purpose of releasing them (Mojazi *et al*., 2005).

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).One of the important factors in fertilization is quality of eggs obtained by brood stocks. Most important factors on this subject are egg diameter and amount of fecundity in female broods. Other influential factor in percent and survival of larvae are broods age and weight. With increase in age, weight and with stripping, some changes will occur gradually in ovarian fluid composition and egg contained. Presumably these morphological, physiological and biochemical changes are responsible for decrease in egg quality, fertilization percent, eyeing rate, hatching, incidence of abnormality and mortality in later phases (Lahnsteiner, 2000). Gall (1974) has shown in studies of hatchery- reared trout that older and heavier females produce larger eggs than younger and smaller fish. The availability of food also affects egg size (Springate *et al*., 1985). Pitman (1979) studied influence of female brood age and egg size on growth and mortality of rainbow trout (*Oncorhynchus mykiss*). A similar study was performed by Shamspour *et al*. (2009) on 3 to 5 years old rainbow trout. Alp *et al.* (2003) studied reproduction biology in brown trout (*Salmo trutta macrostigma*) in 2 and 5 year olds. Kayam (2004) studied the effect of mating different age groups of brood stocks on the reproductive performance, sex ratio, growth and survival rate of rainbow trout. In this paper we aim to determine the relationship between age in female broods and reproduction efficiency parameters in *Salmo trutta caspious*.

MATERIALS AND METHODS

The experiment was carried out at the Kalardasht Salmonid Reproduction Center (KSRC), Iran. The brood stocks were captured from spawning regions during spawning season at 2009. The brood stock of this fish species were captured from Cheshmeh Kileh River during upstream migration, and then transferred to the brood stock pond at (KSRC). A number of nine mature females (Table 1) were selected and transferred to the hatchery for collecting genital material. All brood stocks were anaesthetized in 100 ppm of MS_{222} (tricaine methane sulfonate) for subsequent experiments. The age of the salmon was determined from scale samples taken between the adipose fin and lateral line (Heinimaa & Heinimaa 2004). Sperm and eggs were collected by manual stripping. After stripping sperm were obtained from male stocks and mixed to equal condition for all treatments. Eggs obtained from each age class were transformed to 3 divisions. Equal amount of sperm mixture of male broods was added to each class. Eggs and sperms were fertilized through dry method. After hardness, fertilized eggs were transformed in hatchery. Fecundity and egg size was evaluated in nine females. Fecundity was determined by weighing method (Bozkurt *et al.*, 2006) and egg size was determined by using a caliper (at 0.02 mm sensitivity). The relative fecundity was calculated by dividing the total egg number by the total body weight.

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) times. The fertilization rate was determined according to Bromage and Cumaranatunga (1998) where the 80 eggs were sampled randomly from each tray and then fixed in formalin solution (5 ml formalin (40%) + 45 ml water). Then, eggs were examined under a stereomicroscope (Meiji EMZ-1). The eggs with visible nervous cord in the back of the larval body were considered as fertilized eggs and others without that state were considered as unfertilized eggs. The eyeing stage was determined according to Aas *et al.* (1991). Eyeing rate was defined as the number of eyed eggs divided by the initial number of eggs used for fertilization. The embryos reaching the hatching and absorption of yolk sack stage were recorded as an index of fertility (Billard & Gillet 1981). Hatching rate was defined as the number of hatched larvae divided by the initial number of eyed eggs. When larvae absorbed about two third of yolk sack, amount of larvae survival until absorption of yolk sack was calculated by counting lost larvae. Healthy larvae were poured into trays to manual nutrition (Billard & Gillet 1981).

The SPSS software was used for data analysis. The values of age with fecundity, egg size, number of ovules in each gram of body weight and fertilization factors (fertilization, eyeing, hatching and absorption of yolk sack) were normal according to Shapiro Wilk test. One-Way Analysis of Variance (ANOVA) was employed to camper the means of fecundity, egg size, number of ovules in each gram of body weight and fertilization factors. Where significant F-ratios were calculated by ANOVA, The Tukey test was applied to identify which means was different.

RESULTS

The absolute fecundity and egg size (Table 1, $P<0.05$) as well as fertilization rate (Fig 1, $P<0.05$) and absorption of yolk sack stage (Fig 4, $P<0.05$) significantly increased with increasing female brood age. The relative fecundity and number of ovules in each gram of body weight (Fig 1, $P<0.05$) significantly decreased with increasing female brood age. There was no significant relationship between the female brood age and eyeing (Fig 2) or hatching rates (Fig 3) ($P>0.05$). Maximum mean eyeing rate was 92 ± 1.17 % (mean ± SD) and the hatching rate was 93 ± 1.41 % which belonged to 6 year old female broods.

Table 1. Fecundity, egg size and number of ovules in each gram of body weight in female Caspian brown trout

Female broods age Parameter	4 years old	5 years old	6 years old
Total length (cm)	46.67 ± 1.53[c]	50.0 ± 1[b]	59 ± 2[a]
Weight (g)	1033.33 ± 57.74[c]	1466.67± 57.74[b]	2150.0 ±86.6[a]
Absolute fecundity (eggs)	2150 ± 233.33 [b]	2296.67 ± 75.06 [b]	3060 ± 366.15 [a]
Egg size (mm)	4.83 ± 0.06 [c]	5.17 ± 0.06 [b]	5.40 ± 0.10 [a]
Number of ovules (g)	16 ± 0.58 [a]	13 ± 0 [b]	12 ± 0 [c]
Relative fecundity	2.08 ± 1.12 [a]	1.57 ± 0.01 [b]	1.42 ± 0.14 [b]

Values marked with a similar letter did not differ significantly from each other at p<0.05.

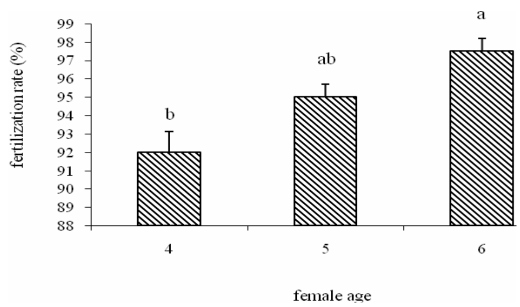

Fig 1. Relationship between fertilization rate and female broods age (P<0.05)

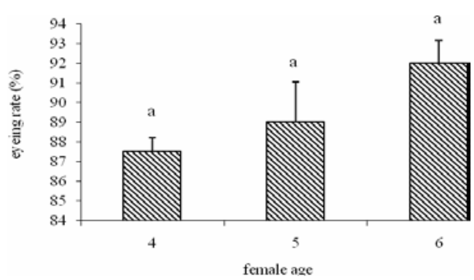

Fig 2. Relationship between eyeing rate and female broods age (P>0.05)

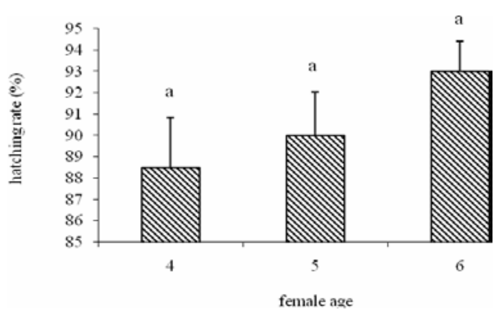

Fig 3. Relationship between hatching rate and female broods age (P>0.05)

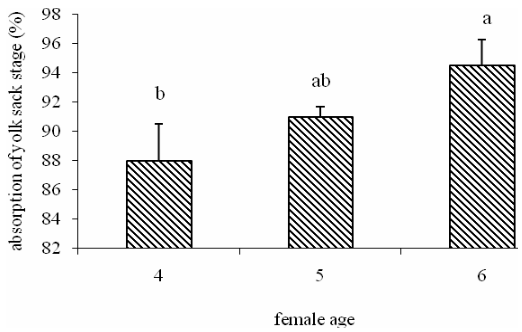

Fig 4. Relationship between absorption of yolk sack stage and female broods age (P<0.05)

DISCUSSION

In the present study 6 year old brood stock produced larger eggs then 4 and 5 year old fish and fecundity in older fish was greater. These results are in agreement with pervious reports on rainbow trout (*Oncorhynchus mykiss*) (Pitman, 1979 and Shamspour *et al*., 2009) and brown trout (*Salmo trutta macrostigma*) (Alp *et al*., 2003). The positive effect of female size on fecundity has been shown previously in wild and hatchery- reared Atlantic Salmon (Thorpe *et al*., 1984; Brannas *et al*., 1985; Eskelinen & Ruohonen 1989; Erkinaro *et al*., 1997; Heinimaa & Heinimaa 2004), in other salmonid species in many studies (Bagenal, 1969; Beacham & Murry 1985; Quinn & Bloomberg 1992; Ojanguren *et al*., 1996; Lobon-Cervia *et al*., 1997; Morita *et al*., 1999; Olofsson & Mosegaard 1999) as well as in this study.

According to the results of present study, 6 year old broods had more egg size than younger brood stocks. Other studies of other species (Alp *et al*., 2003 and Shamspour *et al*., 2009) support this finding.

Although the positive correlation between female size and egg size has been reported in many previous studies in wild Atlantic salmon (Kazakov, 1981; Thorp *et al*., 1984; Brannas *et al*., 1985; Heinimaa & Heinimaa 2004), brown trout (*Salmo trutta* L.) (Ojanguren *et al*., 1996; Lobon-Cervia *et al*., 1997; Lahnsteiner *et al*., 1999; Olofsson & Mosegaard 1999), Oncorhynchus species (Beacham & Murray 1985) and Salvelinus species (Morita *et al*., 1999), many other things also influence the egg size. Egg quality according to diameter and total weight could have positive influence on fertilization rate and improving quality of egg incubation. It depends on female brood stock size. Results revealed that 6 year old broodstock produced larger egg with more survival rate (97.5%) and larger larvae (111.5 mg). It could be summarized that egg size had a positive influence on their incubation period. These results are in agreement with those reported for some teleost species such as herring (*Cluplea harengus*; Blaxter & Hempel 1963), Atlantic salmon (*Salmo salar*; Thorpe *et al*., 1984), Arctic charr (*Salvelinus alpinus*; Wallace & Aasjord 1984), Rainbow trout (*Onchorhynchus mykiss*; Gall 1974; Springate & Bromage 1985 and Shamspour *et al*., 2009), Brown trout (*Salmo trutta abanticus*; Bozkurt *et al*., 2006), Iceland cod (*Gadus morhua*; Marteinsdottir & Steinarsson 1998) and Siberian sturgeon (*Acipenser beari*, Gisbert *et al*., 1999). Also positive correlation between the embryo mortality and the egg size has been observed in earlier studies (Flower, 1972; Beacham & Murray 1985) as well as in this study.

Four year old brood stock revealed higher number of ovules per gram body weight. This can be explained by the relationship between produced egg size and number of ovules in each gram of body weight in that 4 year old brood stock produced smaller eggs and hence showed a greater number of ovules. Similar results were observed by Shamspour *et al.* (2009) in rainbow trout.

When the egg size increases, the relative fecundity has been reported to decrease, either with female age (Belding, 1940; Baum & Meister 1971), female size (Lobon-Cervia *et al*., 1997, Springate, 1990; Kunin & Markevich 1978; Heinimaa & Heinimaa 2004) or with female weight (Springate, 1990). The relative fecundity is higher in small females than in large ones (Lobon-Cervia *et al*., 1997). The present study confirmed these results.

CONCLUSION

Brood stock age has effective influence on development stages after fertilization. The present study demonstrated how the age and size of brood fish can effect fertilization and survival of larvae until the yolk sack absorption stage. The results of this study can be used in artificial breeding programs to produce suitable larvae for breeding and reproduction. Thus using 6 years old brood stock from 4 to 6 year old group, could be the most appropriate alternative to breeding in Caspian salmon hatcheries in the country.

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اثر سن بر عملکرد تولید مثلی مولدین ماده ماهی آزاد دریای خزر
(*Salmo trutta caspious*, Kessler 1877)

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چکیده :

ماهی آزاد دریای خزر (*Salmo trutta caspious*) از جمله ماهیان با ارزش دریای خزر می‌باشد. یکی از مهمترین مسائل در پرورش مقدماتی این ماهی، تکثیر مصنوعی و تولید لارو است. در این مطالعه اثر توان باروری مولدین ماده این گونه در فصل تکثیر ۱۳۸۷، بر روی عوامل مؤثر در تکثیر مصنوعی ضروری به نظر رسید. برای این منظور ۳ گروه سنی از مولدین ماده (۴، ۵ و۶ ساله) با اسپرم ۹ عدد مولدین نر بصورت جداگانه لقاح داده شد. بر اساس نتایج بدست آمده، ماهیان ۶ ساله بیشترین میانگین وزن (۸۶/۶ ± ۲۱۵۰/۰ گرم)، طول کل (۲ ± ۵۹ سانتی متر)، وزن تخمک استحصالی (۳۰/۵۱ ± ۲۵۵/۰ گرم)، قطر تخمک (۰/۰۵۸± ۵/۳۷ میلی متر) و هماوری مطلق (۳۶۶/۱۵ ± ۳۰۶۰ عدد تخمک) را نشان دادند. در حالیکه بیشترین تعداد در گرم تخمک (۰/۵۸ ± ۱۶/۳۳ گرم) و هم آوری نسبی (۰/۱۲ ± ۲/۰۸) مربوط به ماهیان ۴ ساله بود. بین تیمارهای مورد بررسی از نظر میانگین درصد لقاح و درصد بازماندگی لارو تا مرحله جذب کیسه زرده اختلاف معنی دار آماری مشاهده شد ($p \leq 0.05$). نتایج این بررسی نشان داد که تخم های حاصل از لقاح تخمک های مولدین ماده ۶ ساله با مخلوط اسپرم های مولدین نر بیشترین میانگین درصد لقاح(۹۷/۵ درصد)، درصد بازماندگی تا مرحله چشم زدگی (۹۲ درصد)، درصد ظهور لارو (۹۳ درصد) و درصد بازماندگی تا مرحله جذب کیسه زرده (۹۴/۵ درصد) را داشته است.