Effects of successive milt collections on sperm quality and reproduction in wild and cultured endangered Caspian brown trout, *Salmo trutta*

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Abstract

In the present study, the effect of successive milt collections on spermatological parameters and reproduction efficiency in wild and cultured endangered Caspian brown trout was investigated. The milt collections were done 3 times at two week intervals. After milt collection, a small amount of milt was allocated for milt quality evaluation and the reminder was used for fertilization. Based on the results obtained, in the wild fish samples, the values of fertilization rate, eyeing rate, hatching rate, milt volume and duration of sperm motility decreased during successive stripping ($p<0.05$) while results for spermatocrit, and sperm density did not show significant differences ($p>0.05$). Also, the percentage of abnormal spermatozoa increased during successive stripping in the wild fish ($p<0.05$). The survival rate of larvae did not show significant changes during successive strippings ($p>0.05$). In cultured fish, similar results were found for fertilization rate, milt volume and duration of sperm motility ($p<0.05$), although the eyeing rate, hatching rate, spermatocrit and sperm density values did not show significant changes during successive strippings ($p>0.05$). Also, the survival rates of larvae were statistically different between the second and third strippings ($p<0.05$). In conclusion, our results showed that successive milt collections have a significant influence on milt quality and reproductive efficiency in male Caspian brown trout, with the best milt being available in the first stripping.

Keywords: Successive milt collection, Sperm quality, Reproduction, Caspian brown trout

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Introduction

The natural population of Caspian brown trout is seriously declining due to overfishing and environmental pollution so that this species has been categorized as an endangered species by IUCN (1996) (Kiabi *et al*., 1999). Artificial propagation of Caspian brown trout and its culture under controlled conditions might be a successful method to prevent their populations from becoming extinct. In recent years, to overcome the shortage of the milt needed for artificial breeding, milt collection is carried out repeatedly from each male brooder during the spawning season. The most important aspect of male reproductive biology is milt quality, which includes the ability of the sperm to fertilize eggs. Nowadays, cryopreservation of milt is a commonplace method for conservation of endangered fish. In this regard, the quality of milt is a key parameter for the success of cryopreservation process. Sperm motility and sperm density determine the fertilization capability of spermatozoa and are used for the evaluation of milt quality (Suquet *et al*., 1992; Billard *et al*., 1993; Linhart *et al*., 1994; Krol *et al*., 2006). Several studies have reported that successive milt collections during the spawning season affect the milt quality (Büyükhatipoglu and Holtz, 1984; Kruger *et al*., 1984; Piironen, 1985; Munkittrick and Moccia, 1987). Most of theses studies have reported that successive strippings decrease the milt quality during the spawning season although a few studies have shown different results. To our knowledge, there are no data about the successive stripping impacts on efficiency of reproductive parameters (fertilization, hatching, eyeing and survival rates) especially in the Caspian brown trout. In this study, we investigated the changes in milt quality and reproductive parameters in Caspian brown trout in relation to successive strippings during the spawning season. This study helped to determine the ability of brooders in the production of healthy milt, eggs and larvae.

Materials and methods

Broodfish

The experiment was carried out at the Kalardasht Salmonids Reproduction Centre, Iran, during the spawning season. Altogether, 30 mature males (as milt donors) and 13 females (as egg donors) of Caspian brown trout were selected randomly for the experiment from brooder ponds after spermiation and ovulation. To identify mature fish, the brooders were checked every day. The collection of milt and ova was done in both wild stocks and cultured stocks by hand stripping three times during the spawning season as follows: first stripping (n=30 males, n=13 females), second stripping (n=25 males, n=10 females) and third stripping (n=15 males, n=7 females).

Sperm quality parameters assessment

A little amount of the milt was allocated for analysis of sperm quality parameters and the remainder was used

for fertilization. The milt volume was measured by scaled vials. For spermatocrit assay, microhaematocrit capillary tubes (75 mm in length and 1.1–1.2 mm in diameter) were filled with milt 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 milt × 100.

The sperm density was measured according to Caille *et al*. (2006). In this regard, milt 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 the spermatozoa were counted using light microscopy. After 5 min, the number of spermatozoa was counted in 16 individual cells, and then calculated according to Caille *et al*. (2006).

Fertilization assay

At each stripping, artificial fertilization was done to evaluate the reproductive efficiency. Before fertilization, the milt and egg samples were pooled separately in order to minimize variations in gamete quality. Fertilization was done by mixing pooled egg and milt samples for 4-5 min. The fertilized eggs were distributed equally into three trays in the hatchery for incubation.

Six to seven days after fertilization, a batch of 80 eggs from each tray was sampled to calculate fertilization percent according to the formula below:

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

The eggs with neural cord were considered as fertilized eggs.

After 14 days of incubation, the eyeing eggs were discerned from dead eggs by shocking (Aas *et al*., 1991). In this regard, the eggs were shed into a tray from a height of 20 cm. In such situation, the dead eggs became white but 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.

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

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

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

Survival rate= (total number of alevins / total number of alevins) × 100.

Data analysis

The SPSS software was used for analyses of data. Because percentage data (% spermatocrit, % abnormal spermatozoa, % fertilization, % eyeing,

% hatching and % survival) did not have a normal distribution, proportional data were converted by angular transformation (arcsin √p). 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.

Results

In wild stocks of Caspian brown trout, the values of milt volume (Fig. 3) and duration of sperm motility (Fig. 1) decreased during successive strippings (p<0.05) while the spermatocrit (Fig. 1) and sperm density (Fig. 1) did not show significant differences (p>0.05). The percent of fertilization, eyeing and hatching (Fig. 4) reduced during successive strippings while abnormal spermatozoa percent (Fig. 1) increased during this period (p<0.05). The values of survival rate did not show significant changes during successive strippings (Fig. 4, p>0.05). In cultured stocks of Caspian brown trout, similar results were observed for fertilization rate (Fig. 5), milt volume (Fig. 3) and duration of sperm motility (Fig. 2) (p<0.05) although the eyeing rate (Fig 5), hatching rate (Fig. 5), spermatocrit (Fig. 2) and sperm density (Fig. 2) values did not show significant changes during successive strippings (p>0.05). Also, the survival rates of larvae were statistically different between the second and third strippings (Fig. 5) (p<0.05).

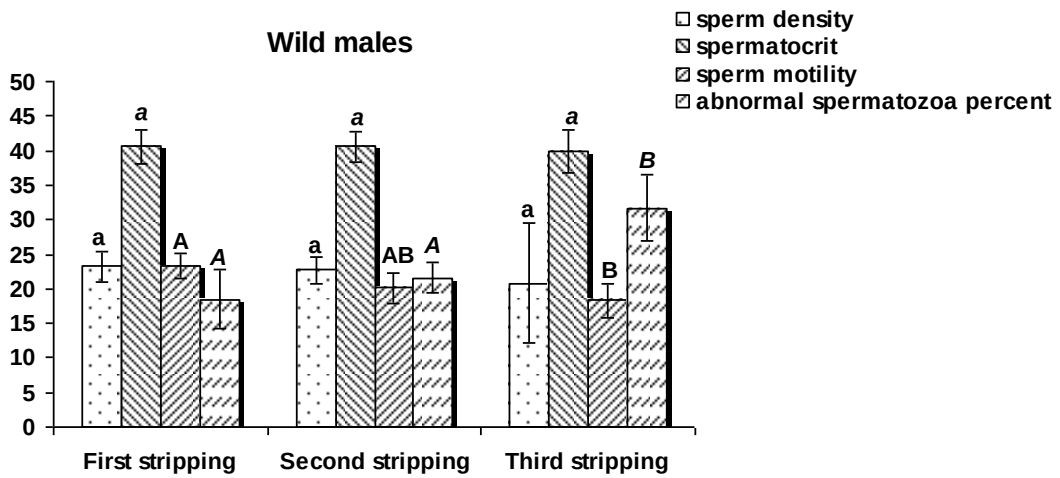

Figure 1: Changes in sperm density, spermatocrit, sperm motility and percentage of spermatozoa abnormality of wild males of caspian brown trout during successive stripping. The values with different letters in the figure are significantly different (p<0.05).

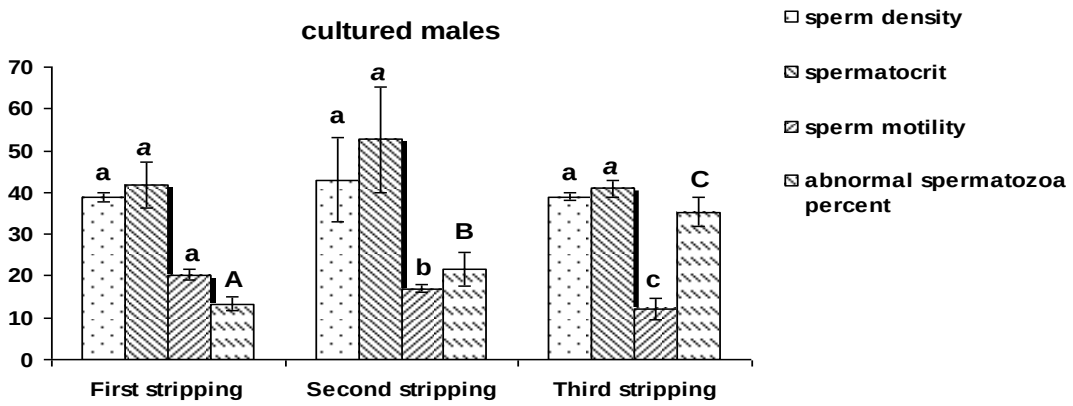

Figure 2: Changes in sperm density, spermatocrit, sperm motility and percentage of spermatozoa abnormality of cultured males of caspian brown trout during successive stripping. The values with different letters in the figure are significantly different (p<0.05).

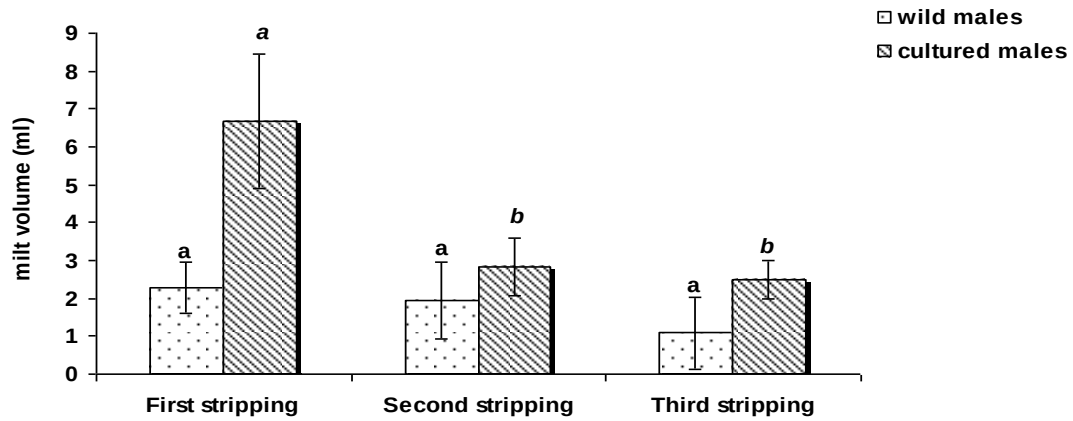

Figure 3: Changes in milt volume of cultured and wild males of caspian brown trout during successive stripping. The values with different letters in the figure are significantly different (p<0.05).

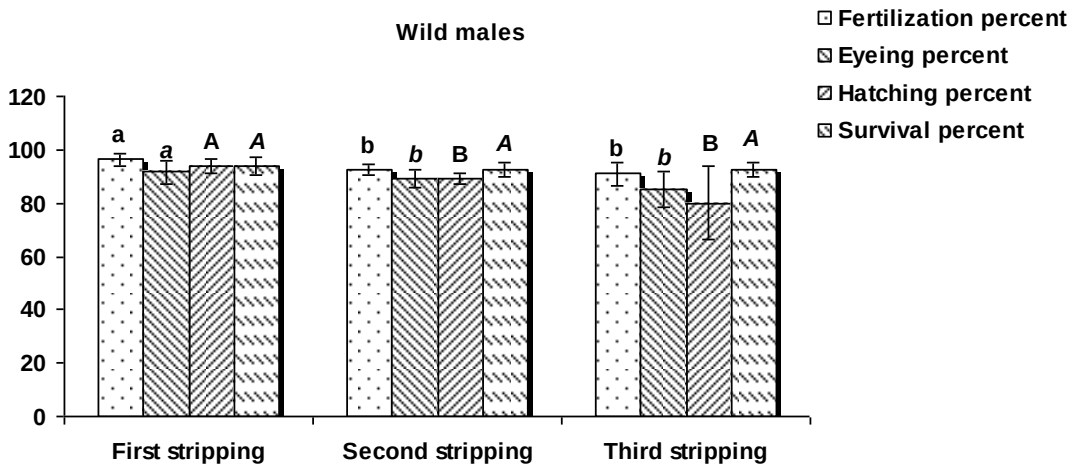

Figure 4: Changes in fertilization percent, eyeing percent, hatching percent and survival percent of wild males of caspian brown trout during successive stripping. The values with different letters in the figure are significantly different (p<0.05).

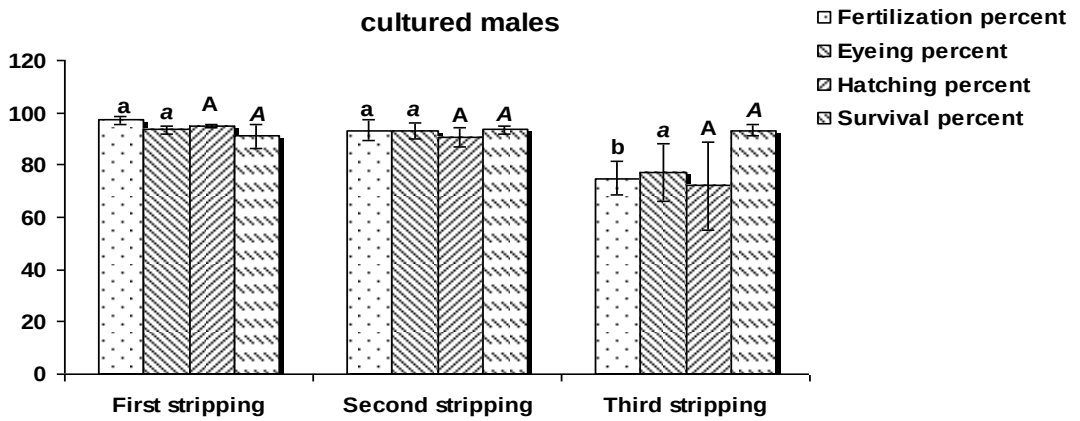

Figure 5: Changes in fertilization percent, eyeing percent, hatching percent and survival percent of cultured males of caspian brown trout during successive stripping. The values with different letters in the figure are significantly different (p<0.05).

Discussion

In our study, some spermatological parameters of cultured and wild males such as milt volume and duration of sperm motility reduced during successive strippings while other parameters remained unchanged. Also, it was found that all reproductive characteristics reduced during successive strippings although eyeing and hatching rates in the cultured Caspian brown trout did not show significant changes over the course of the experiment. These results clearly confirm that reproduction capability and sperm quality of cultured and wild stocks of Caspian brown trout decrease with increasing of milt collection during the spawning season. Several studies have reported the decline in milt quality during the successive milt collections (Sanchez-Rodriguez *et al*., 1978; Buyukhatipoglu and Holtz, 1984; Gjerde, 1984; Piironen, 1985; Suquet *et al*., 1992). In the present study, although the spermatological parameters changed, the survival values of larvae showed no changes during successive strippings in both cultured and wild males. This demonstrates that the viability of eggs is more under the influence of egg quality than sperm quality. Generally, understanding variations in milt production of brooders could be useful to estimate the number of males needed in the hatchery as well as optimize the milt to egg ratios at the time of fertilization. For example, in this study, the higher sperm density in milt collected at the first stripping could fertilize more eggs than when sperm density is lower in the third stripping. Our results showed that the values of milt volume and sperm density in cultured males were higher than that in wild individuals. We used two brooder types whose life cycles had been spent in two different environments. In this respect, the cultured males had completed their life cycle in freshwater while the wild individuals had spent it in salt water of the sea. Morisawa *et al*. (1979) reported the dilution of milt and decreasing of

sperm density in relation to the hypotonicity of freshwater environment. This report is contrary to our results that showed higher sperm density in cultured Caspian brown trout. It seems that the cultured males of Caspian brown trout excrete the excess water of their body in response to hypotonicity of freshwater environments using efficient osmoregulation. In addition to differences in the surrounding water, the nutritional condition has also been different between wild and cultured brooders. Throughout the life cycle of cultured males, commercial dry feed was used while the wild brooders had used the live foods in the sea during their life cycle. Several studies have shown that fish gamete quality varies depending on quality of food composition (Labbe *et al*., 1995; Ciereszko *et al*., 1996; Dabrowski and Ciereszko, 1996; Astuarino *et al*., 2001). In conclusion, our study showed that the milt quality parameters and reproductive efficiency of male Caspian brown trout reduced in relation to successive stripping during the spawning season.

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