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## Full Length Research Paper

# Comparative analysis of milt quality in the cultured and wild stocks of endangered Caspian brown trout, *Salmo trutta caspius*

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**The sperm motility characteristics (percentage of motile spermatozoa and duration of motility) and sperm production (spermatocrit, milt volume and sperm concentration) were measured in order to compare the milt quality between cultured and wild stocks of Caspian brown trout, *Salmo trutta caspius*. Our results showed that cultured brooders produce more dense milt than wild individuals. In contrast, the milt volume, percentage and duration of spermatozoa motility were higher in wild brooders than in cultured individuals. The aim of the present study was to assess the effect of captivity condition on milt quality of cultured males of Caspian brown trout.**

**Key words:** Sperm density, sperm motility, Caspian brown trout.

## INTRODUCTION

Sperm motility and sperm density determine the fertilization capability of spermatozoa and are often used to estimate milt quality (Suquet et al., 1982; Billard et al., 1993; Linhart et al., 1994; Krol et al., 2006; Hajirezaee et al., 2010a, b). The Caspian brown trout, *Salmo trutta caspius* is a critically endangered anadromous species that has been considered for a biological conservation program in the southern basin of the Caspian Sea (Kiabi et al., 1999; Niksirat and Abdoli, 2009). During the last decade, the production of cultured brooders in the hatchery conditions has been considered as a strategy to overcome the shortage of needed milt for artificial breeding. In this regard, as a national plan, the fries taken from wild brooders were reared in the hatchery until sexual maturation. In such situations, it is obvious that this plan involves time and expenditure. The determination of the quality of gametes from these cultured brooders would be essential for evaluation of their reproductive efficiency in captivity conditions. Such

evaluation could prevent the probable waste of time and expenditure

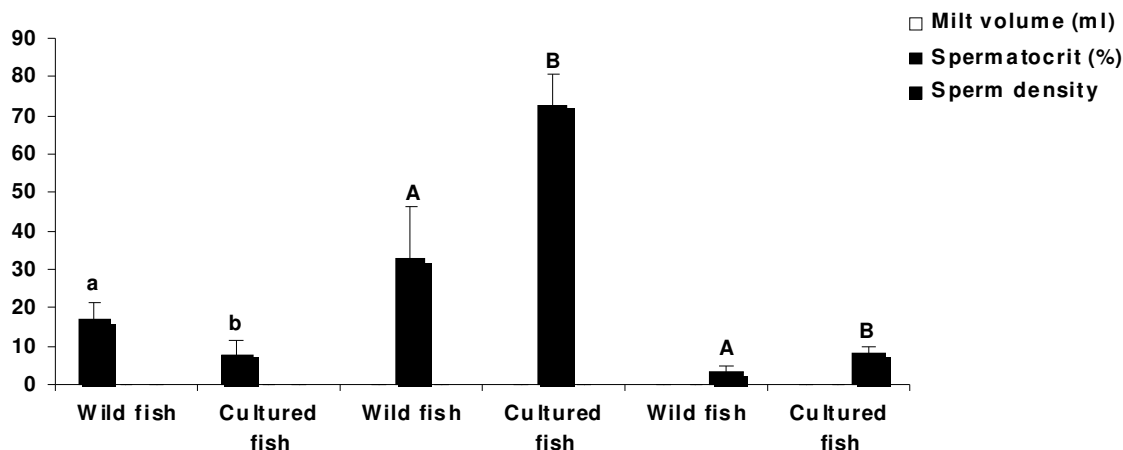
## MATERIALS AND METHODS

Two groups of Caspian brown trout males were considered for experiment: Group A (n = 10, TW = 256.3 ± 22.3 g), the cultured fish: these brooders were reared under captivity conditions in the hatchery for a period of 4 years. Group B (n = 12, TW = 1050 ± 45.4 g), the wild fish: these brooders were captured from the Sardabrood and Tonekabon Rivers during their up-stream reproductive migration, and then transferred to the hatchery. With the onset of spermiation, milt samples were taken from each male.

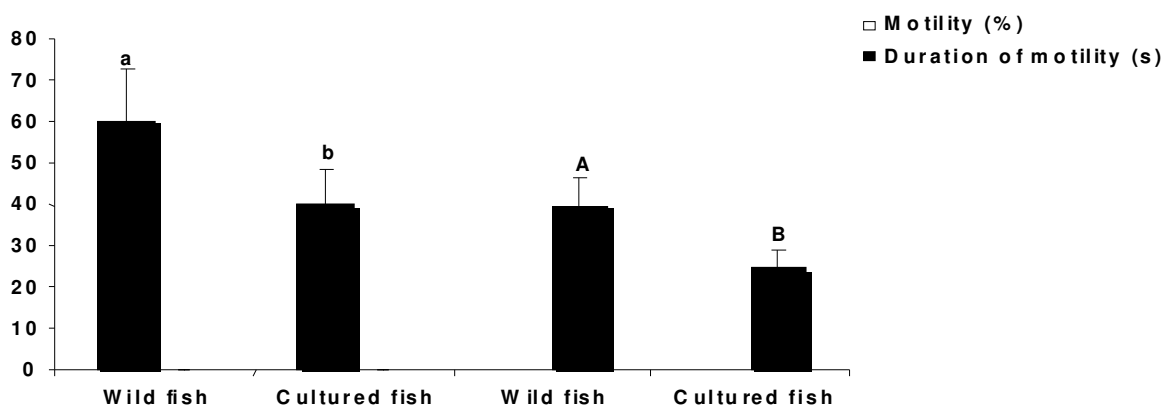
For milt collection, fishes were anaesthetized in 100 ppm of MS222 (tricaine methane sulfonate) and belly was massaged from the anterior portion of the testis towards the genital papilla. Care was taken to avoid contamination of the milt with water, mucus, blood cells, faeces and urine. Milt volume was measured in scaled vials. Spermatocrit was determined by centrifuging milt for 10 min at 5000 rpm in a haematocrit centrifuge (D-78532, Tuttingen Zentrifugen, Germany) according to Piironen (1985) and sperm density in a haemocytometer counting chamber according to Caille et al. (2006).

A two-step dilution was used for motility activation according to the method suggested by Billard and Cosson (1992). Firstly, the milt

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**Figure 1.** Comparison of milt parameters between wild and cultured males of Caspian brown trout. Sperm density: ( $\times 10^9$  spz/ml milt), spermatoцит (%) and milt volume (ml). The values with the different letters are significantly different ( $P < 0.05$ ).



**Figure 2.** Comparison of milt parameters between wild and cultured males of Caspian brown trout (duration and percentage of motile spermatozoa). The values with the different letter are significantly different ( $P < 0.05$ ).

was prediluted to a saline solution (composed of 80 mM NaCl, 40 mM KC1, 1 mM CaCl<sub>2</sub> and 20 mM Tris-HCl per one liter of distilled water (final pH = 9)) in a ratio of 1:100 and secondly, the prediluted milt was subjected to a second dilution in a physiological serum at a videocamera coupled with the optical lens of microscope. At the end, the video recordings were reviewed and the motility was recorded as the percentage and duration of motility. Only forward-moving sperm were judged motile, those simply vibrating or turning on their axes were considered immotile (Aas et al., 1991).

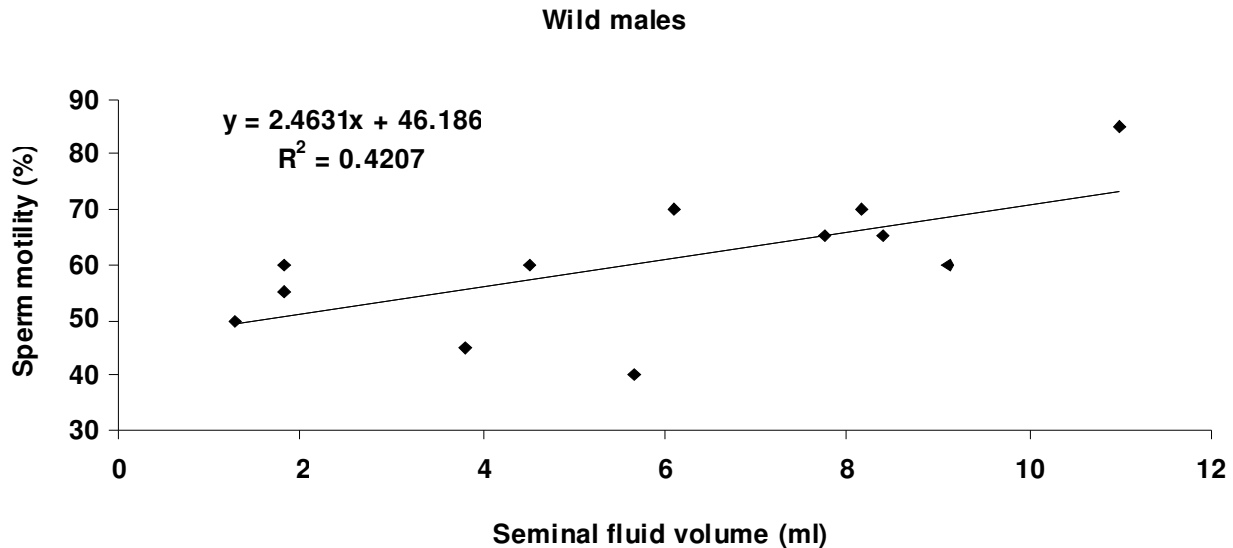
#### Statistical analysis

The Statistical Package for Social Sciences (SPSS) software was used for data analysis. The values of milt volume and duration of sperm motility were normal according to Kolmogorov Smirnov test ratio of 1:20 and immediately, 1  $\mu$ l of solution was placed on the microscope stage and motility was analyzed according to the methods of Rurangwa et al. (2004) in which motility is recorded by a but because percentage data (percentage of sperm motility and spermatoцит) did not have a normal distribution, proportional data

were converted by angular transformation ( $\arcsin\sqrt{p}$ ). The independent samples t-test was used for the comparison of the means between wild and cultured fish. All correlations were tested using the bivariate correlation coefficients of Pearson. Then, linear and non-linear regression models were investigated using regression fits.

## RESULTS AND DISCUSSION

In the cultured Caspian brown trout males, the values of spermatoцит (%) and sperm density ( $\times 10^9$  spz/ml milt) were higher than in wild brooders (Figure 1,  $P < 0.05$ ). In contrast, the values of percentage of sperm motility, duration of sperm motility (s) and milt volume (ml) were higher in wild brooders than in cultured individuals (Figures 1 and 2,  $P < 0.05$ ). Also, a positive correlation was found between seminal fluid volume and percentage of sperm motility in the wild males ( $P > 0.05$ ) (Figure 3).



**Figure 3.** Relationship between seminal fluid volume and percentage of sperm motility in the wild males of the Caspian brown trout.

Our results showed that the means of spermatocrit and sperm density were higher in cultured males than in wild fish. The spermatocrit is defined as the ratio of volume of white packed material (mostly spermatozoa) to the total volume of milt  $\times 100$  (Rurangwa et al, 2004). Therefore, it is obvious that with increase of spermatocrit percentage, the volume of seminal fluid decreases. Contrary to wild males of Caspian brown trout, the cultured fish had entirely spent their life cycle (fry to adult) in a hypotonic medium (freshwater). Morisawa et al. (1979) have reported that hypotonicity of freshwater environment establishes the hydration of testis, possibly causing the dilution of milt and leading to a higher milt volume and subsequently lower sperm density and spermatocrit. In this study, the spermatocrit and sperm density were higher in cultured males than in wild individuals which is contrary to the results reported by Morisawa et al. (1979) on the role of freshwater environment on milt parameters. It is likely that cultured males of Caspian brown trout with the application of an efficient osmoregulation, excrete the excess water of the body in response to hypotonicity of freshwater environment. It is essential to say that the weight of wild males of Caspian brown trout was approximately 4 times of cultured individuals. Suquet et al. (1994, 1998) have reported that milt volume increases with increase of weight in turbot (*Scophthalmus maximus*). Thus, the higher milt volume of wild males than cultured fish may also be due to the higher weight of wild fish.

In this study, the values of percentage and duration of motile spermatozoa were higher in wild males than in cultured individuals. Several studies demonstrated that sperm motility could be influenced by seminal fluid composition (Lahnsteiner et al., 1996, 1998; Ingermann

et al., 2002; Perez et al., 2003). In a previous study on Caspian brown trout, interesting relationships were found between the percentage and  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{Na}^+$ , total protein as well as between duration of motility and  $\text{K}^+$ ,  $\text{Cl}^-$ , total protein and milt pH (Hajirezaee et al., 2010b, c). Fish seminal fluid has a unique composition regarding the presence of the organic and inorganic components which support the viability of spermatozoa (Stoss, 1983; Morisawa et al., 1983; Piironen, 1985; Lahnsteiner et al., 1993, 2004; Ciereszko et al., 2000) till spawning or stripping. In this regard, interactions of ions present in the seminal fluid with the sperm membrane do influence the membrane potential (Ciereszko et al., 2000) and represent a mechanism of inhibition of spermatozoa in the seminal fluid or sperm duct (Boitano and Omoto, 1991), allowing the maintenance of the potential of motility before release to the surrounding medium (Morisawa et al., 1983). In the milt samples of cultured males of Caspian brown trout, the amount of seminal fluid was lower than wild individuals. On the other hand, a positive relationship was found between seminal fluid volume and sperm motility in the wild males. It is likely that with decrease of seminal fluid secretion by spermatoc duct epithelium (Marshall, 1986, 1989; Lahnsteiner et al., 1993, 1994), the quantity of materials involved in sperm motility decreases as well, although the proof of this claim needs to be given in detail for all the materials in the seminal fluid of wild and cultured fish groups.

## Conclusion

In conclusion, although the cultured males of Caspian

brown trout produce more dense milt than wild individuals, the quality of milt was lower in the cultured males than wild individuals in terms of percentage and duration of sperm motility. A detail analysis of seminal fluid composition of wild and cultured fish groups may provide good information on such differences.

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