

## The Effects of Time of Reproductive Migration on Semen Spermatological Parameters in Migrated Kutum *Rutilus frisii kutum* to Valiabad River

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**Abstract:** In this study, the effect of reproductive migration of kutum on semen spermatological parameters (sperm duration, percentage of sperm motility, spermatocrit, sperm density and volume of sperm) was investigated. For this purpose, time of reproductive migration in these brood stocks were divided to three periods (i.e. treatment 1 = February, treatment 2 = March and treatment 3 = April) and in each period we sampled from those male fishes which had approximately the same sizes. The results showed that the spermatocrit, sperm density and volume of sperm were significantly different at different times of the reproductive migration ( $p < 0.05$ ). But, significant differences were not observed among treatments in percentage of sperm duration and the percentage of motile sperms. In general, male brood stocks of kutum in terms of mentioned parameters are more appropriate in March compare to February and April months.

**Key words:** *Rutilus frisii kutum* • Spermatological parameters • Reproductive migration

### INTRODUCTION

Caspian kutum, *Rutilus frisii kutum* Kamenskii 1901, is an endemic fish of Caspian Sea and its populations generally recorded along near the coast, from the Trek River the north to the southern part [1]. It consist more than 70% of fishermen catch in Iran coastal of the Caspian Sea [2].

Since good quality of sperm influences on the survival of produced larvae and on improving the generation, quick assessment of sperm quality can be useful to select the appropriate brood stocks which have sperms with the best quality [3]. Sperm quality is a criterion for measuring the sperm ability in fertilizing the ovum. Therefore, parameters which influence on the fertilization of ovum are sperm quality parameters. The most important parameters which deal with the sperm quality are spermatocrit, sperm density, seminal plasma composition, duration of sperm movement and percentage of motile sperms [3].

A few researches were done on the physiology and biochemistry of sperm structure, sperm mobility and changes in characteristics of milts. Munkittrick and Moccia [4] reported that rainbow trout (*Oncorhynchus*

*mykiss*), spermatocrit, sperm mobility and seminal plasma ions decreased during the reproduction season. Rurangwa *et al.* [3] reported that concentrations of spermatozoa in the rainbow trout and common carp (*Cyprinus carpio*) decreased at the end of spawning season.

The purpose of this study was to determine the effects of different reproductive migration period on semen spermatological parameters of kutum. Therefore, according to the information about characteristics of sperm we can understand that in which month of reproduction season we can have sperms with better quality.

### MATERIALS AND METHODS

**Broodstocks and Samples Preparation:** The experiment was carried out during February until April 2010 in kutum propagation farm located in Valiabad River, Tonekabon, Iran. For this study 20 male breeders with the same size (mean total length  $33.05 \pm 1.75$  cm and mean weight  $382.73 \pm 21.82$  g) were obtained from Valiabad River. Samples were experimented in the three treatments (four fish in each treatment), i.e. beginning (February), middle

(March) and end of reproduction season (April). The males were wiped ventrally with paper towels and the milt was expelled using gentle pressure on the abdominal region without any pollution [5]. Milts were collected by 5 ml syringe (4 volume of air and 1 volume of sperm).

**Spermatological Parameters Measurement:** After adding distilled water (50 times of sperm volume, to stimulate sperm mobility) to this solution, it was put on the stereomicroscope device (microscope equipped with a CCD camera attached to computer, Panasonic WV-CP240, Japan) [6] with magnification number 10 to measure the duration of sperm movement and percentage of motile sperms (time started in less than 7 seconds) and a digital camera recorded the sperm movement by high resolution. The shelf life of sperm for each prototype was measured by stopwatch. The stopwatch started to work once milt was activated by water and stopped when sperm movement stopped [7,8]. Then Adobe premier software (Version 6) was used to get pictures every 10, 20, 30, 40 seconds after the activation of sperms. These pictures changed to 30 forms (slides) and we randomly selected four forms (i.e. Form 1, 4, 7 and 10). Positions of 10 spermatozoa in these randomly selected pictures and also the percentage of motile sperms were calculated. All treatments were done thrice and to avoid experimental error, all measurements were observed by a viewer [6].

For measuring the spermatocrit, some tubes containing semen were centrifuged in the 3000 round of centrifugal machine for 8 minutes (Eppendorf AG 22311 Hamburg, centrifuge 5415D) then hematocrit reader was used to determine the percentage of sperm to seminal fluid (percentage of white space to the total milt volume) [9].

Furthermore, sperm density was measured by haemocytometry standard method with diluted sperm in ratio of 1:2000 by water, using a microscope with 10 magnification phase contrast black background and it was written units per  $\times 10^9$  mL semen.

**Statistical Analysis:** Statistical analysis of data was done by One-Way ANOVA with Duncan test at the level of 95% using SPSS 16. Statistically significance was set at the level of  $p < 0.05$  with  $\pm$  standard deviation (SD).

## RESULTS

As seen in the figures 1 and 2, differences were not significant among different times of reproductive migration in sperm duration and percentage of sperm motility. Nevertheless, spermatocrit, sperm density and

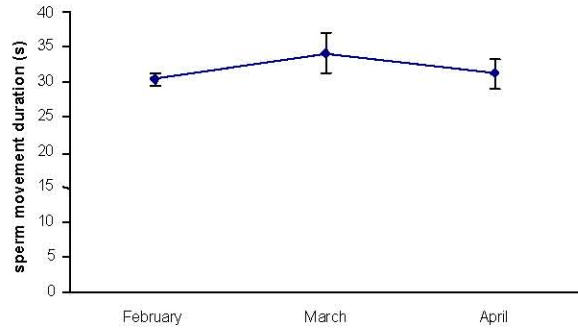


Fig. 1: Sperm movement duration in different periods of reproductive migration

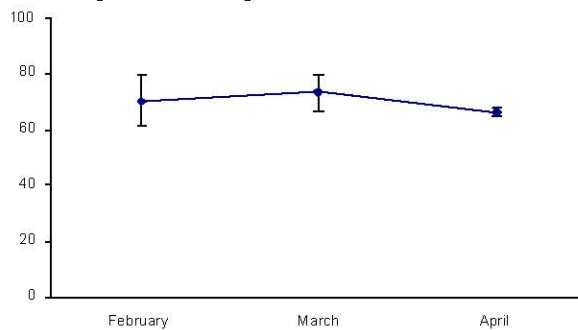


Fig. 2: Sperm motility (%) in different periods of reproductive migration

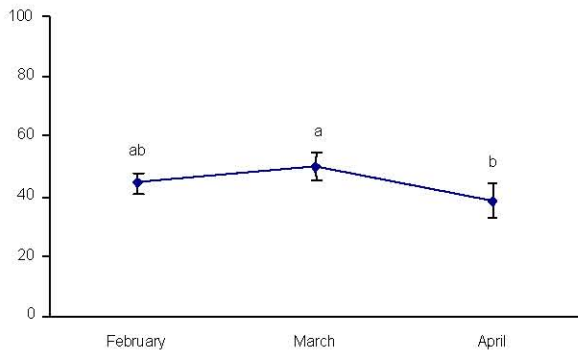


Fig. 3: Spermatocrit (%) in different periods of reproductive migration

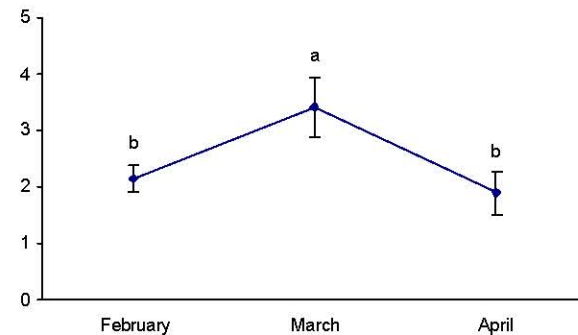


Fig. 4: Sperm density in different periods of reproductive migration

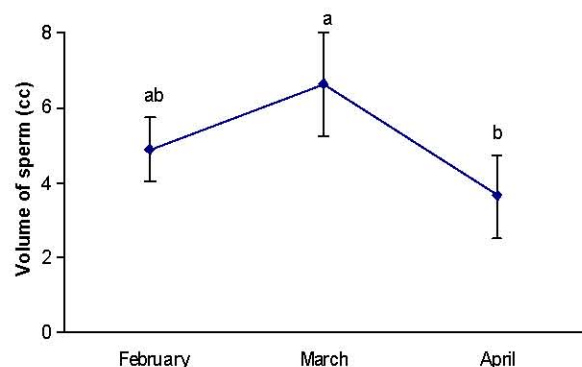


Fig. 5: Volume of sperm in different periods of reproductive migration

volume of sperm showed significant differences among different periods of reproductive migration, as the highest results were observed in March in comparing to another month (Figures 3, 4 and 5).

## DISCUSSION

Sperms of different fish species are different regarding the start of sperm mobility [10], duration of sperm movement, the percentage of motile sperms [11] and the pattern of sperm movement [12].

Suquet *et al.* [13] and Rouxel *et al.* [14] presented that the percentage of motile sperm in turbot (*Scophthalmus maximus*) and cod (*Gadus morhua*) at the beginning, middle and end of migration didn't show the significant differences. Their results agree with our findings. But, Kabir *et al.* [15] in the study on Persian sturgeons (*Acipenser persicus*) found that the duration of sperm movement and the percentage of motile sperm showed differences, significantly.

Spermatocrit and sperm density in seminal fluid are generally used to evaluate the quality of fish sperm [3]. In the present study there were significant differences in spermatocrit among treatments during the spawning season. Suquet *et al.* [13], Rouxel *et al.* [14] and Kabir *et al.* [15] in their studies revealed that there was no significant difference in spermatocrit among different periods of reproductive migration season. But, in our study the highest amount of spermatocrit was obtained in March. As well as, sperm density and volume of sperm were higher in this month in comparing two other months.

In conclusion, according to our results, we can claim that spermatological characteristics is more appropriate in middle of reproductive migration season (March) and with approaching to the end of reproductive migration season, sperm quality decreases.

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