

[Research]

## Effect of age on reproductive performance of Kutum, *Rutilus frisii* (Nordmann, 1840) in Shirood River, the southern coast of the Caspian Sea

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### ABSTRACT

In this study, we investigated the age-dependent changes in reproductive efficiency of Kutum, *Rutilus frisii* caught from Shirood River, the Southern Caspian Sea (Mazandaran Province, Iran). Age-dependent reproductive performance of brooders was assayed on two age and sex groups. Results revealed that there were no significant differences in sperm characteristics between age groups. All female characteristics revealed change except relative fecundity between two age groups. The higher fertilization rate (87%) and also survival rate (91%) were found when the 4 year-old males were crossed with 4 year - old females ( $P < 0.05$ ). Our results confirmed the age-dependent changes of reproductive efficiency in Kutum. So that, the cross between 4 year-old males and 4 year - old females could be useful for enhancement of reproductive efficiency in Kutum .

**Key words:** Kutum, *R. frisii*, Survival, Reproduction traits, Fertilization

### INTRODUCTION

Broodstock productivity clearly represents the most significant constraint on commercial fish farming. Increased knowledge of the factors regulating broodstock productivity is therefore of great importance to the further development of fish culture (Coward & Bromage 2000). Maximizing seed productivity in hatcheries is the ultimate aim of broodstock management. Most important factors on this subject are egg diameter and amount of fecundity in female broods. Other influential factors in rate (%) and survival of larvae include brood age and weight. By increase in age, weight and by stripping, some changes will gradually occur in the ovarian fluid composition and egg content. Presumably these morphological, physiological and biochemical changes are responsible for decrease in egg quality, fertilization rate (%), eyeing rate, hatching, incidence of abnormality and mortality in later phases (Lahnsteiner 2000). Gall (1974) has shown in studies on

hatchery- reared trout that the older and heavier females produce larger eggs than the younger and smaller fish. The availability of food also affects egg size (Springate *et al.* 1985). Sperm quality can be influenced by factors such as size of individuals (Aas *et al.* 1999). Often, older, more experienced males produce higher semen volume with higher sperm density and greater fertilization capacity as compared to the younger, less mature fish (Aas *et al.* 1999). Larval quality also is affected by sperm and egg quality (insemination ratio, spermatocrit etc.), which is taken from available broodfish. So, the high - quality egg or semen mean the high - quality larval fish. Improvements in our understanding of the appropriate culture conditions and management procedure for the brood-fish are essential if we are programming reproductive development to produce reliably the numbers of eggs and fry required by grow-out farms. *Rutilus frisii* belonging to family Cyprinidae, is the most commercially-

important teleost in the Caspian Sea. It only inhabits the Caspian Sea and its main habitat is related to the south of the Sea, especially in Iranian shore (Razavi Sayad 1995). Artificial reproduction and husbandry of this fish in land pool and renewal of its stocks in the Sea started since 1982 (Emadi 1995). The aim of the present work therefore was to investigate the effects of broodstock age on gametes quality and the reproductive performance of *R. frisii* broodstock to find out the possible broodstock management strategies which may be adopted by hatcheries to improve reproductive indices.

## MATERIALS AND METHODS

### Brood fish

The experiment was carried out at Tajan Cyprinid Fish Complex, Sari, Iran. kutums were captured from the Shirood River estuary during spawning migration (water temperature 9-12 °C). So, 30 mature females and 30 male Kutum with 3, 4, 5 and 6 years old were used, respectively. The age of Kutum was determined using scale samples taken between the adipose fin and lateral line (Heinimaa & Heinimaa 2004). Sperm and eggs were collected by manual stripping. Care was taken to avoid contamination of the semen with water, mucus, blood cells, faces or urine. Semen of each male was collected and sperm batches were transported to the laboratory under cold conditions (4 °C) and kept for further analysis and fertilization procedures. After stripping, sperms were divided into equal amounts for all the treatments.

### Sperm quality parameters assessment

An activating solution of 0.3% NaCl was used for estimating sperm motility. So, about 1 µl of semen was placed on a test tube, then 1000 µl of activation solution was added to it and thoroughly mixed with the tip of a pipette. About 10 µl of semen diluted was placed on a glass microscope slide and then the sperm motility was recorded using a camera (Nikon 50i Japan) mounted on a phase contrast microscope (Leica, USA). Each motility determination was performed in triplicate for

each semen sample. The duration of sperm motility was measured immediately after initiation of sperm activation until 100% spermatozoa were immotile and expressed as sperm movement duration. The rate (%) of motile spermatozoa was defined as the percentage of progressively-motile spermatozoa within each activated sample. these spermatozoa were defined as actively - swimming sperms in a forward motion. Only forward - moving sperm was judged motile, while sperms vibrated in place, were not considered to be motile. Observations were made within two hours of semen collection. Semen was drawn into glass microhaematocrit capillary tubes (75 mm length, 1-1-1-2 mm internal diameter) until 60 -80% of the tube volume were occupied by semen. One end of the tube was then sealed with clay and the tubes were centrifuged for 8 min at 3,000 g (Eppendorf-5415D Germany). Spermocrit was defined as the ratio of the total volume of white package material to the total volume of semen  $\times 100$  (Rurangwa *et al.* 2004). Measurements were taken in triplicate for each sample, and the average of the three measurements was used for the results.

### Female gamete parameters assay

During stripping, female characteristics were measured including egg diameter (mm), total weight of stripped eggs, number of eggs per gram, absolute fecundity and relative fecundity. Fecundity was determined by weighing method (Bozkurt *et al.* 2006), while egg size using a caliper (at 0.02 mm accuracy). The relative fecundity was calculated by dividing the total egg number by the total body weight. The fertilization trials were designed as follow: T<sub>1</sub>: 3 year-old males Vs. 3 year-old females; T<sub>2</sub>: 3 year-old males Vs. 4 year-old females; T<sub>3</sub>: 4 year-old males Vs. 3 year-old females and T<sub>4</sub>: 4 year-old males Vs. 4 year-old females. The pooled eggs from each age class were distributed equally to plastic dishes. To control variation among the qualities of egg, those from each age class were pooled separately in order to minimize variations in

gamete quality. Fertilization took place in a dry plastic dish. Afterward, the pooled semen samples were added equally to dishes containing pooled eggs and then mixed. The fertilization solution (3 g urea, 4 g NaCl in 1 L distilled water) was used according to the dry fertilization technique. The spermatozoa egg ratio was approximately  $2 \times 10^9$  (Aas *et al.* 1991). Following fertilization, the eggs were stirred for 1 h, then eggs were rinsed with water and placed into the incubators. Fertilization rate was determined as the rate of the eyed eggs about 6 h after the fertilization. Hatching occurred between 3 - 4 days at water temperature 12 - 17°C. Following equations was used to calculate fertilization capacity.

Fertilization rate=

Number of fertilized egg/ total eggs  $\times$  100 (Brommage & Cumalantunga 1998).

Hatching rate = (number of healthy fertilized eggs / number of fertilized eggs)  $\times$  100 (Hanjavanit *et al.* 2008).

**Data analysis**

Because the data about the sperm quality parameters did not have a normal distribution, Mann Whitney U test was used for normality of data distribution and homogeneity of variance, and then data were statistically analyzed using Student-pair tests.

All statistical analyses were performed using SPSS ver. 16.0. Data were presented as mean  $\pm$  SD.

**RESULTS**

Sperm quality parameters of two age groups are shown in Table 1. There were no significant differences in sperm quality parameters between the age classes.

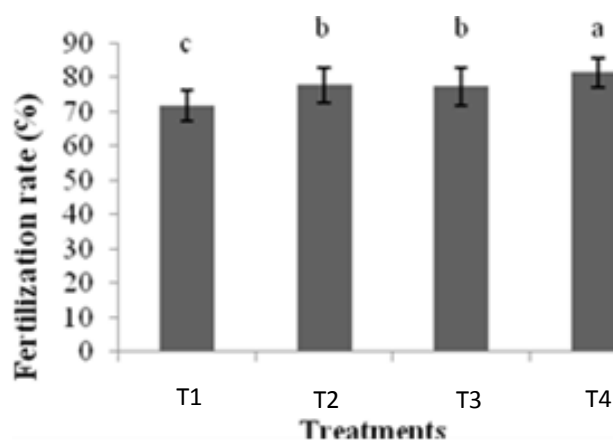
The female characteristics of two age groups during spawning season are presented in Table 2. Among evaluated parameters, only relative fecundity did not show significant difference between the two age classes.

The highest fertilization and hatching rate were found when the 4 year-old males were crossed with 4 year - old female (Figs. 1 and 2).

The highest survival rate was observed in the male (4 years old)  $\times$  female (4 years old) group (Fig. 3).

**Table 1.** Sperm quality parameters in Kutum at different age classes.

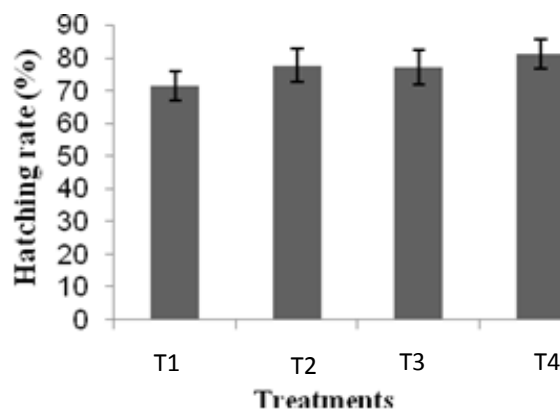
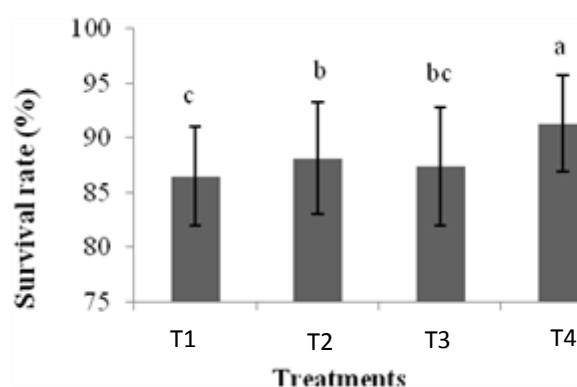
Parameters	Age			
	3 years old	4 years old	5 years old	6 years old
Duration of sperm motility (sec)	32 $\pm$ 7.2	32 $\pm$ 3.6	34 $\pm$ 1.1	29 $\pm$ 3.7
Percentage of motile sperm (%)	77 $\pm$ 1.9	77.4 $\pm$ 2.6	74.5 $\pm$ 3.1	74 $\pm$ 2.8
Sperm density (ml $\times$ 10 <sup>-9</sup> )	32.3 $\pm$ 8.8	35.2 $\pm$ 13.9	32 $\pm$ 2.4	30 $\pm$ 12.7
Spermatocrit (%)	32.7 $\pm$ 5.4	30.3 $\pm$ 5.8	30 $\pm$ 4.2	33 $\pm$ 2.4
Sperm volume (ml)	4.3 $\pm$ 1.7	4.5 $\pm$ 1.2	5.2 $\pm$ 0.7	5.5 $\pm$ 1.3



**Fig. 1.** Fertilization rate in the experimental treatments.

**Table 2.** Some properties of female Kutum at different ages.

Parameters	Age			
	3 years old	4 years old	5 years old	6 years old
Total weight of stripped eggs	96.2 ± 28.1 <sup>a</sup>	142.7 ± 47.09 <sup>ab</sup>	168.2 ± 55.2 <sup>b</sup>	217 ± 57.4 <sup>c</sup>
Number of eggs per gram	360 ± 17.8 <sup>a</sup>	337 ± 23.1 <sup>ab</sup>	321 ± 24.9 <sup>b</sup>	293 ± 22.6 <sup>c</sup>
Egg diameter (mm)	1.48 ± 0.06 <sup>a</sup>	1.54 ± 0.07 <sup>ab</sup>	321 ± 24.9 <sup>b</sup>	1.63 ± 0.06 <sup>c</sup>
Absolute fecundity	34279.9 ± 87.15 <sup>a</sup>	45167.7 ± 126.2 <sup>ab</sup>	25838.7 ± 130.15 <sup>b</sup>	62656.8 ± 458.2 <sup>c</sup>
Relative fecundity	54.1 ± 3.3	54.2 ± 3.2	52.2 ± 3.3	59.2 ± 4.7

**Fig. 2.** Hatching rate in the experimental treatments.**Fig. 3.** Survival rate in the experimental treatments.

## DISCUSSION

In this study, sperm characteristics did not significantly influenced by age. The relationship between age and variation in the sperm quality parameters has been investigated in fishes (Khodzher 1981; Buyukhatipoglu & Holtz 1984; Vuthiphandchai & Zohar 1999; Liley *et al.* 2002; Mordenti *et al.* 2003; Alynia *et al.* 2013). No study has been demonstrated correlation between sperm traits and male age in *R. frisii*. In captive - bred striped bass *Morone saxatilis*, compared to the 1 and 12 year-old fish, the 3 year-old produced

the greatest number of spermatozoa, sperm concentration and spermatocrit (Vuthiphandchai & Zohar 1999). Khodzher (1981) reported that there was no change in the duration of sperm motility of Baikal Omul in the age range of 6-14 years. In Atlantic salmon *Salmo salar*, duration of sperm motility of precocious male parr was longer than that of adult males (Daye & Glebe 1984). The differences may be due to feeding conditions, husbandry procedures, age, environmental factors, spawning time or dilution ratio. Several

lines of evidence suggest that spermatozoa deteriorate as they get older (Vishwanath & Shannon 1997) through damage to the DNA or to the cell membrane (Irvine *et al.* 2000). In fish species, ageing of sperm has also been reported and resulted in changes on sperm quality as the spawning season progresses (Suquet *et al.* 1998). Consequently, a spermatozoa age effect could have confounded an effect of age per se. In recent years the importance of brood fish stocks have been known and it has also been understood that good quality brooders mean good quality fry and production. Egg quality according to diameter and total weight could have positive influence on fertilization rate and improving the quality of egg incubation. In this study, the 3 year - old females produced bigger eggs. Most researchers have pointed out that once the brood fish size and age rise, egg size will increase (Bromage *et al.* 1992). 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, *Clupea harengus* (Blaxter & Hempel 1963), Arctic charr, *Salvelinus alpinus* (Wallace & Aasjord 1984), rainbow trout, *Oncorhynchus mykiss* (Springate & Bromage 1985), Siberian sturgeon, *Acipenser baeri* (Gisbert *et al.* 1999), Brown trout, *Salmo trutta abanticus* (Bozkurt *et al.* 2006), and common carp, *Cyprinus carpio* (Alynia *et al.* 2013). In our study, the number of eggs per gram was higher in 3 year-old females compared to other age classes. This can be explained by the relationship between produced egg size and number of ovules in each gram of body weight. So that, 3 year - old brood stock produced smaller eggs and hence showed a greater number of ovules. Similar results were observed by Alynia *et al.* (2013) in common carp, *C. carpio*. Fish fecundity is known to increase with the age of brooders (Reznick *et al.* 2002). Once the egg size increases, the relative fecundity has been reported to be decreased, either with female age (Baum & Meister 1971), female size (Lobon-Cervia *et al.* 1997; Kunin & Markevich, 1978; Heinimaa & Heinimaa 2004) or with female

weight (Springate 1985). The relative fecundity is higher in small females than in large ones (Lobon-Cervia *et al.* 1997). In our experiment this trend were not observed in absolute fecundity and relative fecundity with age classes. In this study two age classes of male and female kutum broodstocks (i.e. 3 and 4 year-old ones) were crossed together to identify the best age with maximum reproductive performance. Understanding the variation in the sperm and egg quality among individuals is particularly relevant to commercially-important fish species, for whom supportive breeding programs exist, such as kutum used in this study. A common practice in supportive breeding is the sequential or simultaneous addition of sperm from males of different age classes. Thus, if age correlate with sperm traits, supportive breeding might increase the variance in reproductive success among males through higher fertilization success of particular individuals (Wedekind *et al.* 2007). This might in turn result in an undesired decrease in the effective number of brooders and consequently in reduced genetic diversity among the offspring that are released in the wild.

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## اثر سن مولدین بر کارایی تولید مثلی ماهی سفید (*Rutilus frisii* (Nordmann, 1840) رودخانه شیروود، سواحل جنوبی دریای خزر

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### چکیده

در این مطالعه، تغییرات وابسته به سن روی کارایی تولید مثلی ماهی سفید رودخانه شیروود در جنوب (ایران - مازندران) را بررسی و همچنین، عملکرد تولید مثلی وابسته به سن مولدین با استفاده از دو گروه سنی نر و ماده مورد آزمایش قرار گرفت. برای این منظور، مولدین به دو گروه سنی نر و ماده (یعنی مولدین ۳ و ۴ سال) به طور تصادفی از گروه‌های سنی انتخاب شدند. نتایج نشان داد که اختلاف معنی‌داری در ویژگی‌های اسپرم بین سنین وجود ندارد. تمام خصوصیات ماده‌ها بجز هم‌آوری نسبی بین دو گروه سنی تفاوت نشان دادند، به طوری که با توجه به نتایج بدست آمده، نرخ بالاتر لقاح (۸۷٪) و درصد بازماندگی (۹۱٪) در زمانی دیده شد که ماهیان نر ۴ ساله با ماهیان ماده ۴ ساله لقاح داده شده بودند ( $P < 0.05$ ). نتایج ما تغییرات وابسته به سن روی کارایی تولید مثلی در ماهی سفید را تایید کرد. در این رابطه، لقاح بین ماهیان مولد ۴ ساله و ماهیان نر ۴ ساله برای افزایش بازده تولید مثلی می‌تواند مفید باشد.

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