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Influence of broodstock age on reproductive performance in Kutum, *Rutilus kutum* in the Shirood River of the southern Caspian Sea (Iran-Mazandaran-Tonekabon)

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ABSTRACT

In this study, we investigated the age-dependent changes of reproductive efficiency of Kutum in the Shirood River of the southern Caspian Sea (Iran-Mazandaran-Tonekabon). As well as, age-dependent reproductive performance of brooders was tested using two age groups of male and female. For this purpose, the brooders were divided to age classes and then two age groups of male and female (i.e. 3 and 4 years old brooders) selected randomly from age classes. Results revealed that there were no significant differences in sperm characteristics between ages. All female properties revealed change except relative fecundity between two age groups. According to our results, the higher fertilization rate (%87) and survival rate (%91) was found when the 4 years old males were crossed with 4 years old female ($P<0.05$). Our results confirmed the age-dependent changes of reproductive efficiency in Kutum. In this respect, the cross between 4 years old males and 4 years old female of Kutum could be useful for enhancement of reproductive efficiency.

Key words: Kutum, *R. kutum*, 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 factor in percent and survival of larvae are broods age and weight. With increase in age, weight and with stripping, some changes will occure 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). 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 with 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, high quality egg or semen means 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. This species, *Rutilus kutum*, belongs to cyprinids, which is the most famous, valuable, commercial and economic teleost in the Caspian. It just finds only in Caspian Sea and the main habitat is relating to south of the Caspian Sea, especially in Iran's beaches (Razavi Sayad 1995). Artificial reproduction and culture of this fish in land pool and renewal of *Rutilus kutum* stocks in Caspian Sea started since 1982 (Emadi 1995). The aim of the present work therefore was to investigate the effects of broodstock age on gametes quality parameters and the reproductive performance of *R. kutum* broodstock to see whether this identifies possible broodstock management strategies that 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 inlets to the Caspian Sea during spawning migration (water temperature 9-12 ºC). For this purpose 30 females and 30 males mature Kutum with 3, 4, 5 & 6 years old were used respectively. The age of the Kutum was determined from 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 transported to the laboratory under cold conditions (4 ºC) until used for analysis and fertilization. After stripping, sperm divided into equal condition for all treatments.

Sperm quality parameters assessment

An activating solution of 0.3% NaCl was used for estimating motility. For the evaluation of motility, about 1 µl of semen was placed on a test tube and 1000 µl of activation solution was added and thoroughly mixed with the tip of a pipette, about 10 µl of semen diluted placed on a glass microscope slide and 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 Percentages of motile spermatozoa was defined as the percentage of progressively motile spermatozoa within each activated sample. Progressively motile spermatozoa were defined as actively swimming in a forward motion. Only forward moving sperm was judged motile and sperm cells that 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 (Eppendrof-5415D Germany). Spermatocrit was defined as the ratio the total volume of white package material to the total volume of semen ×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's properties including egg diameter (mm), total weight of stripped eggs, number of eggs per gram, absolute fecundity and relative fecundity were measured. 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. The fertilization trials were designed as follow: T_1: 3 years old male *Vs.* 3 years old female; $T_{2:}$ 3 years old male *Vs.* 4 years old female; $T_{3:}$ 4 years old male *Vs.* 3 years old female and $T_{4:}$ 4 years old male *V.* 4 years old female.

The pooled eggs from each age class were distributed equally to plastic dishes. To control variation among the qualities of egg, eggs from each age class were pooled separately in order to the minimizing of variations in gamete quality.

Fertilization took place in dry plastic dishes. Afterward, the pooled semen samples were added equally to dishes containing pooled eggs and then mixed. The fertilization solution (3 g of urea, 4 g of NaCl in 1 L distilled water) was used according to the dry fertilization technique. The spermatozoa egg ratio was approximately 2×10^9.

Following fertilization, the eggs were stirred for 1 h and then eggs rinsed with hatchery water and placed into the incubator. Fertilization rate was determined as the percent 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× 100 (Brommage and Cumalantunga, 1998).

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

Data analysis

Because of data about sperm quality parameters did not have a normal distribution, Mann Whitney 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 the statistical program SPSS 16.0. Data are presented as mean ± SD.

RESULTS

Sperm quality parameters of two age groups are shown in Table 1. There were no significant differences in sperm quality parameters between ages. The females' properties of two age groups during spawning season are presented in Table 2. Among evaluated parameters just relative fecundity did not show significant difference between two age classes. The highest fertilization rate and hatching rate were found when the 4 years old males were crossed with 4 years old female (Fig 1 & 2). The highest survival rate was observed in male (4 years old) × female (4 years old) (Fig 3).

Table 1. Sperm quality parameters in Kutum at different ages.

	Age			
Parameters	**3 years old**	**4 years old**	**5 years old**	**6 years old**
Duration of sperm motility (sec)	32 ± 7.2	32 ± 3.6	34 ± 1.1	29 ± 3.7
Percentage of motile sperm (%)	77 ± 1.9	77.4 ± 2.6	74.5 ± 3.10	74 ± 2.8
Sperm density (ml×10^{-9})	32.3 ± 8.8	35.2 ± 13.9	32 ± 2.4	30 ± 12.7
Spermatocrit (%)	32.7 ± 5.4	30.3 ± 5.8	30 ± 4.2	33 ± 2.4
Sperm volume (ml)	4.3 ± 1.72	4.5 ± 1.21	5.2 ± 0.74	5.5 ± 1.34

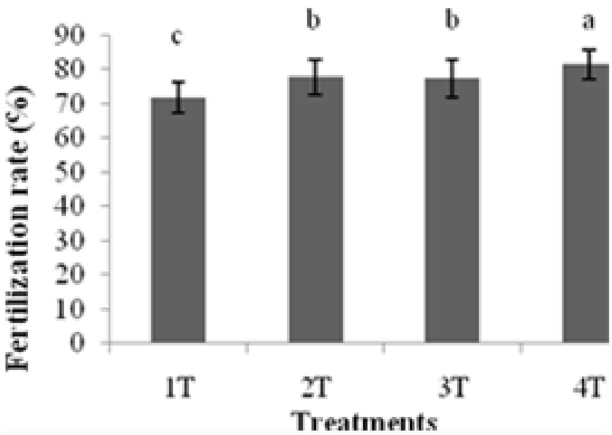

Fig. 1. Fertilization rate in experimental treatments.

Table 2. Some properties of females Kutum at different ages.

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

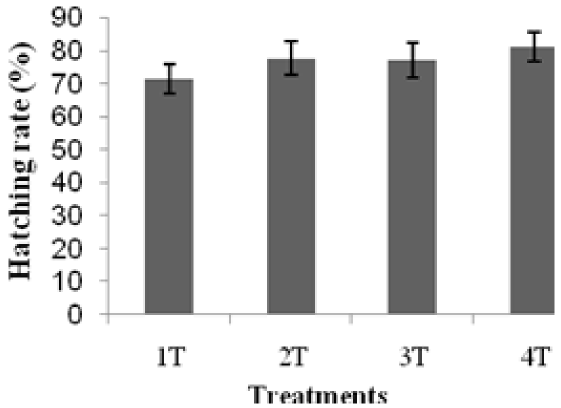

Fig. 2. Hatching rate in experimental treatments.

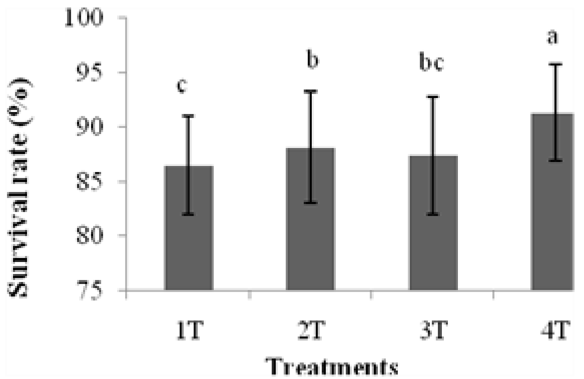

Fig. 3. Survival rate in experimental treatments.

DISCUSSION

In this study, sperm characteristics did not significantly influenced by age. The relationship between age and variation in 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 the Kutum, *R. kutum*. In captive striped bass *Morone saxatilis*, compared to the 1 and 12 years old fish, the 3 years old fish 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 results in changes in 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 means good quality fry and production. Egg quality according to diameter and total weight could have positive influence on fertilization rate and improving quality of egg incubation. In this study, the 3 years old females produced bigger eggs. Most researchers have stated that when 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, *Cluplea harengus* (Blaxter & Hempel 1963), Arctic charr, *Salvelinus alpinus* (Wallace & Aasjord 1984), Rainbow trout, *Onchorhynchus mykiss* (Springate & Bromage 1985), Siberian sturgeon, *Acipenser beari* (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, number of eggs per gram was higher in 3 years old females compared to other ages. This can be explained by the relationship between produced egg size and number of ovules in each gram of body weight in 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 it is known to increase with the age of breeders (Reznick *et al.* 2002). When the egg size increases, the relative fecundity has been reported to decrease, 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. In the present study two age classes of male and female carp broodstocks (i.e. 3 & 4 years old) were crossed to identify the best age with maximum reproductive performance. Understanding the variation in sperm and egg quality among individuals is particularly relevant in economically important species for which supportive breeding programmes exist, such as the Kutum used in this study. A common practice in supportive breeding is the sequential or simultaneous addition of sperm from males of different age. 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 turns result in an undesired decrease in the effective number of breeders and consequently in reduced genetic diversity among the offspring that are released in the wild.

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اثر سن مولدین روی کارایی تولید مثلی ماهی سفید رودخانه شیرود، جنوب دریای خزر (ایران- استان مازندران)

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چکیده

در این مطالعه، ما تغییرات وابسته به سن روی کارایی تولید مثلی ماهی سفید رودخانه شیرود در جنوب (ایران – مازندران - تنکابن) را بررسی نمودیم. 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 به جزء هم‌آوری نسبی بین دو گروه سنی تفاوت نشان دادند. بطوری‌که با توجه به نتایج بدست آمده، نرخ بالاتر لقاح (۸۷ درصد) و درصد بازماندگی (۹۱ درصد) 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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