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# Influence of Age of Common Carp (*Cyprinus carpio*) Broodstock on Reproductive Traits and Fertilization

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**Abstract**

In this study, we examined the age-dependent changes of sperm quality parameters and female properties in common carp. As well as, age-dependent reproductive performance of brooders was tested using two age groups of male and female (i.e. 2 and 3 years old brooders). For this aim, the brooders were crossed randomly from age classes. There were significant differences in sperm density and percentage of motile spermatozoa between ages, but duration of sperm motility and spermatocrit did not differ from two age groups. Some seminal plasma composition such as pH, chloride, acid phosphatase (ACP) and alkaline phosphatase (ALP) showed significant differences between two age classes. Female properties revealed change in egg diameter between two age groups. No significant differences were observed in other female properties between two age groups. According to our results, the higher fertilization rate (%91) and survival rate (%90) was found when the 2 years old males were crossed with 3 years old female ($P<0.05$). Also, highest hatching rate (%96) and larvae length during hatching were found when the 3 years old males were crossed with 2 years old female ($P<0.05$), while larvae length at initiation of active feeding (after yolks soc absorption) was higher when the combination of 2 and 3 years old males were crossed with 2 years old females.

*Keywords*: reproduction traits, fertility ability, fecundity, Common carp.

## Introduction

The carp culture industry is currently the most important sub-sector of fisheries in Iran and its rapid development has attracted considerable attention in recent years. Since the 1970s carp farming has spread along the Caspian coast. In commercial fish farming the evaluation of sperm quality is of interest to increase the efficiency of artificial propagation. High individual variations of sperm quality are frequently reported (Rana, 1995). This may be due to sex ratio, stocking density, age, size, nutrition and feeding regime (Ridha and Cruz, 1989; Smith *et al*., 1991; Izquierdo *et al*., 2001; Chong *et al*., 2004; Tahoun *et al*., 2008). Sperm quality is a measure of the ability of sperm to successfully fertilize an egg. One of the most common parameters employed to study sperm biology is the motility parameters of the spermatozoa (Billard *et al*., 1995). The fish farming industry has been more focused on the quality of eggs or larvae rather than that of sperm, even though the quality of both gametes may affect fertilization success and larval survival (Rurangwa *et al*., 2004). In teleost species, evaluation of quality of male gametes has been primarily dependent on sperm motility, sperm concentration and spermatocrit (Rana, 1995; Suquet *et al*., 1992). Understanding the sperm quality of different ages of broodstock is a prerequisite for hatchery management. Females gamete quality in fish has concentrated on relating egg characteristics to fertilization rate and offspring success. For example, egg size has been positively correlated with fertilizing capacity, measured as the percentage of eggs fertilised in a batch (Buckley *et al*., 1991), and to larval size (Blaxter and Hempel, 1966; Marteinsdottir and Able, 1992). Good quality eggs are known as those which exhibit low levels of mortality at fertilization, eying, hatching and first feeding and those which produce the fastest growing and healthiest fry (Brooks *et al*., 1997). Usually, the sperm motility, sperm concentration, egg diameter, fertilization rate and hatching rate were used as indices of gamete quality. It is well recognized that the size of eggs of fish shows considerable intra- and inter- specific variation. Even parental fish of the same strain, weight and length have eggs that in different size (Bagenal,

1969). Number of eggs produced per broodfish, when it is sometimes referred to as total or absolute fecundity or more usually just as fecundity. Alternatively fecundity may be expressed per unit body weight of post-stripped fish. A few studies showed that fish age influence quality of gametes such as Atlantic salmon *Salmo salar* (Kazakov, 1981), *Coregonus autumnalis migratorius* (Khodzher, 1981), rainbow trout *Oncorhynchus mykiss* (Schmidt-Baulain and Holtz, 1991), striped bass *Morone saxatilis* (Vuthiphandchai and Zohar, 1999), turbot *Scophthalmus maximus* (Suquet *et al*., 1998) and koi carp ornamental carp, (Mordenti *et al*., 2003 ). The aim of the present work was to investigate the effect of broodstock age on gamete quality, fertilization, survival rates of the carp.

## Material and Methods

### Brood Fish, Sperm and Egg Collection

This study was conducted in a small commercial carp farm in Rasht, Iran during 2010-2011 spawning seasons. For this purpose 16 females and 16 males mature carp with two and three years old were used respectively. The average of total weight and total length for two and three years of famales and males were 4350±43.5 g, 61.3±1.41 cm and 5000±81.7 g, 63.5±1.29 cm respectively. To semen collection males were injected intramuscularly with Carp Pitatury Gland Hormone (cPG) at dose of 3 mg $kg^{-1}$. In addition, females' fish were injected intramuscularly with a double injection of 3 mg $kg^{-1}$ cPG. In first injection, 10% (0.3 mg/kg) cPG was given 12 h before the second injection (1.7 mg/kg). Semen was collected after spermiation, approximately 10 h after spawning induction. Semen of each male was collected and sperm batches transported to the laboratory under cold condition (4 ºC) until used for analysis and fertilization. Care was taken to avoid contamination of the semen with water, mucus, blood cells, faeces or urine. Semen from each fish was collected into 5 mL syringe. The females stripping were carried out 10 h after second injection.

### Evaluation of Sperm

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 (Panasonic WV.CP 240 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, 1x1–1x2 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 (Sigma, 13 USA). 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.

### Seminal Plasma Characteristics

To analysis the chemical components of seminal plasma, the seminal plasma was separated from the semen by centrifugation (Eppendorf AG, Hamburg, Germany) and then seminal plasma was collected. Plasma was centrifuged twice to avoid possible contamination with spermatozoa and then samples were frozen at -20°C until analysis. The pH of seminal plasma was immediately determined using a laboratory pH meter (pH meter, Iran 762). The minerals ($Ca^{+2}$, $Mg^{+2}$ and $Cl^{-}$) were measured spectrophotometrically (S2000-UV/VIS England). The concentrations of $Na^{+}$ and $K^{+}$ were determined with flame photometer (Jenway PFP, England) (Standard kits from Parsazmoon, Tehran, Iran). The ACP and ALP were measured by auto-analyzer (Caretium-XI-921, Germany) using enzymatic procedures with a diagnostic kit (Pars Azmoon Co, Tehran, Iran).

### Female Properties and Fertilization Assay

During stripping, females properties including egg diameter (mm), total weight of stripped eggs, number of eggs per gram, total weight of ovary, absolute fecundity, relative fecundity and gonadosomatic index (GSI) 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 gonadosomatic index is given by GSI= GW/W x 100, where GSI = gonadosomatic index; GW = gonad weight, and W = total weight. The fertilization trials was designed as follow: $T_1$: 2 years old male *Vs.* 2 years old female; $T_2$: 3 years old male *Vs.* 2 years old female; $T_3$: 2 years old male *Vs.* 3 years old female; $T_4$: 3 years old male

*V.* 3 years old female; $T_{5:}$ combination of 2 and 3 years old males *Vs.* 2 years old female; $T_{6:}$ combination of 2 and 3 years old males *Vs.* 3 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 $2\times10^9$. 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 1–2 days at water temperature 20-24.5°C. Following equations was used to calculate fertilization capacity. Larvae were saved to examine longitudinal growth until starting the active feeding. Then larvaes were separated and examined to measure the length in initiation of active feeding.

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)

Survival rate were calculated during initial feeding according to the following formulae:

Survival Rate = 100 (live larvae – dead larvae) / live larvae

### 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 10.0. Data are presented as mean±SD.

## Results

Sperm quality parameters of two age groups are shown in Table 1. There were significant differences in sperm density and percentage of motile spermatozoa between ages, but duration of sperm motility and spermatocrit did not differ from two age groups. Seminal plasma composition such as pH, chloride, ACP and ALP significantly changed between two age classes, whereas sodium, potassium, calcium and magnesium did not show significant differences. The females' properties of two age groups during spawning season are presented in Table 2. Among evaluated parameters just egg diameter showed significant difference between two age classes. The highest fertilization rate and hatching rate were observed in $T_2$ and m $T_3$ respectively (Figure 1 and 2). The highest survival rate was found in $T_3$ (Figure 3). Larvae length during hatching and initiation of active feeding were higher in $T_2$ and m $T_6$ respectively (Figure 4 and 5).

## Discussion

In commercial fish farming has been more focused on the quality of eggs and larvae rather than that of sperm, even though the sperm quality of male broodstock also affects the production of healthy larvae. Sperm quality is a very important variable in aquaculture broodstock management (Billard *et al*., 1995; Bromage, 1995), as it can influence the percentage of egg fertilization and thus the total production of viable eggs from given broodstock. Sperm quality is an important factor that increases the efficiency of artificial fertilization (Billard *et al*., 1995; Bromage, 1995). In our study, 3 years old

**Table 1.** The mean±SD of the sperm quality parameters in *Cyprinus carpio*

| Parameters | Age | |
|---|---|---|
| | 2 years old | 3 years old |
| Duration of sperm motility (sec) | 42±7.2 | 44±1.1 |
| Percentage of motile sperm (%) | 75±5.7 | 60±5.1 |
| Sperm density (ml×$10^{-9}$) | 17.6±5 | 24.6±6.4 |
| Spermatocrit (%) | 97±1.26 | 96±0.5 |
| Sodium ($mmol^{-1}$) | 24.7±12.4 | 26.7±12.6 |
| Potassium ($mmol^{-1}$) | 60.7±7.8 | 69±4.16 |
| Chloride ($mmol^{-1}$) | 215.2±14.4 | 74±10.4 |
| Calsium ($mmol^{-1}$) | 2.6 ±0.66 | 2.02±0.17 |
| Magnesium ($mmol^{-1}$) | 4.1±0.35 | 3.9±0.18 |
| Acid phosphatase (IU/L) | 385.7±28.1 | 182.7±21.3 |
| Alkaline phosphatase (IU/L) | 257±15.7 | 207.5±40.1 |
| pH | 7.7±0.26 | 8±0.26 |

**Table 2.** The mean±SD of the some properties of females carp

| Parameters | Age | |
|---|---|---|
| | 2 years old | 3 years old |
| Total weight of stripped eggs | 776±23.9 | 1031±20.5 |
| number of eggs per gram | 6875±21.06 | 638±27.7 |
| Total weight of ovary | 776±237.9 | 1031±205.7 |
| Egg diameter (mm) | 1.14±0.11[b] | 1.31±0.09[a] |
| Absolute fecundity | 523500±171.08 | 657701±133.2 |
| Relative fecundity | 123.6±52.1 | 131.4±52.02 |
| GSI (%) | 18.1±7.4 | 20.6±3.8 |

*The values with different letters are significantly different (P< 0.05) (n= 3).

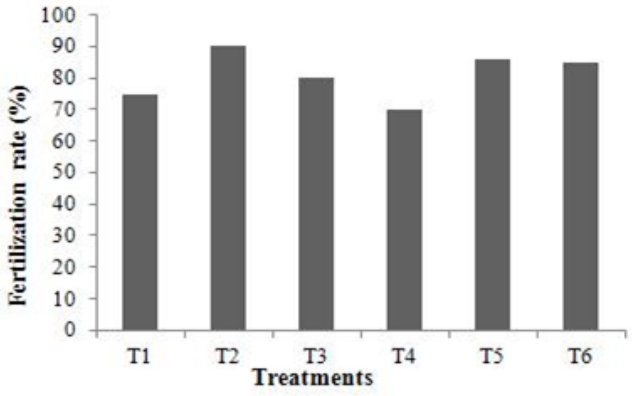


**Figure 1.** Fertilization rate in experimental treatments.

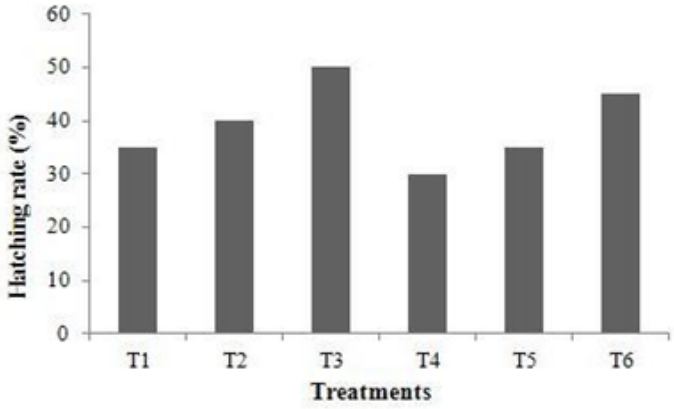


**Figure 2.** Hatching rate in experimental treatments.

males showed higher duration of sperm motility than in 2 years old individuals (P>0.05), but this trend was reverse for percentage of motile spermatozoa. The percentage of motile spermatozoa showed significant difference between two age classes and higher values were detected in 2 years old individuals. The percentages of motile sperm were not similar among the three groups of striped bass *Morone saxatilis* (Vuthiphandchai and Zohar, 1999) and smallmouth yellow fish *Barbus aeneus* (Vlok and Vuren, 1988). A similar tendency was observed in value of spermatocrit, but it was not statistically different between two age groups. Similar results were observed in sockeye salmon, *Oncorhynchus nerka* and Atlantic salmon, *Salmo salar* where the spermatocrit values were higher in 3 years old males than in other age classes (Hoysak and Liley, 2001; Daye and Glebe, 1984). Some authors confirmed that spermatocrit will decrease with increasing male broodstocks age (Tekin *et al*., 2003; Liley *et al*., 2002). In our experiment, sperm density decreased with age. An explanation for this is that sperm volume increased in larger fish and as the relationship between sperm volume and sperm density is reverse, therefore with increasing age, sperm volume will increase, but its concentration will decreases (Tekin *et*

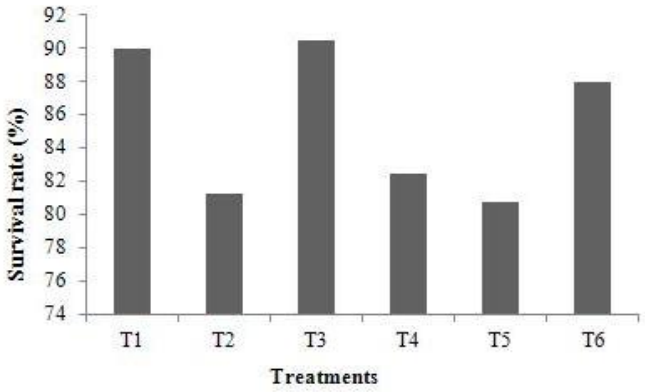


**Figure 3.** Survival rate in experimental treatments.

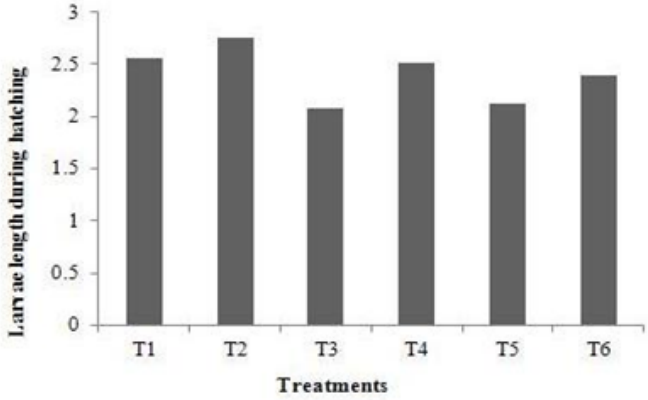


**Figure 4.** Larvae length during hatching in experimental treatments.

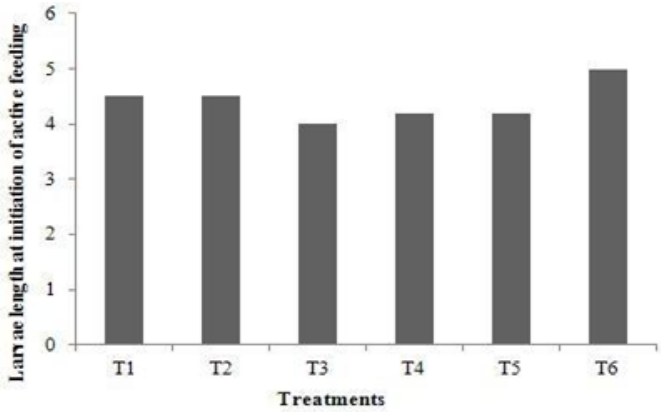


**Figure 5.** Larvae length at initiation of active feeding in experimental treatments.

*al*., 2003). 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 and 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 and Glebe, 1984). The differences may be due to feeding conditions, husbandry procedures, age, environmental factors, spawning time or dilution ratio. For successful production in aquaculture systems, it is necessary to have basic knowledge of the physical and chemical characteristics of the sperm to determine the reproductive ability of cultivated fish. The ionic and organic composition of the seminal plasma can indicate fish fertilization capacity (Ciereszko *et al*., 2000). Also, composition of seminal plasma has a great influence on the biological quality of the semen and these factors are directly related to the

fertilization success (Rurangwa *et al*., 2004; Alavi and Cosson, 2005). This may be also leads to better understanding of the mechanisms of fertilization. In our experiment chloride, ACP and ALP concentrations were higher in 2 years old males than those measured in 3 years old individuals ($P<0.05$), while seminal plasma pH was higher in 3 years old males than in 2 years old individuals ($P<0.05$). 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. Therefore, these have made it inevitable that brood fish are selected more carefully and raised in more suitable conditions. The egg diameter has good impact on fertilization rate and improvement 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 and Cumaranatunga, 1998; Bromage *et al*., 1992). In our study, number of eggs per gram were higher in 2 years old females compared to 3 years old individuals, but their values were not important statistically ($P>0.05$). This could be due to the smaller size of eggs in these females than in 3 years old females. Fish fecundity it is known to increase with the age of breeders (Reznick *et al*., 2002). Ridha and Cruz (1989) reported that, 1 year old Nile tilapia *Oreochromis niloticus* broodstock had a higher fecundity than in 2 and 5 years individuals. Total fecundity and egg size increase with age, while contrary relative fecundity decreases with age (Springate *et al*., 1984; Bromage and Cumaranatunga, 1988). The same researches have been demonstrated this increase, depending on fish age and fish size increase. Relative fecundity has been expected to decline with age (Siraj *et al*., 1983; Ridha and Cruz, 1989). In our experiment similar trends were observed in absolute fecundity and relative fecundity with age. In the present study two age classes of male and female carp broodstocks (i.e. 2 and 3 years old) were crossed to identify the best age with maximum reproductive performance. Brood stock age has effective influence on development stages after fertilization. The present study demonstrated how the age of brood fish can effect fertilization. The crossing fish with different ages can be used as a simple procedure to achieving better results of fertilization capacity in carp hatcheries. The results of this study can be used in artificial breeding programs to produce suitable larvae for breeding and reproduction.

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