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## The influence of the broodstock's age in propagation efficiency and reproduction ability of grass carp (*Ctenopharyngodon Idella Valenciennes, 1844*)

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| Article Info  | Abstract  |
|---|---|
| <p><b>Article history:</b></p> <p>Received: 3 December 2022</p> <p>Accepted: 23 May 2023</p>                        | <p>Grass carp is known as one of the most important species among warm water aquaculture in polyculture fish farming in Iran. The aims of this study were to evaluate the influence of broodstock's age on propagation efficiency and reproduction ability. In this study, 4 and 5-year-old male and female broodstocks (4 from each group) were used. The quality of ovule and sperm in grass carp broodstocks were studied. The results showed statistically significant difference in female fishes in terms of fork length, weight, egg diameter, and fecundity (<math>p &lt; 0.05</math>). In addition, significant difference was found respecting the factors of sperm motility and spermatocrit (in 4-year-old broodstocks, the maximum of <math>93.75 \pm 4.03</math>) (<math>p &lt; 0.05</math>). There was also statistically significant difference in terms of the sperm biochemical compositions between the 4 and 5-year-old groups (<math>p &lt; 0.05</math>). There was statistically significant difference in terms of hatching and larvae viability (<math>p &lt; 0.05</math>). The eggs produced from fertilization of 4-year-old males and 5-year-old females had the maximum average of fertilization rate (98%), hatching (94%), and larvae viability (90%). It was concluded that the broodstock's age is effective on the stages after fertilization. Consequently, using 5-year-old females and 4-year-old males in grass carp can improve the stages after fertilization until the incubation stages and the fish propagation centres can benefit from the results of this research in order to increase the quality of produced larvae.</p> <p>© 2024 Published by Amol University of Special Modern Technologies Press.<br/>This is an open-access article under the CC-BY 4.0 license. (<a href="https://creativecommons.org/licenses/by/4.0/">https://creativecommons.org/licenses/by/4.0/</a>)</p> |
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## Introduction

This research was conducted in July of 2013 at Garmabi Cooperative Fish Breeding Farm No. 12, located in Agha Seyed Sharif Village, Rasht City, Gilan Province, Iran. This center is private and cooperative and was built in 1358. The main activities of this center are the reproduction of Amur, carp, phytophagous, and bighead carp and the breeding of fattening fish. For many years, this cooperative has been known in the province and the country as a breeding center in the tropical fish sector. In order to carry out this research, a total of 16 male and female breeders of Amur fish in two age groups of 4 and 5 years (4 male and 4 female breeders from each group) were randomly selected from the breeding population. Their gender was determined by observing and touching their pectoral fins, so that the male broods had rough and jagged pectoral fins, but the female broods had soft pectoral fins, large abdominal fins, and red and swollen genital pore. Their gender was determined by observing and touching their pectoral fins, so that the male broods had rough and jagged pectoral fins, but the female broods had soft pectoral fins, large abdominal fins, and red and swollen genital pore. Three spawners were selected from each age group and to ensure their age, 3-4 scales were separated from the lower part of the dorsal fin and above the lateral line with tweezers and placed on a piece of paper, and the number of the fish under study was recorded on it. These scales were in the laboratory and after removing the layer of fat and mucus on it, it was placed on a slide and the age was determined by counting annual growth rings with a loupe (Prasenkende Haqit, 2019). Then, the fish used are caught slowly with a net from inside the relaxation ponds, and the head is usually covered with a cloth or bag to reduce stress on them, and then they are weighed with a scale. After that, based on the weight of the fish, we prepare the dried pituitary glands to be used.

Hormone injection in herbivorous carp was done by using a combination of 2 mg of pituitary gland, 0.4 µg of LHRH-A2 hormone, and 0.2 ml of physiological serum per kg of body weight. The use of 0.5 mg of pituitary gland, 0.4 µg of LHRH-A2 hormone along with 0.25 ml of physiological serum per kg of body weight at the same time as the second injection (about eight hours after the first injection) was done. About eight hours after the second calving, ovulation, and sperm extraction of the broodstock was done by the stripping method.

After spawning and sperm collection from the broodstock, all the eggs obtained from each broodstock were first weighed and then poured into three separate containers at the rate of 250 g per egg pan (with three repetitions). The eggs were olive green in color and immediately the fish was transferred to fresh water; the diameter of 10 eggs of each spawner was measured using calipers with an accuracy of 0.02, and the eggs obtained from all spawners of the same age were mixed together until the breeding conditions for fish of the same age were the same and the drop the quality of one of the generators does not have much effect on conception. Before the egg and sperm combination operation, the trays and incubators used were numbered. Then, the fertilized eggs were transferred to the incubator. Incubation of Amur eggs in the workshop, we used Zuk-type cone-shaped incubators. Water flows under the incubator and the eggs are rotated so that enough oxygen reaches all the eggs and the possibility of losses in them is minimized. At this stage, the amounts of oxygen and water temperature were also measured.

## Materials and Methods

### Measurement of cognitive sperm parameters

#### Spermatoctrit measurement

To calculate the amount of spermatoctrit from the sperm of producers of each age group before mixing them (individual sperm of male producers), sampling was done. Sampling by microhematocrit tube it was done (Aas *et al.*, 1991; Suquet *et al.*, 1992; Rakitin *et al.*, 1999; Tvedt, 2001). Sperm was pulled into the microhematocrit tube based on capillary characteristics and then the end of the capillary tube was blocked with a special paste (Suquet *et al.*, 1992). Then, the samples were centrifuged with a microcentrifuge device (Aas *et al.*, 1991; Rakitin *et al.*, 1999; Liley *et al.*, 2002) for five minutes at 14000 revolutions per minute (Vladi *et al.*, 2002) and then, the spermatoctrit level of each sample was read using a special ruler for spermatoctrit percentage (Suquet *et al.*, 1999). Measurement of the percentage and length of the movement period to check the movement of sperm due to the high concentration of sperm, it must first be diluted to determine its form and activity. Dilution is usually done in a ratio of one to twenty. For this purpose, we first poured 40 ml of water or physiological serum on the slide and then added 2 ml of sperm to it and then made a spread and examined the movement of sperm under the microscope with a magnification of 400 (Suquet *et al.*, 1992).

## Measurement of sperm density

In order to count and determine the density of the spermatozoa collected from the broodstock, we first diluted them and then counted them using a special hemacytometer slide and a microscope. Special pipettes for red blood cell counting, called red melangor, were used for sperm dilution (Suquet *et al*, 1992).

## Examining sperm ion parameters

To check the amount of sodium using the flame measurement method and using the Jenwa film photometer, and to evaluate the amount of calcium ions (Man Company kit) was used using the colorimetric method (methyl thymol blue method). Also, to calculate the amount of magnesium ion (Pars Azmun Company kit) by colorimetric or photometric method (using Xylidyl Blue dye) and the amount of glucose of sperm (Pars Azmun Company kit) by enzymatic method was used. pH was measured with a PHS30 pen pH meter made in China. To determine the amount of acid phosphatase, autoanalyzer model BR made by Italian company and RA1000 - Technicon model made by American company were used and the quantitative detection of alkaline phosphatase (Pars Azmun Company kit) was used by DGKC (German Biochemical Society Standard) method or photometric method. To measure the amount of chloride, potassium, sodium, and phosphorus, an electrode analyzer with the model Caretium-XI-921 Series of German technology and colorimetric method was used.

Determination of fertilization percentage, hatching rate and survival percentage of larvae after carrying out fertilization and transferring the fertilized eggs to zooc type incubators, we should measure the rate of fertilization, hatching and survival of the resulting larvae to check the success of the work. For this purpose, about six hours after fertilization, we removed about 100 eggs from each incubator and separated and counted the hatched eggs with embryos from the eggs that were probably white or cloudy and the embryo was not visible inside them (Razavi). Sayad, 1374 (of course, we used a loop to ensure the work) then the percentage of conception was calculated from the following formula: Fertilization percentage = (total amount of eggs/number of fertilized eggs) 100

Hatching percentage = number of fertilized eggs / number of larvae) 100

**Table 1.** Treatment table for fish breeders affairs.

| Age | Standard deviation<br>± Average | Minimum | Maximum |
|-----|---------------------------------|---------|---------|
| 4   | 25.86 ± 63.2                    | 84      | 90      |
| 5   | 29.1 ± 5.95                     | 94      | 97      |

## Results

### Comparison of the total length of female genitalia

According to the obtained results, in 4-year-old female fish, the mean and standard deviation of the total length factor was equal to  $86.25 \pm 2.63$  cm (minimum 84 and maximum 90 cm) and in 5-year-old female fish it was equal to  $5.29 \pm 1.5$  cm (minimum 94 and maximum 97 cm). Thus, the total length in 5-year-old female reproductive was more than the total length in 4-year-old female reproductive. (Table 2). According to the t-test, there was a statistically significant difference between the ages of 4 and 5 years in terms of total length ( $p < 0.05$ ) ( $t = -6.315$ ,  $df = 6$ ,  $Sig.=0.001$ ).

**Table 2.** The values (mean ± standard deviation) of the total length of female reproductive organs based on age.

| Age | Standard deviation<br>± Average (cm) | Minimum<br>(cm) | Maximum<br>(cm) |
|-----|--------------------------------------|-----------------|-----------------|
| 4   | 82 ± 2.83                            | 80              | 86              |
| 5   | 91.5 ± 1.25                          | 90              | 93              |

### Comparison of fork length of female reproductive

According to the obtained results, in 4-year-old female fish, the mean and standard deviation of the fork length factor was  $82 \pm 2.83$  cm (minimum 80 and maximum 86 cm) and in 5-year-old female fish it was  $91.5 \pm 1.29$  cm (minimum 90 and maximum 93 cm). Thus, the fork length in 5-year-old female breeders was higher than the length of the fork in 4-year-old female breeders (Table 3). According to the t-test, between the ages of 4 and 5 years, a statistically significant difference was observed in terms of fork length ( $p < 0.05$ ) ( $t = -6.11$ ,  $df = 6$ ,  $Sig.=0.001$ ).

**Table 3.** values (mean and standard deviation) of the fork length of female producers based on age.

| Productive male and female                    |
|---|
| Female 4 years old × Male 5 years old         |
| 4-year-old female × 4-year-old male           |
| 5-year-old female × 4-year-old male           |
| 4-year-old female × 5-year-old male           |
| Female 4 years old × Male (4 and 5) years old |
| Female 4 years old × Male 5 years old         |

**Comparison of body weight of female producers**

According to the obtained results, in 4-year-old female fish, the mean and standard deviation of weight was equal to  $9025 \pm 250$  g (minimum 8700 and maximum 9300 g) and in 5-year-old female fish, the mean and standard deviation of weight was equal to  $10450 \pm 129.1$  g (minimum 10300 and maximum 10600 g). Thus, the body weight of 5-year-old females was higher than that of 4-year-old females (Table 4). According to the t-test, there was a statistically significant difference between the age factor of 4 and 5 years in terms of body weight ( $p < 0.05$ ) ( $t = -10.129$ ,  $df = 6$ ,  $Sig.=0.000$ ).

**Table 4.** Values (mean  $\pm$  standard deviation) of body weight of female reproductive based on age.

| Age | Standard deviation $\pm$ Average (g) | Minimum (g) | Maximum (g) |
|-----|--------------------------------------|-------------|-------------|
| 4   | $9025 \pm 250$                       | 8700        | 9300        |
| 5   | $10450 \pm 129.1$                    | 10300       | 10600       |

**Comparison of total egg weight**

According to the obtained results, in 4-year-old female fish, the mean and standard deviation of the total weight of extracted eggs was equal to  $455.32 \pm 10.79$  g (minimum 440.33 and maximum 465.28 g) and in 5-year-old female fish, the mean and standard deviation of the total weight of retrieved eggs was equal to  $530.09 \pm 4.83$  g (minimum 525.08 and maximum 536.61 g). Thus, the retrieved egg weight was observed in 5-year-old female breeders than in 4-year-old female breeders (Table 5).

According to the t-test, there was a statistically significant difference between the age factor of 4 and 5 years in terms of the total weight of the extracted eggs ( $p < 0.05$ ) ( $t = -12.641$ ,  $df = 6$ ,  $Sig.=0.000$ ).

**Table 5.** The values (mean  $\pm$  standard deviation) of the total weight of the extracted eggs of female producers.

| Age | Standard deviation $\pm$ Average (g) | Minimum (g) | Maximum (g) |
|-----|--------------------------------------|-------------|-------------|
| 4   | $455.32 \pm 10.79$                   | 440.33      | 465.28      |
| 5   | $530.09 \pm 4.83$                    | 525.08      | 536.61      |

**Comparison of extracted egg diameter**

According to the obtained results, in 4-year-old female fish, the mean and standard deviation of the diameter of the retrieved eggs was equal to  $1.47 \pm 0.054$  mm (minimum 1.4 and maximum 1.52 mm), in 5-year-old fish, the mean and standard deviation was

equal to  $1.64 \pm 0.035$  mm (minimum 1.6 and maximum 1.68 mm). Thus, the diameter of the extracted egg in 5-year-old female producers was more than the diameter of the extracted egg in 4-year-old female producers (Table 6). According to the t-test, there was a statistically significant difference between the ages of 4 and 5 years in terms of egg diameter ( $p < 0.05$ ) ( $t = -5.299$ ,  $df = 6$ ,  $Sig.=0.002$ ).

**Table 6.** The values (mean  $\pm$  standard deviation) of the diameter of the extracted egg of female reproductive based on age.

| Age | Standard deviation $\pm$ Average (mm) | Minimum (mm) | Maximum (mm) |
|-----|---------------------------------------|--------------|--------------|
| 4   | $1.47 \pm 0.054$                      | 1.4          | 1.52         |
| 5   | $1.64 \pm 0.035$                      | 1.6          | 1.68         |

**Comparison of the number of eggs per gram**

According to the obtained results, in 4-year-old female fish, the mean and standard deviation of the number of eggs per gram was equal to  $527.5 \pm 21.02$  g (minimum 500 and maximum 501 g) and in 5-year-old female fish, the mean and standard deviation was equal to  $507.5 \pm 4.66$  g (minimum 505 and maximum 512 g). Thus, the number of eggs per gram of body weight in 4-year-old females was more than that of 5-year-old females. (Table 7). According to the t-test, no statistically significant difference was observed between the number of eggs per gram based on the age of 4 and 5 years ( $p > 0.05$ ) ( $t = 1.858$ ,  $df = 6$ ,  $Sig.=0.112$ ).

**Table 7.** The values (mean  $\pm$  standard deviation) of the number of eggs per gram of fertile females based on age.

| Age | Standard deviation $\pm$ Average (g) | Minimum (g) | Maximum (g) |
|-----|--------------------------------------|-------------|-------------|
| 4   | $527.5 \pm 21.02$                    | 500         | 501         |
| 5   | $507.5 \pm 4.66$                     | 505         | 512         |

**Comparison of the total length of male progenitors**

The results showed that in 4-year-old male fish, the mean and standard deviation of the total length factor was  $88.5 \pm 3.42$  cm (minimum 84 and maximum 92 cm), in 5-year-old male fish it was  $90.75 \pm 3.4$  cm (minimum 86 and maximum 94 cm). So, the total length of 5-year-old males was more than that of 4-year-old males (Table 8). According to the t-test, there was no statistically significant difference between the ages of 4 and 5 years in terms of total length ( $p > 0.05$ ) ( $t = -0.933$ ,  $df = 6$ ,  $Sig.=0.387$ ).

**Table 8.** Values (mean  $\pm$  standard deviation) of the total length of male reproductive based on age.

| Age | Standard deviation $\pm$ Average (cm) | Minimum (cm) | Maximum (cm) |
|-----|---------------------------------------|--------------|--------------|
| 4   | 88.5 $\pm$ 3.42                       | 84           | 92           |
| 5   | 90.75 $\pm$ 3.4                       | 86           | 94           |

### Comparison of fork length of male progenitors

The results showed that in 4-year-old male fish, the mean and standard deviation of the fork length factor was 84.5  $\pm$  2.65 cm (minimum 81 and maximum 87 cm), in 5-year-old male fish it was 87  $\pm$  3.74 cm (minimum 82 and maximum 91 cm) So, the fork length in 5-year-old males was more than that of 4-year-old males (Table 9).

According to the t-test, there was no statistically significant difference between the ages of 4 and 5 years in terms of fork length ( $p > 0.05$ ) ( $t = -1.091$ ,  $df = 6$ ,  $Sig.=0.317$ ).

**Table 9.** The values (mean  $\pm$  standard deviation) of the fork length of male breeders based on age.

| Age | Standard deviation $\pm$ Average (cm) | Minimum (cm) | Maximum (cm) |
|-----|---------------------------------------|--------------|--------------|
| 4   | 84.5 $\pm$ 2.65                       | 81           | 87           |
| 5   | 87 $\pm$ 4.74                         | 82           | 91           |

### Comparison of body weight of male reproductive

The results showed that in 4-year-old male fish, the mean and standard deviation of the weight factor was equal to 6900  $\pm$  1432.9 g (minimum 5500 and maximum 8900 g), in 5-year-old male fish it was 8525  $\pm$  499.2 g (minimum 8000 and maximum 9000 g). So, the body weight of 5-year-old males was more than that of 4-year-old males (Table 10).

According to the t-test, no statistically significant difference was observed between the body weight factor based on the age of 4 and 5 years ( $p > 0.05$ ) ( $t = -2.142$ ,  $df = 6$ ,  $Sig.=0.076$ ).

**Table 10.** Values (mean  $\pm$  standard deviation) of body weight of male breeders based on age.

| Age | Standard deviation $\pm$ Average (g) | Minimum (g) | Maximum (g) |
|-----|--------------------------------------|-------------|-------------|
| 4   | 6900 $\pm$ 1432.9                    | 5500        | 8900        |
| 5   | 8525 $\pm$ 499.2                     | 8000        | 9000        |

### Investigating sodium factor in fish sperm

According to the results, in 4-year-old male fish, the

mean and standard deviation of the sodium factor in sperm was 40.75  $\pm$  1.71 mEq/l (minimum 39 and maximum 43 mEq/l), and in a 5-year-old male, the mean and standard deviation of the sodium factor in sperm was 33.75  $\pm$  2.06 mEq/l (minimum 31 and maximum 36 mEq/l). So, the average sodium in 5-year-old male breeders was more than that of 4-year-old male breeders (Table 11).

According to the t-test, a statistically significant difference was observed between the ages of 4 and 5 years in terms of sodium in sperm ( $p < 0.05$ ) ( $t = 5.23$ ,  $df = 6$ ,  $Sig.=0.002$ ).

**Table 11.** Values (mean  $\pm$  standard deviation) of sperm sodium of male producers according to age.

| Age | Standard deviation $\pm$ Average (mEq) | Minimum (mEq) | Maximum (mEq) |
|-----|--|---------------|---------------|
| 4   | 40.75 $\pm$ 1.71                       | 39            | 43            |
| 5   | 33.75 $\pm$ 2.06                       | 31            | 36            |

### Investigating potassium factor in fish sperm

According to the results, in 4-year-old male fish, the average and standard deviation of the potassium factor in sperm was 50.75  $\pm$  3.1 (minimum 48 and maximum 55), and in 5-year-old male fish, the average and standard deviation of potassium factor was 35.25  $\pm$  7.00 (minimum 28 and maximum 45). Five-year-old male progenitors showed lower amount of potassium in sperm compared to 4-year-old male (Table 12).

According to the t-test, a statistically significant difference was observed between the ages of 4 and 5 years in terms of potassium in sperm ( $p < 0.05$ ) ( $t = 3.921$ ,  $df = 6$ ,  $Sig.=0.008$ ).

**Table 12.** Values (mean  $\pm$  standard deviation) of sperm potassium of male reproductive according to age.

| Age | Standard deviation $\pm$ Average | Minimum | Maximum |
|-----|----------------------------------|---------|---------|
| 4   | 50.75 $\pm$ 3.1                  | 48      | 55      |
| 5   | 35.25 $\pm$ 7.27                 | 28      | 45      |

### Investigation of calcium factor in fish sperm

According to the obtained results, in 4-year-old male fish, the mean and standard deviation of the calcium factor in sperm was equal to 3.013  $\pm$  1.54 mg/dl (minimum 1.8 and maximum 5 mg/dl) and in 5-year-old male fish, the average and standard deviation of calcium in sperm was equal to 5.3  $\pm$  4.1 mg/dl (minimum 1.1 and maximum 9.8 mg/dl). So, the amount of calcium present in 4-year-old male fish showed more sperm compared to a 5-year-old male

(Table 13). According to the t-test, there was no statistically significant difference between the ages of 4 and 5 years in terms of calcium in sperm ( $p > 0.05$ ) ( $t = -1.045$ ,  $df = 3.826$ ,  $Sig.=0.336$ ).

**Table 13.** Values (mean ± standard deviation) of sperm calcium of male reproductive according to age.

| Age | Standard deviation ± Average (mg/dl) | Minimum (mg/dl) | Maximum (mg/dl) |
|-----|--------------------------------------|-----------------|-----------------|
| 4   | 3.013 ± 1.54                         | 1.8             | 5               |
| 5   | 5.3 ± 4.1                            | 1.1             | 9.8             |

### Investigating the chloride factor in fish sperm

According to the obtained results, in 4-year-old male fish, the mean and standard deviation of factor chloride in sperm was equal to  $71.75 \pm 1.26$  mEq/l (minimum 70 and maximum 73 mEq/l) and in 5-year-old male fish, the mean and standard deviation of chloride in sperm was equal to  $60.75 \pm 3.3$  mEq/l (minimum 57 and maximum 65 mEq/l). So, in 4-year-old male progenitors, the amount of chloride in sperm was more than that of 5-year-old male (Table 14).

According to the t-test, there was a statistically significant difference between the ages of 4 and 5 years in terms of chloride in sperm ( $p < 0.05$ ) ( $t = 6.223$ ,  $df = 6$ ,  $Sig.=0.001$ ).

**Table 14.** Values (mean ± standard deviation) of sperm chloride of male reproductive according to age.

| Age | Standard deviation ± Average (mEq/l) | Minimum (mEq/l) | Maximum (mEq/l) |
|-----|--------------------------------------|-----------------|-----------------|
| 4   | 71.75 ± 1.26                         | 70              | 73              |
| 5   | 60.75 ± 3.3                          | 57              | 65              |

### Investigating the magnesium factor in fish sperm

According to the obtained results, in 4-year-old male fish, the mean and standard deviation of magnesium factor in sperm was  $4.5 \pm 0.87$  mEq/l (minimum 3.9 and maximum 5.8 mEq/l) and in 5-year-old male fish, the mean and standard deviation of magnesium in sperm was  $7.05 \pm 3.8$  mEq/l (minimum 3.1 and maximum 11.6 mEq/l). So, in 4-year-old males, the amount of magnesium in sperm was lower than that of 5-year-old males (Table 15).

According to the t-test, there was no statistically significant difference between the ages of 4 and 5 years in terms of the magnesium factor in sperm ( $p > 0.05$ ) ( $t = -1.306$ ,  $df = 3.317$ ,  $Sig.=0.275$ ).

**Table 15.** Values (mean ± standard deviation) of sperm magnesium of male reproductive according to age.

| Age | Standard deviation ± Average (mEq/l) | Minimum (mEq/l) | Maximum (mEq/l) |
|-----|--------------------------------------|-----------------|-----------------|
| 4   | 4.5 ± 0.87                           | 3.9             | 5.8             |
| 5   | 7.05 ± 3.8                           | 3.1             | 11.6            |

### Investigating the glucose factor in fish sperm

According to the results, in 4-year-old male fish, the mean and standard deviation of the standard factor of glucose in sperm was  $6.45 \pm 2.53$  mg/dl (minimum 4.5 and maximum 10 mg/dl) and in 5-year-old male fish, the mean and standard deviation of glucose in sperm was  $5.125 \pm 0.63$  mg/dl (minimum 4.5 and maximum 6 mg/dl). So, in 4-year-old males, the amount of glucose in sperm was more than that of 5-year-old males (Table 16).

According to the t-test, there was no statistically significant difference between the ages of 4 and 5 years in terms of the glucose factor in sperm ( $p > 0.05$ ) ( $t = 1.018$ ,  $df = 6$ ,  $Sig.=0.348$ ).

**Table 16.** Values (mean ± standard deviation) of sperm glucose of male reproductive according to age.

| Age | Standard deviation ± Average (mg/dl) | Minimum (mg/dl) | Maximum (mg/dl) |
|-----|--------------------------------------|-----------------|-----------------|
| 4   | 6.45 ± 2.53                          | 4.5             | 10              |
| 5   | 5.125 ± 0.63                         | 4.5             | 6               |

### Investigation of acid phosphatase factor in fish sperm

According to the obtained results, in 4-year-old male fish, the mean and standard deviation of the acid phosphatase factor present in sperm was equal to  $425.5 \pm 96.48$  IU/l (minimum 361 and maximum 569 IU/l) and in 5-year-old male fish, the mean and standard deviation of acid phosphatase was equal to  $451.5 \pm 101.76$  IU/l (minimum 326 and maximum 570 IU/l). Thus, the amount of acid phosphatase in sperm was lower in 4-year-old males than in 5-year-old males (Table 17). According to the Mann-Whitney non-parametric test, no statistically significant difference was observed between the ages of 4 and 5 years in terms of acid phosphatase in sperm ( $p > 0.05$ ) (Mann-Whitney  $U=6$ ,  $Sig.=0.564$ ).

**Table 17.** Values (mean  $\pm$  standard deviation) of sperm acid phosphatase of fertile males according to age.

| Age | Standard deviation $\pm$ Average (IU/l) | Minimum (IU/l) | Maximum (IU/l) |
|-----|---|----------------|----------------|
| 4   | 425.5 $\pm$ 96.48                       | 361            | 569            |
| 5   | 451.5 $\pm$ 101.76                      | 326            | 570            |

### Investigation of alkaline phosphatase factor in fish sperm

According to the results, in 4-year-old male fish, the mean and standard deviation of the alkaline phosphatase factor in sperm was  $200 \pm 140.22$  IU/l (minimum 120 and maximum 410 IU/l) and in 5-year-old male fish, the mean and standard deviation of alkaline phosphatase in sperm was equal to  $454.75 \pm 702.96$  IU/l (minimum 85 and maximum 1509 IU/l). Thus, the amount of alkaline phosphatase in the sperm of 4-year-old male progenitors was lower than that of 5-year-old male (Table 18). According to the Mann-Whitney non-parametric test, there was no statistically significant difference in terms of the alkaline phosphatase factor present in sperm between the ages of 4 and 5 years ( $p > 0.05$ ) (Mann-Whitney U=4, Sig.=0.248).

**Table 18.** Values (mean  $\pm$  standard deviation) of sperm alkaline phosphatase of fertile males according to age.

| Age | Standard deviation $\pm$ Average (IU/l) | Minimum (IU/l) | Maximum (IU/l) |
|-----|---|----------------|----------------|
| 4   | 200 $\pm$ 140.22                        | 120            | 410            |
| 5   | 454.75 $\pm$ 702.96                     | 85             | 1509           |

### The results of sperm concentration in fish

According to the results, in 4-year-old male fish, the mean and standard deviation of density factor was equal to  $29881250000 \pm 1123302683$  mm<sup>3</sup> (minimum 28520000000 and maximum 31220000000 mm<sup>3</sup>) and in 5-year-old male fish, the mean and standard deviation of density factor was  $4092312916 \pm 27797500000$  mm<sup>3</sup> (minimum 24120000000 and maximum 33210000000 mm<sup>3</sup>). So, in 5-year-old male progenitors, the amount of density in sperm was lower than that of 4-year-old male (Table 19).

According to the t-test, there was no statistically significant difference between the ages of 4 and 5 years in terms of density factor in sperm ( $p > 0.05$ ) ( $t = 0.982$ ,  $df = 6$ , Sig.=0.364).

**Table 19.** Values (mean  $\pm$  standard deviation) of sperm density of male reproductive based on age.

| Age | Standard deviation $\pm$ Average (mm <sup>3</sup> ) | Minimum (mm <sup>3</sup> ) | Maximum (mm <sup>3</sup> ) |
|-----|---|----------------------------|----------------------------|
| 4   | 29881250000 $\pm$ 1123302683                        | 28520000000                | 31220000000                |
| 5   | 27797500000 $\pm$ 4092312916                        | 24120000000                | 33210000000                |

### The results of spermatocrit in fish sperm

According to the results, in 4-year-old male fish, the mean and standard deviation of spermatocrit was  $93.75 \pm 4.03\%$  (minimum 89 and maximum 98%), and in 5-year-old male fish, the mean and standard deviation was  $75 \pm 3.3$  (minimum 81 and maximum 89%). So, the average percentage of spermatocrit and standard deviation was found to be higher in 4-year-old males than in 5-year-old males.

According to the t-test, a statistically significant difference was observed in terms of the spermatocrit factor between the ages of 4 and 5 years ( $p < 0.05$ ) ( $t = 3.453$ ,  $df = 6$ , Sig.=0.014).

### Results of sperm motility duration in fish

According to the obtained results, in 4-year-old male fish, the mean and standard deviation of the movement duration factor was  $29.25 \pm 11.79$  seconds (minimum 17 and maximum 45 seconds) and in 5-year-old male fish, the mean and standard deviation was  $40.5 \pm 7$  seconds (minimum 33 and maximum 48 seconds). So, the average duration of sperm motility in 5-year-old males was less than that of 4-year-old males (Table 20).

According to the t-test, there was no statistically significant difference between the ages of 4 and 5 in terms of the sperm motility duration factor ( $p > 0.05$ ) ( $t = -1.605$ ,  $df = 6$ , Sig.=0.16).

**Table 20.** Values (mean  $\pm$  standard deviation) of sperm motility duration in male offspring according to age.

| Age | Standard deviation $\pm$ Average (second) | Minimum (second) | Maximum (second) |
|-----|---|------------------|------------------|
| 4   | 78 $\pm$ 4.32                             | 72               | 82               |
| 5   | 87 $\pm$ 4.24                             | 82               | 91               |

### Results of sperm pH in fish

According to the results obtained in 4-year-old male fish, the mean and standard deviation of sperm pH factor was  $8.08 \pm 0.52$  (minimum 7.6 and maximum 8.8) and in 5-year-old male fish, the mean and standard deviation of sperm pH was equal to  $8.17 \pm 0.42$  (minimum 7.7 and maximum 8.62). Thus, sperm pH

was higher in 5-year-old male producers than in 4-year-old male producers (Table 21). According to the t-test, there was no statistically significant difference between the ages of 4 and 5 years in terms of sperm pH ( $p > 0.05$ ) ( $t = -0.254$ ,  $df = 6$ ,  $Sig.=0.808$ ).

**Table 21.** values (mean ± standard deviation) of sperm pH of male producers according to age.

| Age | Standard deviation ± Average | Minimum | Maximum |
|-----|------------------------------|---------|---------|
| 4   | 8.08 ± 0.52                  | 7.6     | 8.8     |
| 5   | 8.17 ± 0.42                  | 7.7     | 8.62    |

### Fertilization factor of fish

According to the results obtained from the study of the treatments, the treatment of 5-year-old female and 5-year-old male had the highest average percentage of conception with a value of  $94.33 \pm 4.04\%$ , and the treatment of 4+5-year-old male and 4-year-old female has the lowest average percentage of conception with the value of a was  $85\% \pm 5$  (Table 22).

According to the one-way analysis of variance test, we concluded that there is no statistically significant difference between the studied treatments (age combination) in terms of the percentage of conception ( $p > 0.05$ ) ( $F (5,12) = 1.373$ ,  $Sig. = 0.301$ ).

**Table 22.** Values (mean ± standard deviation) of fertilization percentage in the studied treatments.

| Treatment | Treatment           | Standard deviation ± Average | Minimum | Maximum |
|-----------|---------------------|------------------------------|---------|---------|
| 1         | 4 male x 4 female   | 90 ± 5 <sup>a</sup>          | 85      | 95      |
| 2         | 5 male x 4 female   | 90 ± 5 <sup>a</sup>          | 85      | 95      |
| 3         | 4 Male x 5 female   | 90 ± 5 <sup>a</sup>          | 85      | 95      |
| 4         | 5 male x 5 female   | 33.94 ± 4.4 <sup>a</sup>     | 90      | 98      |
| 5         | 4+5 male x 5 female | 91 ± 1 <sup>a</sup>          | 90      | 92      |
| 6         | 4+5 male x 4 female | 85 ± 5 <sup>a</sup>          | 80      | 90      |

### Fish spawning rate factor

According to the observed results, the treatments (4+5-year-old male and 5-year-old female) had the highest average spawning percentage with a c value of  $80 \pm 5\%$ , and the treatment (4-year-old male and 4-year-old female) had the lowest average spawning percentage with a value of 52 It was  $32.67 \pm 2.0\%$  (Table 23).

According to the one-way analysis of variance test, we concluded that there is no statistically significant difference between the studied treatments (age combination) in terms of mean percentage ( $p < 0.05$ ) ( $F (5,12) = 36.185$ ,  $Sig. = 0.000$ ) and Tukey's test showed that statistically significant differences are observed between the following treatments in pairs in terms of the mean percentage.

**Table 23.** Values (mean ± standard deviation) percentage of spawning rate in the studied treatments.

| Treatment | Treatment           | Standard deviation ± Average | Minimum | Maximum |
|-----------|---------------------|------------------------------|---------|---------|
| 1         | 4 male x 4 female   | 32.67 ± 2.52 <sup>a</sup>    | 30      | 35      |
| 2         | 5 male x 4 female   | 70 ± 5 <sup>ab</sup>         | 65      | 75      |
| 3         | 4 Male x 5 female   | 65 ± 5 <sup>b</sup>          | 60      | 70      |
| 4         | 5 male x 5 female   | 70 ± 5 <sup>ab</sup>         | 65      | 75      |
| 5         | 4+5 male x 5 female | 80 ± 5 <sup>c</sup>          | 75      | 85      |
| 6         | 4+5 male x 4 female | 60 ± 5 <sup>b</sup>          | 55      | 65      |

The results showed that the treatment (5-year-old female and 4-year-old male) has the highest mean percentage of survival until the yolk sac absorption stage with b value of  $90.14 \pm 1.02\%$  and the treatment (5-year-old female and 5+4-year-old male) had the lowest mean percentage of survival until the yolk sac absorption stage with a value of  $80.78 \pm 0.67\%$ .

According to the one-way analysis of variance test, we concluded that there is a statistically significant difference between the studied treatments in terms of the average percentage of survival ( $p < 0.05$ ) ( $F (5,12) = 45.929$ ,  $Sig. = 0.000$ ) and Tukey's test showed that between the following treatments in pairs, statistical significance is observed in terms of the mean survival percentage.

### The results of the initial length of fish larvae

According to the observed results, the treatment (5-year-old male and 5-year-old female) had the highest average initial larval length with value of  $4.7 \pm 0.25$  mm (minimum 4.45 and maximum 4.95 mm) and treatment (female 5 years old and male 5+4 years old) had the lowest mean initial larval length with a value of  $3.9 \pm 0.23$  mm (minimum 3.7 and maximum 4.15 mm) (Table 24).

According to the one-way analysis of variance test, we concluded that there is a statistically significant difference between the investigated treatments in terms

of the average initial length of larvae ( $p < 0.05$ ) ( $F(5,12) = 5.987$ , Sig. = 0.005) and Tukey's test showed that between the following treatments in pairs in terms of the average initial length of Ahnlaf larvae, statistically significant is observed.

**Table 24.** Values (mean  $\pm$  standard deviation) of the initial length of larvae in the studied treatments.

| Treatment | Treatment           | Standard deviation $\pm$ Average | Minimum | Maximum |
|-----------|---------------------|----------------------------------|---------|---------|
| 1         | 4 male x 4 female   | 4.22 $\pm$ 0.197 <sup>ab</sup>   | 1.4     | 4.4     |
| 2         | 5 male x 4 female   | 4.4 $\pm$ 0.18 <sup>ab</sup>     | 25.4    | 6.4     |
| 3         | 4 Male x 5 female   | 4.25 $\pm$ 0.2 <sup>ab</sup>     | 5.4     | 4.45    |
| 4         | 5 male x 5 female   | 4.7 $\pm$ 0.25 <sup>b</sup>      | 4.45    | 95.4    |
| 5         | 4+5 male x 5 female | 3.9 $\pm$ 0.23 <sup>a</sup>      | 7.3     | 15.4    |
| 6         | 4+5 male x 4 female | 4.05 $\pm$ 0.09 <sup>a</sup>     | 96.3    | 14.4    |

### Results of the secondary length of fish larvae

According to the observed results, the treatment of 5-year-old female and 5+4-year-old male had the highest average length of secondary larvae with a value of  $4.98 \pm 0.03$  mm (minimum 4.95 and maximum 5 mm) and the treatment of 4-year-old male and female 5-year-old had the lowest mean length of secondary larvae with a value of  $4.52 \pm 0.11$  mm (minimum 4.41 and maximum 4.63 mm) (Table 25).

According to the one-way analysis of variance test, we concluded that there is no statistically significant difference between the studied treatments in terms of the average length of the secondary larvae ( $p > 0.05$ ) ( $F(5,12) = 1.64$ , Sig. = 0.223).

**Table 25.** The values (mean  $\pm$  standard deviation) of the secondary length of larvae in the studied treatments.

| Treatment | Treatment           | Standard deviation $\pm$ Average | Minimum | Maximum |
|-----------|---------------------|----------------------------------|---------|---------|
| 1         | 4 male x 4 female   | 4.52 $\pm$ 0.11 <sup>a</sup>     | 4.41    | 63.4    |
| 2         | 5 male x 4 female   | 4.58 $\pm$ 0.44 <sup>b</sup>     | 12.4    | 5       |
| 3         | 4 Male x 5 female   | 4.85 $\pm$ 0.17 <sup>a</sup>     | 68.4    | 1.5     |
| 4         | 5 male x 5 female   | 4.82 $\pm$ 0.19 <sup>b</sup>     | 63.4    | 5       |
| 5         | 4+5 male x 5 female | 4.98 $\pm$ 0.03 <sup>ab</sup>    | 95.4    | 5       |
| 6         | 4+5 male x 4 female | 4.52 $\pm$ 0.41 <sup>a</sup>     | 12.4    | 93.4    |

## Discussion

### Investigating the reproductive capacity of female producers and their effect on the efficiency of artificial propagation of Amur fish

Investigating the fertility effect of female breeders in the process of incubation and survival of the produced larvae requires the examination of the quality of the eggs obtained from them, and the use of high-quality gametes from breeding breeders is very important in ensuring the production of better larvae (Kjordvik *et al.*, 1990). Based on the results obtained in the present research, 5-year-old females produced larger eggs compared to 4-year-old females, and mating was more at older ages. This result has been proven in many past studies including Alp *et al.* (2002) on brown trout (*Salmo trutta macrostigma*) that 5-year-old spawners were more fertile than younger spawners. Also, this result has been proven by Pitman (1979) and Shamspour (2007) on rainbow trout (*Oncorhynchus mykiss*) that 5-year-old female spawners produced larger eggs with greater larval emergence ability compared to younger spawners. It led to the production of larger larvae with faster growth and finger heights with fewer losses. The positive effect of female reproductive size on mating was shown by Quinn and Bloomberg (1992) on Chinook salmon and in the case of wild and farmed Atlantic salmon. Also, the results of the studies were similar to this research.

The result obtained by Zhatkosh (1389) which was carried out on Sargandeh carp proved that 5-year-old female spawners have larger eggs (with an average value of  $1.53 \pm 0.06$  mm) than 4-year-old female spawners (with an average diameter of  $0.04 \pm 1.48$  mm) and the total weight of eggs obtained from 5-year-old female progenitors was more than that of 4-year-old progenitors (the total egg weight of 5-year-old fish was  $1.86 \pm 0.06$  on average and  $1.73 \pm 0.06$  in 4-year-old fish). The aggregation was more in 4-year-old spawners, which is equal to 689,166 ( $67.67 \pm 763.76$ ), and this number is equal to  $670500 \pm 1322.88$  in 5-year-old children.

In 2008, Rahbar showed that 6-year-old female spawners of the Caspian sea salmon produced larger eggs compared to 4- and 5-year-old female spawners, and the amount of spawning was also higher at older ages. According to his research, the egg diameter in this fish at the age of 6 years was equal to 5.4 mm, which has been observed at different ages, and the absolute accumulation at the age of 6 years was equal to 3060 eggs was more and had a statistically significant difference.

The result obtained by Alinia (2010), which was

done on common carp proved that the 3-year-old female spawners have larger eggs (with an average value of  $1.31 \pm 0.09$  mm) than the 2-year-old female spawners (with an average diameter of  $0.11 \pm 1.14$  mm) and the total weight of eggs obtained from 3-year-old females was higher than that of 2-year-old females. (the total egg weight of 3-year-old fish was on average  $1031.25 \pm 205.52$  g and in 2-year-old fish it was  $766.75 \pm 237.95$  g) was a number and this number in 2-year-old offspring was equal to  $171089.7 \pm 1.523500$  numbers.

Zahmatkesh (2010) showed that the number of eggs per gram of 4-year-old spawners in bighead fish ( $405 \pm 4/g$ ) was more than that of 5-year-old females (with an average of  $395 \pm 3$  g). In Rahbar's research (2008), 4-year-old Caspian Sea salmon females had the highest number of eggs per gram compared to 5- and 6-year-olds, which is 6 eggs per gram in 6-year-old females and 16 eggs in 4-year-old females in grams, and there is a positive relationship between the diameter of the retrieved eggs and the number of eggs in grams.

In this research, 5-year-old female breeders had larger egg diameter than younger breeders. Similar to this result, Alp *et al.* (2020) on brown trout (*Salmo trutta macrostigma*) proved that 5-year-old breeders compared to with younger age, they had more egg diameter.

Depending on the size of the female producer, the quality of the egg can have a positive effect on the rate of fertilization and improvement of the egg incubation process, depending on its diameter and total weight. In this research, the 5-year-old female breeder produced larger eggs with a higher larval survival rate (91.12%), it can be stated that the size of fish eggs had a positive effect on their incubation process. In similar research by Shamspour (2008) on rainbow trout and Gisbert *et al.*, (1999) on Siberian fish (*Acipenser baeri*) found a positive correlation between egg size, total fish length, body weight, and the size of newly emerged larvae. According to previous studies, there has been a positive correlation between embryonic losses and egg size in Chinook (*Oncorhynchus tshawytscha*) (Fowler, 1972) and Keta (*Oncorhynchus keta*) (Beacham and Murray, 1985).

According to the results obtained between the studied ages, in terms of the number of eggs per gram, 4-year-old female producers showed the highest amount. The reason for this is the relationship between the diameter of extracted eggs and the number of eggs per gram, that 4-year-old breeders with smaller eggs had the highest number of eggs per gram. A similar result was reported in rainbow trout by Shamspour (2007) that 2-year-old female breeders had the highest number of eggs per gram and the smallest egg

diameter compared to 3-year-old breeders.

According to the results obtained in the present study, 5-year-old female producers have a relatively higher average fertility.

In the results of this research, the fertilization from the eggs of 5-year-old female breeders and the combination of sperms extracted from 5-year-old male breeders had the highest percentage of fertilization (91.33%) and produced larvae. The reason for this can be stated as follows: the quality of 5-year-old female eggs in terms of maturity and fertilization with 5-year-old male sperm, due to ensuring that it is not sterile or of inappropriate quality, can preferably result in the best percentage of fertilization.

### **Investigating sperm cognitive parameters of Amur fish spermatocrit and sperm concentration**

There is a big difference between sperm density in different species of fish. Sperm density as one of the ways to determine sperm concentration depends to a large extent on the volume of sperm fluid in one sperm collection (Billard *et al.*, 1995). According to the obtained results, the mean and standard deviation of sperm density in 5-year-old male progenitors was less than 4-year-old. Also, no statistically significant difference was observed in terms of sperm density in these two age groups. It may be concluded that due to the earlier maturation of male mackerel fish compared to female, sperm deposition starts at the end of the third year of the fish and has the maximum sperm concentration in the fourth year. Various studies show that sperms with a high density do not necessarily have the highest motility and fertilization percentage (Geffen and Evans, 2000; Williot *et al.*, 2000).

One of the methods of examining sperm concentration is determining the percentage of spermatocrit, which is done by centrifuging a certain amount of sperm and separating the plasma liquid from sex cells and various substances in semen. Spermatocrit is the number of cells in a specific volume of semen. Hormonal changes during migration and environmental pollution cause differences in the amount of spermatocrit in the species (Irvin *et al.*, 2002). In this research, the mean and standard deviation of spermatocrit in 4- and 5-year-old fishes were  $93.75 \pm 4.2603$  and  $84.75 \pm 3.3$ , respectively, which indicated a decrease in spermatocrit at older ages, and a statistically significant difference was observed. In the research conducted by Shamspour (2007) on rainbow trout, the highest percentage of spermatocrit and sperm concentration was observed in 3-year-old male spawners compared to 5-year-old spawners. This result was obtained by Rakitin *et al.*

(1999) on Atlantic fish oil (*Gadus morhua*). It was reported that there is a positive and statistical relationship between spermatocrit and sperm concentration. Zahmatkesh (2010) observed that the spermatocrit level had a statistically significant difference in relation to the age of the broodstock, and therefore the spermatocrit level has a direct and positive relationship with increasing age. Therefore, it may be possible to state that the reason for the difference between the results of this research and the studies conducted by Zahmatkesh (2010) on male breeders of large-headed carp in the studied age group is temperature conditions, environmental conditions, type of nutrition, and genetic characteristics of the studied fish, while the results of this research were consistent with many studies that showed more spermatocrit in younger ages. Shamspour (2008) in the study conducted on rainbow trout (*Oncorhynchus mykiss*) observed that 3- and 4-year-old males were more suitable in terms of spermatocrit percentage than 5-year-old males, and 4-year-old females had the highest percentage of fertilization, eye color, and percentage of survival of productive larvae. In many studies, the relationship between sperm density and spermatocrit has been confirmed, for example, based on some studies, there is a significant positive correlation between spermatocrit and sperm density in rainbow trout species (*Oncorhynchus mykiss*) (Baynes and Bouck, 1976; Jacobson and Scott, 1985; Munkittrick and Moccia, 1987; Cieriezko and Dabowski, 1993). Coho salmon (*Oncorhynchus kisutch*) (Bouck and Acobson, 1976), Atlantic salmon (*Salmo salar*) (Piironen, 1985), whitefish *Coregonus* and yellow sosses *Perca flavescens* (Cierieszkon and Dabowski, 1993), common carp (*Cyprinus carpio*) and ozone *A. stellatus* (Islambolchi, 1998) has been stated.

### **The percentage of motility and the length of the sperm motility period**

Sperm quality refers to its motility, which is the leading determining factor for the fertilization ability of sex cells (Billard, 1978; Cosson *et al.*, 1991; Lahnsteiner *et al.*, 1997), and also sperm motility is expressed as an important characteristic for male fertility. (Birkhead *et al.*, 1999). The duration of sperm motility in bony fish with external fertilization has been reported as 30 seconds to 1 minute (Billard *et al.*, 1999).

According to the report (Aas *et al.*, 1991; Dillane, 1998; Pool, 1998), the percentage of mobility and its duration have a significant effect on the percentage of conception, and therefore its determination is important in the study of the percentage of conception. Also, Wojtczak *et al.* (2007) studied rainbow trout and

concluded that the percentage of sperm motility is the first factor that guarantees the success of fertilization. In the present study, the maximum length of sperm motility period in 5-year-old male fish was  $40.5 \pm 7.59$ , and the percentage of sperm motility in 5-year-old male fish was  $87 \pm 4.24$ , and the lowest motility period in 4-year-old male fish was  $11.79 \pm 29.25$  and the percentage of motility in 4-year-old male fish was  $78 \pm 4.32$ . In this study, there was no statistically significant difference between sperm motility duration and reproductive age ( $p > 0.05$ ). In 2010, Fallah Shamsi investigated the effect of ionic and biochemical composition of sperm on the efficiency of artificial reproduction of white fish. The results showed that the longest period of sperm motility and the percentage of sperm motility in 4-year-old fish were  $66.3 \pm 6.1$  and 9, respectively. The lowest length of sperm motility period and percentage of sperm motility in 3-year-old fish were  $60.5 \pm 12.8$ ,  $80 \pm 6.7$ , respectively. In this research, there was no statistically significant difference in the duration of sperm motility and the percentage of sperm motility in fish with the age of the spawners. The results obtained from both mentioned studies showed the longest period of sperm motility in older breeders. But there was a difference of opinion on the percentage of sperm motility. This difference in the data may be due to the variety of characteristics of weight, length, age, and many other factors related to the male fish used in each research. Geffen and Evans (1999) stated that the percentage of fertilization has a direct relationship with the primary sperm motility in rainbow trout, but it does not have a positive relationship with sperm concentration or ATP concentration. Liley *et al.*, (2002) research on salmon sperm showed that high spermatocrit decreases sperm motility time. The result of Tekin's study in 2003 on salmon also showed that with a decrease in sperm concentration, the duration of sperm motility increases and with an increase in sperm concentration, the duration of sperm motility in this fish decreases. In the African catfish studied by Adewumi *et al.*, (2005), sperm motility increased with the increase in the amount of semen, and there is a strong relationship between the amount of semen and the percentage of egg fertilization and hatching. Rainis *et al.*, (2005) on three species of fin fish (*Sparus auratus*), brown trout, and rainbow trout proved that the duration of sperm movement in *Sparus auratus* is significantly longer (50 minutes) than the minute was in Azadmahian. Lorestani (2004) also showed that the sperm motility at the age of two years in salmon was more than that of three and four years (there was no significant difference between three and four years). Bahmani *et al.* (2007) compared the average sperm motility rate

(seconds) and the percentage of sperm motility in males of white fish caught from four rivers; Shiroud, Khashkroud, Safidroud, and Chelvund.

This issue has already been confirmed in some carp fishes (Linhardt *et al.*, 1991) and in the case of swordfish (*Rutilus rutilus*) in Finnish waters, a positive correlation has been reported between the duration of sperm motility and the speed of their movement (Kurt *et al.*, 2004). Zahmatkesh (2010) investigated the effect of the age of the breeders on the reproductive efficiency and reproductive capacity of the carp fish. The results showed that there is a significant difference between the two age groups of three and four years old regarding the reproductive capacity between sperm motility, the length of the motility period, and sperm density.

### Sperm pH

pH is one of the important parameters of sperm activation in different species of fishes, which affects sperm fertilization ability. It has been found that intracellular and extracellular pH, like the ionic compounds of activating solutions, have an effect on the start and duration of the sperm motility period. pH has little effect on carp sperm motility (except at high pH), in addition, carp sperm can start moving at pH 6-9. Also, studies have shown that a time-dependent change in the internal pH of carp sperm later the hypoosmotic shock that changes the pH to alkaline causes the movement of carp sperm (Alavi and Cosson, 2006). In the conducted research, the mean and standard deviation of sperm pH in 4 and 5-year-old fish were  $8.08 \pm 0.52$  and  $8.17 \pm 0.42$ , respectively, and there was a statistically significant difference between the 4- and 5-year-old age groups in terms of sperm pH. Some studies indicated that lowering sperm pH affects sperm motility (Kopeika and Kopeika, 2008). Considering these results, it may be said that due to the low pH of sperm at younger ages, it will have an effect on sperm motility. Fallah Shamsi (2011) concluded that the mean and standard deviation of sperm pH in 3- and 4-years old fish were  $7.79 \pm 0.51$  and  $7.61 \pm 0.26$ , respectively. He did not observe a statistically significant difference between the two age groups of 3 and 4 years in terms of sperm pH, which was similar to the results obtained in the present study. Akcay *et al.*, (2004) reported the mean and standard deviation of sperm pH as  $8.06 \pm 0.08$  for mirror carp. In the research conducted by Dadres (2008) on Iranian Dice fish, he did not observe any relationship between sperm pH and sperm motility percentage, while there was a positive and direct relationship with fertilization percentage and spawning percentage and a negative and opposite relationship

with sperm density.

## The effect of ionic compounds on the efficiency of artificial reproduction of Amur

### 1. Effect of calcium ion

The results showed that calcium ion had a strong and positive relationship with spermatocrit, and a strong inverse relationship with the duration of motility and a weak inverse relationship with sperm density, while it had a direct but weak relationship with the percentage of motility. An increase in calcium ion decreased sperm activity and finally Reduction of efficiency is multiplied (Tabares *et al.*, 2007). Metaji (2010) stated that calcium ion has negative effects on the sperm density of Caspian sea salmon, but the relationship between this ion and spermatocrit is positive and direct. Also, the increasing effect of calcium ion and its positive effects on the percentage of fertilization, hatching percentage and hatching percentage in rainbow trout were investigated by Hosseini (2008) and it was proved that the increase of calcium ion in the environment increases the fertilization percentage, hatching percentage, and egg hatching percentage. The difference in the final result of Mataji's research (2010) compared to other studies may be due to the difference in the species, age and size of the fish used.

It was also proved in the Iranian tas fish (*A. presicus*) that the increase of calcium ion is a negative factor in the motility of spermatozoa and as a result the reproduction efficiency (Alavi *et al.*, 2004). Biological sensitivity of sperm to calcium by Tas (1997) and Kasan (1999) was reported. Based on this, the amount of calcium more than 10 mmol has negative effects on sperm motility; as a result, it reduces the reproduction efficiency. It can be concluded that sperm fertilization ability depends on sperm motility and sperm motility depends on It is calcium and magnesium ions, so if it is more than the actual amount (10 mmol), it can have a negative effect on sperm motility, so the reproduction efficiency is reduced.

Rahali Moghadam (2009) in a study that investigated the effect of total hardness (calcium ion) on the fertilization efficiency of white fish concluded that the water hardness of 300 mg/l of calcium carbonate with a fertilization percentage of 96.66 is the best amount of total hardness for reproduction. The fish is white because the hardness of the water includes calcium and magnesium ions, so it can be concluded that the presence of these ions in the water increases the reproduction efficiency.

## 2. Magnesium ion effect

The research conducted on the intracellular mechanisms of sperm movement in bony fish reports the important role of magnesium ion in the initiation of sperm movement activity in bony fish (Cosson *et al.*, 1999). In the present study, the results showed that magnesium ion has a weak inverse relationship with sperm density and a strong inverse relationship with motility duration, and also has a direct and weak relationship with spermatocrit and sperm motility percentage. According to the studies done on Brycon henni fish, the longest period of sperm motility followed by the best fertilization percentage was obtained when the sperm dilution was done in a solution containing magnesium (Tabares *et al.*, 2007).

Also, magnesium ion is considered a positive factor in the Iranian Dice fish (*A. presicus*) and increases the reproduction efficiency, but its excessive increase (10 Mm) causes a drop and decrease in reproduction efficiency (Alavi *et al.*, 2004). The effect of magnesium ion on the rate and duration of sperm motility and ultimately the reproduction efficiency in sea bass was also investigated by (He *et al.*, 2004). The results showed that magnesium ion has a positive effect on reproduction efficiency and its increase will increase the percentage (fertilization and hatching) and its decrease will also result in a decrease in the percentage (fertilization, hatching, and hatch). Sperm motility is dependent on calcium and magnesium ions (Billard and Cosson., 1992). In the current research, the highest amount of magnesium was seen in 2-year-old male reproductive, and according to the results of the fertilization percentage, it can be stated that the magnesium factor has an effect on the proper quality of sperms of 2-year-old male reproductive.

## 3. Effect of sodium ion

Sodium is an effective factor on sperm motility and acts opposite to potassium and activates sperm motility and is one of the dominant ions in seminal plasma (Alavi *et al.*, 2006). In the present study, the results showed that sodium ion had positive and strong effects on cognitive sperm parameters such as sperm motility duration and a weak direct relationship with sperm density as well as an inverse and weak relationship with spermatocrit and sperm motility percentage. Alavi *et al.* (2004) reported that the percentage of motile spermatozoa and the duration of motility will decrease with increasing sodium ion concentration in Iranian Dice fish (*A. presicus*), which is naturally associated with a decrease in the efficiency of artificial reproduction. Hosseini *et al.* (2008) attributed the

maximum sperm motility time to sodium-containing activator solutions and stated that sodium ion enhances sperm motility. In this research, due to the difference in the species, the environmental conditions of the breeders, their age and size, there were differences in the results compared to other studies. But in the end, based on the results obtained in this research and the results of other researches, the key role of sodium ion is undeniable and it is considered to be the sperm activator, but nevertheless, its increase from the normal level causes a decrease in sperm quality and a decrease in efficiency. It will lead to artificial reproduction.

## 4. Effect of potassium ion

Based on the results obtained in this research, it was proved that potassium ion has weak direct effects on spermatocrit and sperm density and has a weak inverse relationship with the duration of motility and the percentage of sperm motility, for example, Alavi *et al.* (2004) in a research on fish dice Iranian *A. presicus* stated that potassium has the role of inhibiting sperm motility and as a result reduces the reproduction efficiency. But Tabares *et al.* (2007), in their research on Brycon henni fish, obtained a completely similar result to the current research on white fish and stated that potassium ion in the sperm of Brycon henni fish caused more spermatozoa activity and as a result, it increases the efficiency of fertilization. The difference in potassium ion concentration between water and seminal plasma initiates sperm motility. Also, the potassium concentration needed to prevent sperm motility depends on the sodium ion concentration. If the sodium ion is high, more potassium is needed to prevent sperm motility (Morisawa, 1983).

## 5. Effect of chloride ion

Based on the results obtained in this research, it was proved that chlorine ion had direct and weak effects with spermatocrit and sperm motility duration, and had a strong direct relationship with the percentage of motility and a weak inverse relationship with sperm density. Also, a statistically significant difference was observed between the two age groups of 4 and 5 years in terms of chlorine ion.

## The effect of biochemical compounds on the efficiency of artificial propagation of Amur fish

### 1. The effect of alkaline phosphatase enzyme

Based on the obtained results and also based on the regression relationship between alkaline phosphatase

and sperm cognitive parameters of common carp sperm, it was determined that there is a direct and strong relationship with spermatocrit and sperm motility percentage, as well as an inverse relationship with sperm motility duration and sperm density. These results indicated that alkaline phosphatase can be effective on sperm motility percentage.

In a research by Metaji (2010) on Caspian sea salmon, the positive relationship between alkaline phosphatase and sperm density was observed, and also the relationship between alkaline phosphatase with fertilization percentage, hatching percentage and hatching is negative and vice versa.

Zahmatkesh (2010) evaluated the relationship of alkaline phosphatase with sperm motility and positive and direct, but with negative and reverse replication efficiency parameters.

## 2. The effect of acid phosphatase enzyme

Based on the obtained results and also based on the regression relationship between alkaline phosphatase and normal carp sperm cognitive parameters, it was determined that there is an inverse relationship with spermatocrit and sperm motility percentage, as well as a direct effect with motility time and a direct effect with sperm density. Zahmatekesh (2010) evaluated the relationship between acid phosphatase with the amount of spermatocrit, density, and length of movement period, and therefore, a positive and direct correlation with reproduction efficiency was proved.

According to the results of this research and comparing it with the results of other research, it can be stated that there is a great difference in terms of quantity and quality between the sperm plasma of bony fishes and cartilaginous fishes. On the other hand, each of the investigated parameters can have different effects on artificial reproduction efficiency in different species and it seems that they do not follow a general rule to express their effects. Based on the hypotheses raised at the beginning of the research, it can be stated that potassium ions, sodium, calcium, magnesium, chloride, and phosphatase, alkaline phosphatase enzymes, as well as the percentage of motility, duration of motility, and density of sperm are to some extent a strengthening factor in reproduction efficiency. Artificial carp has been normal.

According to the analyzes performed on ages, weights and different sizes and the results of reproduction and data on the percentage of fertilization, spawning rate and survival in this research, the largest share of which belongs to 5-year-old females and male breeders (4+5) years old and it can be said that 5-year-old female and 5+4-year-old male breeders of Amur fish

can be the best options for breeding operations in hydrothermal fish breeding centers.

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## Conflict of Interest

The authors do not have any potential conflict of interest to declare.

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