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Artificial Spawning and Early Development of *Acipenser persicus*

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Abstract: Annually, for restocking the fingerlings of *Acipenser persicus* in Caspian Sea, artificial propagation of *A. persicus* is practiced in many farms in North of Iran. The artificial reproductions of sturgeon fish were carried out in two major farms and then their reproduction data were compared. In the present study, based on existing data for the year 2006, a total of 384 females were caught and 93% of them were spawned. The age of sexual maturity for female was 14-16 years. The required weight for these types of fish selection for hormonal treatment and artificial spawning was 14-52 kg. The average eggs produced per female were 52000. The optimum temperature for spawning was in the range of 16-18°C. The percentage of fertilization rate was 65-75 in both farms and with significantly difference. The percentage of hatching rate was 61 percent in both farms. There was significant correlation between diameter of eggs and percent of fertilization (r=0.88). The diameter of eggs and weight of larvae were positively correlated (r=0.81). There were no significant correlation between percent of fertilization and hatching rate (r=0.22) and between percent of fertilization and duration of oocyte development (r=0.17).

Key word: *Acipenser persicus* · Artificial propagation · Hatching rate · Fertilization rate

INTRODUCTION

Most of the populations of sturgeons in Caspian Sea have been diminished. However, the stocks of *Acipenser* in south part of Caspian Sea are self–sustaining and provide a unique population. In every spring, a number of mature *A. persicus* migrated to southern part of Caspian Sea. This abundant spawning population provides an opportunity to catch and carry out study on artificial reproduction and early life development in captivity [1-3].

These are resulted from the performance of restrictive harvest regulation to protect the population and enhancement by releasing millions of fingerlings of this species by artificial propagation. Therefore, further information is needed on the propagation techniques and habitat requirement of eggs and larvae. In the last decade, a number of investigations were conducted on the structure of population, recruitment and endocrine regulation of *A. persicus* [4-9].

An investigation on the basic propagation of *A. persicus* was carried out during period of 2002-2006 at Acipenser Propagation Centers in Mazandaran and Golestan provinces [10]. These sites are the major artificial spawning centers. Mature fish from the bottom of the sea were captured by fisherman using gill-nets. Spring season (March and April), is a suitable catchments time of *A. persicus* for spawning. As not all the fishes caught are able to spawn, hormonal treatment for induce spawning are carried out on *A. persicus* and most of them are based on extraction of pituitary gland hormones of sturgeons. The aim of this study was to illustrate the reproduction of an important fish (*A. persicus*) and their captivity in the world.

MATERIALS AND METHODS

Collection of Fish for Spawning: Females of anadromous *A. persicus*, migrate to southern rivers in Caspian Sea and rivers such as Gorganroud and Tajan. Their early migration from the sea may end up with immature germination. Persian sturgeons were collected in winter and spring (2002-2006) by cooperative organization of fisherman in Iranian fishing zone, southern part of Caspian Sea, with the water depth of 2 to 70 m. The sea water temperature may fluctuate in winter and spring; the temperatures were in the range of 4-14 and 13-20°C, respectively. During spawning season, before fish enter the river with gonad at stages of III-IV, they are caught in sea by gill-net. Those fish at status of incomplete stage IV, maturity are used for artificial spawning.

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Gonad maturity was detected by their shape and appearance. The mature fish have large soft belly and reddish vaginal. Fish were transferred from the sea to the hatchery using truck tanker supplied with fresh water and equipped with oxygenation apparatus. All of them were subsequently kept at out-door circular concrete ponds (3×3 m) at a density of 5-10 kg/m^3. The tanks were supplied with river water at 8°C and were saturated with oxygen.

Selection of Mature Fish and Incubation Process: Gonad maturity was detected by taking sample, using a plastic cylindrical probe. High degree of migration of germinal vesicle (G.V) expressed by polarization index (PI) indicated spawnable females. PI is the ratio of distance between germinal vesicle and the animal pole to the diameter of the oocyte, egg envelope excluded [11]. PI within the range of 5 to 8 was more advanced in maturity than higher or lower PI. A Total of 115 spawning fish were selected out of 158 individuals in S. Rajaei farm, whereas all brooders in S. Marjani was ready for hormonal injection, based on G.V. position. Under hatchery condition, the oocyte maturation, ovulation of sturgeon and natural spawning were processed under the influence of the gonadotropic hypophysial hormones. In order to take place of oocyte maturation and ovulation, the spawner was stimulated by usage of suspension of powdered acetone dried sturgeon pituitaries at a rate of 45-50 mg per female. The pituitary extracted dried in acetone was prepared in autumn (4-6 months before spawning process) from the relative fish. The water temperature during the spawning process ranged between 16 and 20°C. The yield of ovulated eggs was determined as weight of ovulated eggs/body weight. For some of the batches, the mean value of counting was 100 units and then the whole eggs weights were determined. Semen was collected in a beaker using a tygon tube with a 50 ml syringe. Semen study done by other investigator [12] which was selected mainly base on motility.

Fertilization and Embryonic Development: Occurrence of ovulation was checked between 20-24 h after SPG (Sturgeon Pituitary Gland) injection. When eggs could be stripped freely the vaginal pole is closed by cloth. The sample was collected for observation and checking matter under loop, then males were stripped first. After collection of the sperm of 2 males (20 ml per male), quality was estimated by naked eye observation. Then the ripe female was transferred to hatchery. The abdomen was kept dry by hanging the fish, then by cutting a small portion of lower part of abdomen with a sharp knife, the ovulated eggs were collected in plastic dishes (recently the eggs were taken without killing the fish). Current fertilization was carried out with a pool of semen at a rate of 10 ml semen kg^{-1} of ovulated eggs and pool of semen in water diluted (1200, V/V respectively) [13]. Just one min after fertilization stickiness was neutralized by a 30 min treatment in a continuously moving clay water suspension. Then, eggs were rinsed and put in Yoshjinkov incubators. At the same time, the fertilization rate control was organized by putting 100 fertilization eggs into small boxes placed in the same incubator. Fertilization rate were determined at gastrula stages.

Fertilization rate of gastrula stage has the advantage to determine the right fertility by sperm, separation abnormal occur due to polyspermy and parthenogenesis that can be detected by non-straight and non-symmetrical furrows within first cleavages. Incubation periods lasted for 5-7 days at 17-23°C via biometrical studies. During artificial propagation the biometrical data of breeders, incubation information and larval developmental stages were measured and scored.

Statistics: Results were calculated and plotted as mean ± standard deviation. Significant in difference between all the biometrical factors, with analysis of variance was tested by Pearson correlation used software of SPSS, 17 for Windows.

RESULTS

From a total of 427 fish caught from two farms, 384 were injected and 356 were spawned by hypophysation. The artificial spawning start at the middle of May and by that time, numbers of fish were ready for spawning. The best time for selection of spawner was just at the end of winter and lasted for two weeks, when the water temperature was greater than 20°C. In total 41000 g eggs were obtained from the two farms. The average fertilization rate in S. Rajaei and S. Marjani farms were 75.21 and 65.20%, respectively. The hatching rate from both farms was 61.3%. The best result of germinal vesicle (GV) position for propagation was in the range of 7 and 8. The biometrical data gathered from both farms are shown in Table 1.

The biological parameters of *A. persicus* such as "the weights of spawners, the temperature of water during artificial propagation, duration of oocyte maturation, total length, GV position, the weight of ovary, number of eggs per gram, the weight and diameter of eggs, fertilization rate, hatching rate, fungi infection mortality, survival larval rate, absolute fecundity and relative fecundity are described as following:

Table 1: Biological and technological data of *Acipenser persicus* in two Spawning Farms

	Spawning Results	
Criteria of investigation	S.Rajaei farm	S.Marjani arm
Age at sexual maturity of female	14 to 16 years	14 to 16 years
The sexually mature fish length	167 ±2 cm	158.8 ±10 cm
The sexually mature fish weight	28.6±4 kg	29.5±6 kg
The spawning season	March - April	March - April
Optimum temperature requirement for spawning	16-18°C	16-18°C
Females percent selection for artificial propagation	70-75 %	100%
The effectiveness percent of hypophysation of females	75-80 %	00 %
Quantity of eggs per female	243100±7400	219800±7000
Quantity of dry eggs by body weight of female	160.8 g	100.1
Milt production per male with hypophysation	50 ml	50 ml
Milt for fertilization of 1000 g of eggs	10 to 15 ml	10 to 15 ml
Diameter of dry size of the eggs	3.3±0.16 mm	3.4±0.19 mm
Dry weight of the eggs	19.1±2.1 mg	19.9±2.2 mg
Count of dry eggs per kg	52800± 580	50580± 5980
Stocking density of swollen eggs in incubator	1000 per litter	1000 per litter
Number of eggs by body weight of female	8490	7440
Fertility rate of the eggs	75.3±12 %	65.20±23 %
Requirement time for hatching (Day-Degree day)	5-6d (85-100 d.d)	5-6d (85-100 d.d).
Hatching rate of the eggs	61.3±21.3 %	61.3±21.3 %
Span time of yolk absorption (Day or Degree day)	9d (140-160 d. d)	9d (140-160 d. d)
Survival of hatched larvae up to releasing to pond	7.4 %	59%
Weight of fry at time of introducing to pond	60-90 mg	50-60 mg
Three-weeks old production of larvae by 1 kg of dry eggs	18000 to 20 000	18000 to 20 000
Optimum rearing temperature	20°C	2 0°C
Length of four-days old fry	5-7 mm	5-7 mm
Weight of four-days old fry	17.25±3	17.11±2
First feeding start	After 3-4 days	After 3-4 days
Starter feed in fiber glass tanks (2×2×1 m)	Artemia naupli	Artemia naupli
Density of stocking in earthen pond	90000 in ha.	80000-90000 in ha
Survival rate of the fry through the rearing period	40-90 %	40-90 %
Number of fry surviving at 30-65 days, from 1kg dry	7000-8000	12000-13000

The Weights of Spawners: The total weights of eggs that were collected from all spawners were 41kg. The minimum and maximum weight of fish was 14 kg and 52 kg, respectively. The average weight of fish was 28.8 kg. The highest mode of spawner had 26 kg weight. The correlation between the rate of GV and duration of ovulation was moderate and positive value P>0.064 and no significant.

The Temperature of Water During Artificial Propagation: The water temperature is an important factor in oocyte maturation and ovulation. In artificial fish farm, the water temperatures were varied from 15 to 19 °C with mean value of 17°C. The best results were obtained when the water temperature was at 18.7°C. The temperature has shown a significant negative correlation with duration of oocyte maturation (r = -0.48, P<0.00), because of the temperature increase the length of maturation decrease.

Duration of Oocyte Maturation: As mentioned before, the duration of oocyte maturation is dependant on water temperature. The maturation time varied between 18 to 36 h after hormonal injection. The average time for spawning and the highest frequency was 24 h. There was no significant difference between weights of fish and duration of oocyte maturation (P>0.946). The weight of fish had positive correlation with length of fish (r = 0.633, P<0.00) as well as the weight of ovary (r = 0.76, P<0.00).

Total Length: The maximum and minimum total lengths spawned fish were 100.5 and 201.2cm, respectively. The average length and the highest frequently were 163.3 and 165.0cm, respectively. There was no significant differences between selected and unselected breeder (P>0.949). There was positive correlation (r =0.285, P<0.945) between length of fish and weight of ovary.

GV Position: The GV position of propagated fish ranged from 4.4 to 10.96. The average GV and the mode were 7.4 and 7.5, respectively. The best result between them was in the range of 6 to 8. There was a significant difference between the weights of ovary of these two groups ($P<0.00$).

The Weight of Ovary: *A. persicus* in average had 3.9 kg eggs. The maximum and minimum weights of eggs were 12.5 and 1 kg out of 23 kg fish samples. The most spawned had the eggs between 3-4.5 kg. Obviously, the length and weight of the fish had positive correlation with the amount of eggs ($r=0.29$, $P<0.011$ and $r=0.77$, $P<0.00$, respectively).

Number of Eggs per gram: The average number of eggs per gram was 5.1. The maximum and minimum numbers of fish eggs per gram were 40 and 69, respectively. The mode number of eggs belong to the fish had 50 eggs gr^{-1}. There was significant and highly correlation between the number of eggs with the weight and diameter of eggs ($r = 0.85$, $P<0.00$).

The Weight and Diameter of Eggs: The fluctuations of weight per eggs were from 14.5 to 34.0 mg with average value of 19.7 mg. The average diameter of egg was 3.4 mm. The weight, diameter and number of eggs per gram had significant correlation with each others ($P<0.00$).

Fertilization Rate: Fertilization rate is the mean characteristics determining the quality of eggs and sperm. The average fertilization rate during the whole artificial propagation was 67.67% which illustrated from the good quality of eggs. The minimum and maximum fertilization rate was 5% and 97%, respectively. Most of the fishes had 70% fertility. The correlation between fertilization rate and GV increment position was negative ($r =-0.25$, $P<0.03$). It means, selection of lower GV gives the better results of fertilization. The correlation between fertilization rate and duration of oocyte maturation was negative ($r = -0.24$, $P<0.018$).

Hatching Rate: The hatching rate respect to the total egg and fertilization rate was 61.2% and 81.5-94%, respectively. The lowest hatching rate was 20% that was related to a fish of 75% fertility. Therefore, the low hatching rate was due to damaging the eggs during incubation and fungi infection. The highest frequently of hatching rate was related to this group (20% hatching rate). There were no correlation between hatching rate and artificial reproduction parameters.

Fungi Infection Mortality: The fertilized eggs in incubators, under some factors such as polyspermy and parterrogenesis development, might be damaged due to handling or unfavorable condition of incubation. Damage eggs were more prone to fungi infection. The rate of fungi infection and mortality was high (up to 38%). This is most probably due to farmers not applying malachite green chemical to prevent fungi infection.

Survival Larval Rate: The highest rate of survival (80%) occurred during period of absorption yolk eggs in fiberglass tank. The lowest and average rates were 55 and 63.6%, respectively. The high survival rate of larvae in absorption yolk eggs and nursery period was $r = -0.44$ that indicated the good quality of eggs where selected by spawner of *A. persicus* and also depend on the oocyte maturation period. In other word, eggs remaining in ovary for a longer period reduce the quality of eggs for embryonic and larval stages.

Absolute Fecundity: The average absolute fecundity was 1500000 and the average eggs obtained from one kg of fish weight was 52000. The minimum and maximum absolute fecundity was 708000 and 2600000, respectively. Absolute fecundity had significant correlation with the parameters of weight, length, GV position, weight of ovary and fertilization rate (eggs quality) where the correlation values were $r = 0.56$, ($P<0.000$); $r = -0.11$, ($P<0.001$); $r = 0.93$, ($P<0.00$) $r = 0.36$, ($P<0.00$) respectively.

DISCUSSION

The initial spawner condition at the time of catching and the condition fish kept before hypophisial stimulation till maturation are very important for eggs quality in oocyte maturation. The main conditions are water flow, dissolved oxygen and water temperature [13]. The stimulate maturation of sturgeon at the first time was performed without using any hormones [14]. It was placed in condition close to those found in the nature during the migration to spawning grounds: they were kept in a basin with strong circular flow and pebbly bottom. In these conditions, a few females matured and this proved about the effect of proper condition to stimulate oocyte maturation and to ensure the mass propagation of sturgeon. In Shahid Rajaii sturgeon propagation fish farm, all the conditions were well managed for artificial propagation of *A. persicus* during the whole period of spawning time. The circular flow equipped with river water mixed with well water, saturated with oxygen and water temperatures were maintained around $18 \pm 0.2°C$.

Therefore, the fish are in proper condition in holding tank at early and late stage of spawning time. For artificial reproduction of *A. persicus* in 2002 among 157 spawner caught about 50% was good for this purpose. The main criteria for selection were PI. The spawned fish had excellent eggs quality and there was no significant difference between the fertilization rates at different time of propagation. It showed that those fish that were spawned at different time of spawning had equal quality for ripeness. The small fluctuation in hatching rate was due to small change in hatchery condition and infection by fungi and that was not due to the quality of eggs [13]. The main factor for spawning of the spawner is the condition of breeders at the time of catching. PI is an efficient criterion for determining the stage of ripening of female breeders and is reliable and utilizable technique in this respect, but this index in several samples was not enough to show the correct condition of eggs, because of the range of GV position of unspawned fish was within the upper range of spawned fish. Therefore, it is necessary to use an additional technique to complete determination of the final stage of maturation such as using the hormonal kit. A test of follicle was incubated in a ringer solution with hypophysial gonadotropic hormones or in progesterone [14, 15]. One of the main factors for propagation is temperature. Sturgeon fish have a large range of water temperature for its reproduction.

During natural reproduction in the river and after the pituitary injection into the females at sturgeon hatchery, the oocyte begins to mature only at spawning temperature. The range of spawning temperature for *A. persicus* was 11-21°C. The female of *A. persicus* started to react to pituitary injection by oocyte maturation at water temperature above 9-10°C or below 25-26 °C. The range of temperatures reported in the literature [13] was from 12-13 through 25-26°C for *Acipenser stelatus*. In general, the temperature of induced stress in sturgeon fish is 25-26°C. They felt unsuccessful, consequently prevent ovulation. The ovulated eggs are damaged and not good [16 & 17]. The lower temperature (e.g. 8°C) also has considerable effect on reduction of fertilization rate [18].

In Shahid Rajaii farm, the usage of cold underground water with a constant temperature of spawning pool and incubation were in proper range. Therefore, the breeders and embryo are less under unfavorable and changing of environmental condition. The change of temperature at gastrula causes death of embryo and at later stage abnormality in larvae. Controlling system temperature prolongs the propagation of fish and helps in managing production of larvae without any negative environmental effect on production.

The eggs mortality was raised from 8 to 80% due to fungi infection. Therefore it is necessary to improve the hatchery techniques. Although Yoshjinkov and Asutre incubators are widely used at all sturgeon hatcheries, but introducing others modern incubator is necessary.

CONCLUSION

In circular flow with river water mixed with well water, saturated with oxygen and water temperatures maintained around 18±0.2°C are the proper condition for artificial propagation of *A. persicus*. The main criteria for selection are PI. The best results of GV are in the range of 6 to 8. It is also necessary to use an additional technique to complete determination of the final stage of maturation such as using the hormonal kit. The eggs mortality was very high due to fungi infection. Therefore it is necessary to improve the hatchery techniques and introducing others modern incubator instead using Yoshjinkov and Asutre incubators.

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