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Spawning of South Caspian Kutum (*Rutilus frisii kutum*) in Most Migratory River of South Caspian Sea

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Abstract: The objective of this study was to study the status and the normative of reproduction of Caspian Kutum (*Rutilus frisii kutum*). The artificial reproduction in four rivers has investigated and the reproduction normative of this fish was compared. Based on the data of 2002, in total, about 30000 female and male have caught (ratio: 1 female to 2.4 male) and 4000 kg eggs have used for incubation in hatchery. The temperature in spawning time was in the range of 5-21°C but mostly 11°C, which is good for propagation. The number of dried eggs was 320 g^{-1}. The fertilization rate is a good criterion for determination of eggs and sperm quality. The range of fertilization rate in four investigated river was 75 to 92% and it was significantly different ($p<0.05$). The hatchability in the rivers was very high but in those with high turbidity (colloid material) was low, due to damage the eggs during river incubation. The larvae reared in earthen pond riches of phytoplankton and zooplankton.

Key words: *Rutilus frisii kutum*, natural spawning, artificial propagation, Caspian sea

INTRODUCTION

The fish of *Rutilus frisii kutum* is the most important bony fish of South part of Caspian sea that more than 70% of catch fish by fishermen in Iran coastal of Caspian sea consist of this fish.

Spring spawners arrive on the spawning ground in March to May. Due to sharp decrease in stocking of *R. f. kutum* in Caspian sea attempted made in artificial propagation of this fish for enhancing and restocking. Much of the work performed dealed with quantitative purpose and the production has been increased from 2 million in 1981 to 140 million at present time. This effort made the possibility of harvesting up to 10000 ton in 2002 comparing to 1000 ton in 1981. The growing demand for increasing sophisticated information of the effect of artificial propagation of *R. f. kutum* has focused attention on the need for study of status of this fish in different rivers. The present study will contribute toward an improved understanding of some characteristics of breeders and normative of propagation as well as some information about the quality of fingerlings.

The *R. f. kutum* is an order of cypriniforms representative of cyprinid. Males normally mature between their third and fourth year, sometimes earlier, females during their fourth year. Spawners are 3-8 years old and the principal age groups are 4-5 years for males and 5-6 years for females in the Anzali Mordab, older than the 3-4 years of fish reported in 1970-1971. However, recently males are maturing at age 2 and females at age 4 with most spawners at age 3 and 4 years, respectively. The commercial catch in Iran was 3-7 years old, 39.0-57.1 cm long and weighed 613-2525 g (Coad, 1995).

Distinct sex characteristics make it easy to distinguish between mature males and females. In the adult, the female has a large belly. The male, if lightly squeezed around the belly will emit white milt. At spawning time, very small plate covers the body of male and especially in the head that is like a spot white. At this time the male is sufficiently different from the female to allow for easy identification.

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Natural habitat of this fish is ouly in the Caspian sea and is not found in the lakes, rivers or fishponds. Feeding and nutrition of *R. f. kutum* is consisting of zooplankton, phytoplankton, crustacean and mollusks.

In natural environment, the fish spawn in groups, in slow moving rivers, at a temperature of 11-22°C. The eggs attach itself to vegetation and sands and hatches within three days. In spite of high quality meet of this fish, no cultivation of this fish are practice in pond. Economic characteristic of *R. f. kutum* are listed as: high degree of fecundity, high meet quality and rapid growth in natural habitat.

R. f. kutum suitable for spawning is catch in May and July from the river and delta of a river. Most of fish selected from the river reproduce easily but those are catching from delta and sea, are not able to spawn. Presently, hormonal treatments for induced spawning are practiced in *R. f. kutum* and are mostly based on the use of Carp pituitary (CP). Females ovulated after one or two CP doses of 1-2 mg kg^{-1} b.wt. If the whether is cold the female are injected 4-5 mg kg^{-1} b.wt. and males after a single injection of 3-5 mg kg^{-1} b.wt. Spawning of *R. f. kutum* was successful after a single injection of LH-RH analogue (LHRHa), combined with pimozied. Mostly sufficient volnme of sperm is stripped without any hormoual treatment. Storage and sperm motility are priority for successful artificial reproduction. Eggs for artificial insemination were mixed together with sperm and immediately activated in clean water of river. After 5 min, fertilized eggs are washed for 30 min with river or clean water eliminating the stickiness. No clay or talk suspension is added to the fertilized eggs.

Fertilized eggs remain in jar without any shaking during early developmental stage (for 2 h) and then incubated in siscrine incubator (cloth box) in river (for 2-3 days). After that eyed stage eggs are incubated in 10 L Weis jar. Hatching is expected 6 days after fertilization at 15-16°C. The objective of this study are to introduce the biology and some aspects of artificial reproduction of unique bony fish of Caspian sea, process of restocking and the future status of this fish.

MATERIALS AND METHODS

The experiment were conducted at Shahid Rajaei Fish Complex and Ecological Institute of Caspian sea, Department of Fish Genetic and Aquaculture at Sari, Iran. Kutum were captured from four main river of Tajan, Shiroud, Tonkabon, Goharbaran Rivers inlets to the Caspian sea during the spawning migration in March-May 2002-2004 (water temperature 8-12°C).

To determination reproduction normative, about 30% of male and female were sampled, every day during artificial propagation in 4-river station. The brood fish were catching and individual brood fish suitable for stripping were selected, kept separated in 50-tanks. Both male and female were checked for spermiation and ovulation by gentle hand stripping. Female showing large and soft abdomens were selected for spawning. Unripe female were kept in wooden cage for 1-2 days for ripening. In practice, 2 males displaying high sperm quality were used per female.

When eggs could be stripped easily, total eggs were collected in plastic dry jar. Females were stripped ouly once. Oocytes and sperm were mixed with feather. The clean river water was added and the mixture was gently shaken for 1 min. After female ovulation, females were killed and the remaining gonad was weight. All data about male and female, eggs, sperms, fertilization rate, hatchery time and rate are scored.

Statistics

Results were calculated and plotted as Mean±SD. Comparison and significance in difference between the normative of reproduction were tested by one way ANOVA and followed by Duncan Multiple range test. The level of statistical significance was set at $p<0.05$.

RESULTS

A total of approximately 42000 male and 15000 female were catch every year. The breeders are three significant spawning categories involving unripe, preripe, ripped (Fig. 1). A part of pre-ripe are spawned, after keeping them 1-2 days in cage located in the river. Approximately 75% of the immature fish that were transferred to temporary cage without using any hormone reached maturity and spawned. In rivers with mainly flooding current no installation of cage in the river is possible and therefore only approximately 46% male and 58% female were used for spawning. After selection of breeders artificial propagation were done as following steps. The belly of the fish is wiped dry with cloth, then a gentle pressure is applied to the belly to make the roe flow out. The eggs of 3-5 females are released into 10 L plastic dishes, then the milt of 5-10 fishes is added to the dish About 20 mL of fish milt is used to 1 L of eggs. The eggs are fertilized immediately upon their removal from the female. The eggs and milt are thoroughly mixed by a feather, before addition of water

The fertilization is promoted by the use of clean water of the river. Not any fertilizing solution or deadhesive material are used. The mixing action is continuous for 2 min. At 2 min intervals, water is added to the mixture. After 30 min, the water is poured off and fresh water is added, it is repeated several times. The eggs are swell to 4 to 5 times their original volume within 1 h and then are put in safety place for 2 h without any movement or shaking and then after rinsing the eggs are placed in sisgren incubators. In the first 1-2 days the sisgren incubators is to have a moderate flow of water and then at eyed stage, the eggs are placed in hatching 10 L jar. The water temperature during hatching is 15-18°C. After three days of fertilization the flow are increased to supply more oxygen for developing eggs. For preventing the generation of fungus, the eggs are not treated by malachite green or any drug. Hatching of the larvae is expected on the 6th day. After hatching, the hatched larvae are transferred, to 240 L Zug jar. After 3 days, at starting active feeding, by the time of yolk absorption the larvae are transferred to 05-2 ha.

The 3 years data of propagation of *R. f. kutum* are presented in Table 1 biological and technological data.

From the total number of fish catched in the river from 2.1 male and 1.7 female one fish were selected as spawner. The highest number of spawner (2560) was catch from Shiroud and the lowest number (510) from Tonkabon river. The number of breeders catch in river was highly correlated with the condition of weather turbidity of sea and river, the condition of the delta of the river. In this respect, the wind direction, sea weaving, direction of river flow to sea, turbidity of river were significant ($p<0.05$). The average female catched were 37% heavier than male. Also total length, fork length and standard length of male were shorter than female by 26, 28 and 14%, respectively. The smallest fish spawned had a 30 cm length female and 25.7 cm male.

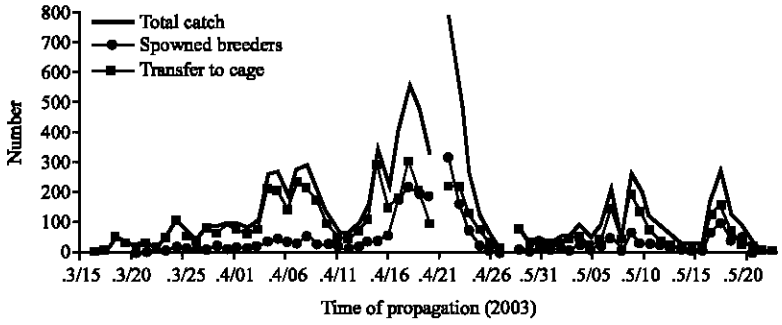

Fig. 1: The diagram shows the total catch, spawned and kept in cage for increase ripening at different time of spawning periods

Table 1: Biological and technological data of *Rutilus frisii kutum*

Criteria of investigation	Spawning results
Propagation period	15 March-30 May
Measure of sexually mature female	35-60 cm and weight of 550 to 2700 g
Ratio of breeders to catch male	1:2.1
Ratio of breeders to catch female	1:1.7
Ratio of optimum female to male sex	1:2.38
Percentage of the females migrate to the river produce eggs	65-75
Count of dry eggs per kg	379342
Count of swollen eggs per liter	67250
Size of the dry eggs	1-1.5 mm diameter
Size of swollen eggs	2-3 mm diameter
Dry eggs by b.wt. of female	130.2 g
Swollen eggs by b.wt. of female	491 g
Swollen eggs per female	520-825g
No. of eggs by b.wt. of female	33370
No. of eggs per female in different rivers	36380-56299
Milt production per male	10 to 20 mL
Fertility rate	80 to 95%
Optimum water temperature	15-18°C
Time required for hatching	4-6 days (80-90°C days)
Time span of the larval stage	3-4 days (60-70°C days)
Survival rate of batched larvae to the first feeding	90-95%
Four-day old larvae/1 kg of dry eggs	300 000 to 400 000
Rate of stocked larva in zog-jar	2000 larvae L^{-1}
Measure of four-day old fry	5-7 mm in length
First feeding	Within 3-4 days
The start feeding in earthen pond	Rotatoria
Density of stocking	500000-600000

Table 2: Comparing the sperm parameters of two main river of South part of Caspian sea

Name of river	Type	Motility	Viscosity	No. (mL^{-1})	Vol. per fish
Tajan	Normal	3±0.7	Fluid	211±27	3.3±0.8
Shiroud	Normal	4±0.3	Viscosity	197±39	3.7±0.9

The temperature of water is an important factor in ripening and spawning of fish. The temperature of the river was from 5 to 21°C and mostly 11°C. The average temperature of river was 11.16°C. Increasing temperature from average 11°C to average 20°C showed significant correlation with ripening of breeders and the correlation was positive but low ($r = 0.174$). With increasing temperature in range of spawning period, the brood stocks had a better potential for propagation. Out of this range number of breeders reduced and the quality of breeders and eggs were reduced as well as the weight of ovary.

The weight of ovary of 148 ovulated female were 165.7 g. The highest amount of eggs (480 g) and lowest amount (42 g) were obtained from the fishes of 3380 and 350 g, respectively. The most frequent breeders were 900 g fishes had a 146.7 g ovary. There was a high correlation between the weight of ovary and weight and length of fish of $r = 0.931$, $r = 0.913$, respectively. Number of dry eggs (eggs before fertilization and incubation) was in the range of 216-434 and in average 320. The correlation between weight of fish and number of eggs are storage and negative ($r = -0.579$). Number of wet eggs per gram (4 h after fertilization) was in average 67. That is in average for 4 river 33270 number per kg fish and 40723 per fish. The fish that have good quality of sperm were used. For testing the quality of sperm 100 fish were selected randomly and the following items are measured (Table 2).

Fertilization is the main criterion for distinction quality of eggs and sperm. The average fertilization rate of fish in four rivers was 85%. The estimation of fertilization in Shiroud river was 92%, Tonkabon river 95%, Tajan river 75% and Goharbaran river 78%. The low rate of fertilization in Tajan and Goharbaran is due to obtain breeders from delta or sea that is not ripped enough. From total eggs for incubation 66% hatched and larvae produced.

The eggs, during incubation, due to damage and unfavorable condition of incubation become weak and having potential to fungi infection. The management of farm and incubation is very important in

this respect. Mortality and fungi infection was 5-34% and in some special unfavorable condition up to 100%. The absolute fecundity from 8604 fish sampling was 40723. The minimum fecundity in Shiroud was 13768 but the average was 37651. The fecundity is highly correlated and significant with length (r = 0.918), weight (r = 0.849), age (r = 0.801) and the size of ovary (r = 0.952). The relative fecundity is number of eggs per kg of fish. The average fecundity in *R. f. kutum* was 33270. This amount is very close to absolute fecundity that indicates the average weight of fish is about 1 kg. Minimum relative fecundity was 22902 and maximum 10738, relative fecundity of 56538 had the highest mode between all the fish spawned. Relative fecundity had a highly negative correlation with total length (r = -0.398), weight (r = -0.422), age (r = -0.657) and the weight of ovary (r = -0.163) and it was significant.

DISCUSSION

There are a series of changes in ecosystem of Caspian sea, e.g., fluctuation of level of water, invention of *Mnemiopsis leidyi* (Yousefian and kideys, 2003), changes in hydrology and hydrobiology of it. In the other hand for several decades peoples have abused rivers using for disposal of the wastes. Most of the rivers have proved unable to absorb the increasing run-off resulting from poor watershed management resulting in increasing flooding maiuly due to change in land use within the basins including deforestation, damming and accelerated drainage of urban and agricultural lands. At the same time there has been a progressive loss of biological diversity in the form of species or sub-species adapted to the former regimes but unable to survive or endangering some of the native and unique fishes such as *R. f. kutom* in the modified conditions.

In this respect we tried to report part of biology reproduction and normative of artificial propagation of *R. f. kutum*, which is the highest value bony fish of Caspian Sea.

Biological Aspect of *R. f. kutum* at Spawning Time

The adult *R. f. kutum* reach an average length of 45 cm, has a slim cyrindial shape and is a vigorous swimmer. Distinct secondary sex characteristics of male is not difficult distingnish between immature males and mature males due to special white spotted spread on head and sides of fishes (named as wedding spot).

The *R. f. kutum* is a semi migrating fish of south Caspian sea, that for spawning migrate to the south river from end of winter to middle of spring. Artificial reproduction is substitute of natural spawning due to improper condition of the rivers. Uulike most of marine fish that can spawn after induction hormones such as GnRHa, (Arabaci and Sari, 2004) , in case of *R. f. kutum* most of breeders are riped enough for propagation at the time of catching and are not to be stimulate with any hormonal treatment except a part that is induce by using carp pituitary extract or recently by using GnRHa combined with domperidone (Heyrati *et al*., 2007). In this respect during 2002-2003 in average 12800 breeders (males and females) are catch for production of 305 million fingerlings in earthen pond.

The survey of migration of breeders showed, up to April temperature in average 10°C and more than 60% of breeders catch in river were not ripped enough, they were kept in cage for further gonad development. By increasing temperature (>11°C in April) most of fish catched was ready for spawning however by increasing temperature from 18°C to 21°C, the quality of eggs decreased. The eggs shell become soft, gelatin from and the rate of active larvae decreased significantly. Catching and harvesting of fish in the river was also under many different factor effects. Sometimes the delta was closed, or the seawater enter to the river, fluctuation of river current and muddy condition was an other problem of *R. f. kutum* migration.

The unstable condition of river and seas make the high variation in fish qnality and quantity in different rivers. The salinity, turbidity, temperature are effect on size, type and qnality of fish migrate

for spawning. Therefore high variation exists in normative of fish propagated in different rivers and among the breeders. High turbidity and flooding of river make impossible catching the breeders in the rivers, therefore the fish are catch by gill net in delta of river, but they are not ripped enough. This reduces the hatchability of eggs to 30-50%. Solving this problem, the fish that are not of final stage of maturation, are transferred to cage fixed in the side of river, provoke fish spawning and up to 65% of immature fish will spawn 1-2 days staying there. Comparing number of dry and wet eggs per kg of fish during last 5 years do not show any significant different while the hatching rate decrease from 95% in 1998 to 85% in 2003, indicating the rate of immature fish have been increased. As stated before a period of incubation of *R. f. kutum* eggs, are spending in the river. Keeping the *R. f. kutum* eggs in improper condition such as muddy condition and salinity in a number of river stop eggs development. The average size of mature females (700 g) has been decreasing.

Fecundity traits should only be considered in a breeding program if the genetic correlation between egg size or egg quality with early survival or growth rate is negative. It is, however, important to keep records of these traits to study whether or not they are changing over a period of time. Survey of absolute and relative fecundity between the years of 1973 and 2003 (Yousefian *et al*., 2003) showed that there are very close number of absolute fecundity (56953 in 2002 versus, 58615 in 1973) and no significant difference between them, while relative fecundity have been changed from 74774 to 47352, it means the fish catch as breeders are smaller in resent years comparing to 1973. Coad (1995) also repeated that the average size of mature females (700 g) has been decreasing. Reduction of average size of fish is due to selection of ripped fish taking the eggs. Actually small fish will ripe earlier than bigger and ovulation takes place faster than bigger fish. Approximately 72% of fish bigger than 85% of fish smaller than 800 g spawn easily. Therefore trend of artificial propagation is toward small fishes. This may affect negative selection of growth rate, therefore selection response for growth rate are negative and diminish some unknown rare gene and genetic drift. Actually losing the bigger fish that need to stay more in the river for their genes characteristics of late ovulation or late spawning genetics patterns is incorrect and displeased part of the study.

With this system these fish are chose only if they ovulate easier than the others and no regard is consider of other characters e.g., growth rate, disease resistance, salinity resistance, etc. For solving the genetic problem we suggested during hole period of spawning time, a port of all breeders whether are small or large, should be propagate. If they are not ripped yet, they will be kept in fibreglasses 2×2×1 near the river. CP stimulates the permeation and ovulation injection intramuscularly at doses 5 mg kg^{-1} b.wt. In this case females are stripped at 300-450 degree-hours post-stimulation.

REFERENCES

Arabaci, M. and M. Sari, 2004. Induction of ovulation in endemic pearl mullet (*Chalcalburnus tarichi*), living in the highly alkaline Lake Van using GnRHa ([D-Ser(tBu)6, Pro9-Net]GnRH) combined with haloperidol. Aquaculture, 238: 529-535.

Coad, B.W., 1995. Freshwater fishes of Iran. Acta Scientiarum Naturalium. Acad. Scientiarum Bohemicae, 29: 1-64.

Heyrati, F.P., H. Mostafavi, H. Toloee and S. Dorafshan, 2007. Induced spawning of kutum, Rutilus frisii kutum (Kamenskii, 1901) using (D-Ala6, Pro9-NET) GnRHa combined with domperidone. Aquaculture, 265: 288-293.

Yousefian, M. and A.E. Kideys, 2003. Biochemical composition of *Mnemiopsis leidyi* in southern Caspian Sea. Fish Physiol. Biochem., 29: 127-131.

Yousefian, M., H.K. Rostami, S. Phyrozkandian, H. Molaei and F. Habibi *et al*., 2003. The quantitative, qualitative and hygienic investigation of Caspian Kutum (*Rutilus frisii* kutum) fingerlings in Shahid Rajaei fish propagation center, Final Report. Iranian Fisheries Research Organizatio Publishers Ed., 83.492, pp: 105 (In Persian).