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Investigation of Reproduction Steps of *Perca fluviatilis* in Anzali Pond (2007-2008)

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ABSTRACT

The present research was conducted on *Perca fluviatilis* fish from May 2007 till June 2008 in Anzali Pond. Sampling was conducted by means of Gill net with 18-20 mm holes in a monthly manner. 254 samples of the fish type were caught. Parameters like total length (mm), fork length (mm) body weight (grams), and gender were measured in all months and parameters like Gonad weight (grams), ovule diameter (mm), and relative and absolute reproduction were measured from October till February. Measurement of the weights of male and female Gonad and Ovule diameter during October till February showed that ovary maturity in this type of fish is simultaneous and in a group form and increase in the size of the weight of Gonadsand increase in ovule diameter starts from October and continues until December in which spawning is done at a time. In the investigated samples, the maximum ovules diameter belonged to February with about 45 Microns which is one month before spawning. It seems that this value is increased until spawning in December. Therefore, the size of ovules was about 1 mm in an ovary of a fish in which several ovules were remaining after spawning. Mean value of fork length and body weight for male and female fishes was equal to 188.4 and 116.78 respectively. Absolute reproduction was measured from November to February and 35942 ovules were obtained on average. Most samples were placed in the first stage of sexual maturity with a frequency of 39.4%. from among 254 fishes, gender of 251 samples were identified. Males constituted 37% and females constituted 63% of prey. Sexual ratio of female to male was 1.7:1. Further, ovaries were also investigated in terms of histology in October to February. The results of the investigation of Gonads sexual maturity stages via histology and the results of investigation of sexual maturity stages were macroscopically consistent.

KEYWORDS: Perca fluviatilis, sexual maturity, reproduction, Anzali Pond.

INTRODUCTION

Perca fluviatilis is a fresh water fish species which lives mainly in European and Asian waters in geographical latitudes between 40 to 70 (Weatherley, 1963). In Iran, it lives in southern Caspian sea in Anzali Pond and rivers which discharge Caspian Sea (Sattari et al, 2002). Most reproduction studies conducted on this type of fish have been implemented in areas with high latitudes (Heibo and Magnhagen, 2005). The present research was conducted in Anzali Pond which is situated in low latitude. Fishes and other animals have different reproduction features in different geographical areas (Bani and Moltschaniwskyj, 2008). Therefore, an investigation of reproduction features of *perca fluviatilis* in low latitudes can explain diversity in this fish reproduction features and variations in reproduction time and spawning. Differences in reproduction features like sexual maturity index, reproduction, the time and way for spawning are affected by biological and non-biological factors (Pankhurst and van Der Kraak, 1997). spawning time and reproduction time depend on environmental factors like light period length, water temperature, nutrition and physic-chemical parameters (Glasser et al, 2004). Local and regional factors can also affect spawning time. These include spring floods in large rivers which enable perca to use flooded areas for spawning (Balon, 1963). Anyway, the start and length of spawning period is different in different geographical populations (Brown and Peterson et al, 2001). Below Table summarizes spawning time in different areas which have been reported by different individuals.

place	Spawning time	Year	researcher
Western europe	Early April to late May	1980	Holliday, Tresurer
Western europe	Early April to late May	1987	Craig
Western europe	Early April to late May	1995	Gubier
Lindre lake in France	Middle April with immediate temperature increase	1998	Sulistyo
Aydat lake	Middle April with immediate temperature increase	1994	Jamet and Dessmols
Windermer lake	Middle April	1951	Lecren
Saarlampi lake in south Finland in 62 degrees of latitude	Middle May	1966	Urho
Suomungarvii lake in Finland	May	1982	Viljanen and Holopainen
Geneva lake in France in 46 degrees of northern latitude	Late April to June	1995	Gillet and Dubois

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Two types of reproduction can be explained in this kind of fish: potential reproduction which includes the number of mature ovules in ovary before spawning. Real reproduction which includes spawned ovules. The numbers of ovules which are attracted in ovary during Gonad evolution affect reproduction.

In *perca fluviatilis* species, ovary is single and testicle is in pairs. This kind of fish spawns collectively (Sulistyo et al, 1998). The present research aims to investigate the reproduction of *perca fluviatilis* fish during a year in Anzali pond and its sexual maturity stages were also studied. Anzali Pond has a surface area equal to 218 square kilometers and is located south Caspian Sea (37 degrees and 28 minutes of northern latitude, and 49 degrees and 25 minutes of eastern longitude) in Guilan Province and is classified into four sections: central, western, eastern and SiahKeshim which is located in the south western section.

MATERIALS AND METHODS

Samples studied in the present research were caught since 25 of June 2007 till 15th of 25th of May 2008 during 12 months in a station in the western part of the Pond (beaches of Anzali Pond). Sampling was conducted by means of Gill et with 18-20 mm net size. In every sampling, 20 to 30 fishes (*Perca fluviatilis*) were randomly selected and were transferred to the laboratory of faculty of Sciences and Marine Technology (Tehran) after freezing. In general, 254 samples were analyzed. The frozen fishes were defrosted first. Then, a ruler with 1 millimeter precision and a balance with 1 milligram precision were used for determination of weight and length parameters of fishes. After biometry and measurement of weight, the fishes ages were determined. Age determination was conducted by means of scales of fishes. First, 2 to 3 scales were removed from the middle section of the rear fin and side line and then the scales were washed by means of lukewarm water and they were placed on slide and were investigated by means of a microscope with 4*10 magnifying power. The procedure was that one dark ring was considered along with a bright area during 1 total year. The fishes were dissected order to determine sexual maturity stages. Kesteven's seven-stage sexual maturity graduation table was used for determination of sexual maturity stages (Bisvas, 1993).

It must be mentioned that the gender of all fishes were identified except for three fishes. Therefore, the 251 fishes were used in the subsequent investigations. In order to measure their weights of Gonads, ovaries and testicles were removed from inside of the bellies of the fishes and were weighed by means of a digital balance with 1 milligram precision. In order to measure ovule diameter in the samples, a number of ovules were removed from different spots of ovary by means of dissection knife (two ends of ovary and its middle) and was stabilized in 10% Formalin. Then, they were spread on a slide. Diameters of 30 ovules were randomly measured and registered by means of a microscope equipped with graduated lens and with 4*10 magnifying power. Then, mean value of this number was calculated as mean value of ovule diameter (Bisvas, 1993). We managed to measure ovule diameter only for 38 female fishes out of 158 fishes.

In order to determine absolute reproduction, the whole ovary was weighed by digital balance with 1 milligram precision. Then, it was put into Gilson solution. The ovary remained in the Gilson solution for two months and it was mixed 2 or 3 times a week by means of a glass mixer. After two months, the solution was screened and ovules were separated. They were exposed to air for about 7 to 8 days and the ovules were separated manually. They were weighed in order to determine the dry weight of the ovary. Then, 0.01 grams of the ovules was taken and the number of ovules existing in 0.01 gram of ovary's dry weight was counted. This was repeated three times. Absolute reproduction was calculated using the following formula:

$$\text{reproduction} = \frac{\text{dry weight}}{\%1} \times \text{average of the three numbers}$$

relative fish reproduction was calculated by the following formulas (Bisvas, 1993):

$$\text{reproduction} = \frac{\text{all ovules lv}}{\text{body weight}} \qquad \text{relative reproduction} = \frac{\text{all ovules}}{\text{body length}}$$

The following formula was used for calculating quality coefficient or quality coefficient or K factor:

$K=Cf=(W*10^5)/L^3$

K=quality coefficient

W= fish weight (gram)

L= fish length

In order to calculate (GSI) index which is in fact an indirect method for estimation of spawning season of species, first we weighed the weight of Gonad of any fish using a digital balance with 1 milligramprecision and then we used the following formula for calculation of GSI (Bisvas, 1993):

$$GSI = \frac{\text{Gonad weight (grams)}}{\text{body weight (grams)}} \times 100$$

CONCLUSION AND DISCUSSION

The main biometric factors of female fishes were investigated in all stages of sexual maturity. Therefore, parameters like fork length, body weight, ovule weight, ovule diameter and absolute reproduction were investigated separately.

Table 1.the main biometric factors of female fishes in the fourth stage of sexual maturity regarding January

SD	Mean	samples	Indices
1.16	20.25	22	fork length (centimeters)
22.43	135.31	22	Fish weight (grams)
3.12	8.12	21	Ovary weight (grams)
4.23	30.72	19	Ovule diameter (microns)
14450.5	38290.64	11	reproduction
63.16	173.25	11	Relative reproduction (with respect to length)
83.08	263.36	11	Relative reproduction (with respect to weight)

Table 2.the main biometric factors in female fishes in the fifth stage of sexual maturity regarding February

SD	Mean	Sample numbers	Indices
0.97	19.46	8	Fork length (centimeters)
26.65	129.86	8	Fish weight (grams)
3.58	14.55	8	Ovary weight (grams)
3.45	37.39	8	Ovule diameter (microns)
9455.6	27334.67	3	Absolute reproduction
44.89	130.61	3	Relative reproduction (with respect to length)
83.93	210.25	3	Relative reproduction (with respect to weight)

Ovule diameter

In the sixth stage of sexual maturity which belongs to March, ovule diameters were not measurable in all samples except for one sample in which a number of ovules remained in the ovary after spawning and ovules diameters were measured to be equal to 1 millimeter. In this research, the diameters of ovules of 38 fishes were measurable from among 154 female fishes. These 38 fishes belonged to October to February months. Mean value of ovule diameter in this research was equal to 28.3± 8.19 except March sample. The maximum diameter of ovules which belongs to February was about 37.39 microns. Furthermore, no ovule was observed in ovary from April to September.

Variance analysis test showed that there is a significant statistical difference between different months in terms of ovule diameter mean ($p<0.05$). Dunkan's test shows that there is a difference between all investigated months in terms of ovule diameter mean value in the paired form. The results showed that average ovule diameter increased from 17.52 ±1.7 microns in October to 37.39 ±3.45 in February. Ovules diameter has a pick point in February.

Sexual maturity index: (GSI)

The results of analysis of GSI average one-way ANOVA on male fishes in different months showed that there is a significant difference between different months in terms of GSI average ($p<0.05$). Dunken test showed that there was a significant difference between different months in terms of GSI average. The results of analysis of GSI average one-way ANOVA on female fishes showed that there is a significant difference between different months in terms of GSI average value ($p<0.05$). Duncan\s test showed that there is a significant difference between different months in terms of GSI mean value. Considering the results of the research on ovule and GSI, it can be concluded that spawning is immediate and its time is late march when the weather warms.

Quality coefficient or (CF): considering the fact that there was one sample in male fishes in April, this sample was set aside for analysis. The results of one-way variance analysis of mean value of quality coefficient in male fishes in different months showed that there is a significant difference between different months in terms of quality coefficient average ($p<0.05$). considering Duncan's test, only March was different from other months in terms of quality coefficient average. The results of one-way variance analysis for average of quality coefficient of female fishes in different months showed that there is a significant statistical difference between different months in terms of quality coefficient average ($p<0.05$). considering Duncan's test, there is a significant difference between August and other months in terms of average quality coefficient.

Absolute reproduction

The following conclusions can be drawn considering absolute reproductiondata in November, December and February:

- average and standard deviation of absolute reproductionin November was equal to 29807+-9071.26 and 22500 and 39960 for maximum and minimum level
- mean value ad standard deviation of absolute reproductionin December was equal to 41742+-15244.25 and 27567 and 71400 for minimum and maximum level
- mean value ad standard deviation of absolute reproductionin February was equal to 27334+-9455.62 and 16456 and 33580 for minimum and maximum level

One-way variance analysis did not show any significant difference between these three months in terms of reproduction average ($p>0.05$).

Microscopic and macroscopic investigation of Gonads

In macroscopic analysis, female and male Gonads showed serious differences in different months in terms of color and size. Ovaries which are single in these fishes did not have any growth during June to September and were in the form of a thin strip in abdominal area which was hardly visible. Since middle September, ovaries size increased and very tiny ovules were visible in October in ovary. As ovaries grew, their colors changed from pink to yellow-ginger and capillarywere observable on the thin membrane which surrounded the ovary. After spawning in March, the ovaries were observed in the form of pink shells with smaller size and weight. In male ones, testicles were in pairs and milky white in color. The results showed that in reproduction season, male fishes become sexually mature earlier than female ones. Further, the size of testiclesis reduced after spawning. An investigation of biometric factors showed that average ovule diameter and average ovary weight increased from step 4 to step 5 (January to February) while they reduced from stage 5 to stage 6 (February to March). Furthermore, in the fifth stage of sexual maturity, an ovary is fully mature and has the maximum weight and ovules diameters are also maximum and the fishes are ready for spawning in this stage. In general, the weight of ovary in female fishes is more than testicle weight in female fishes. This is due to high volume of ovary in female fishes. Stages 3 to 5 of sexual maturity were observed in investigation of texture of ovary and testis of *perca fluviatilis* fish from October to February. Stage 3 of sexual maturity lasts from late September to late October, stage 4 lasts from early November to late January and stage 5 belongs to February and in March, the fishes are ready for spawning. According to observations of this research, maturity stages were the same both in apparent observation and Gonad histology method.

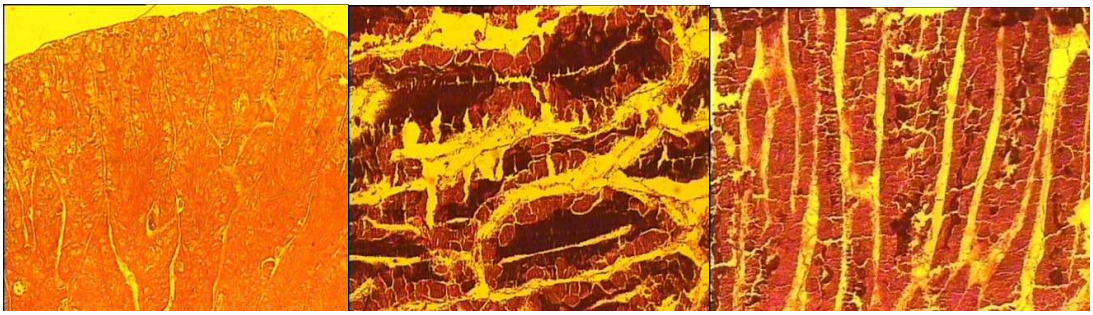

Figure 1.texturesamples related to testicle

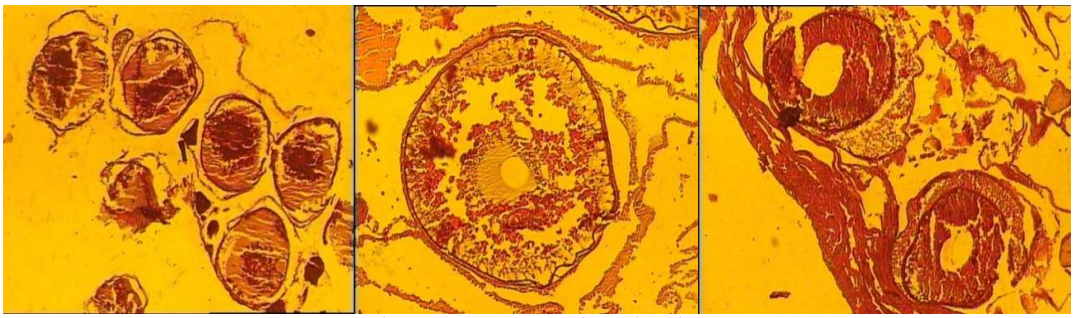

Figure 2.texturesamples related to ovary

In this type of fish, Gonad evolution starts with water temperature reduction and light periodin autumn. In this research, spawning in Anzali Pond took place in low geographical latitude (37 degrees and 28 minutes of northern latitude and 49 degrees and 25 minutes of eastern longitude) in March along with water temperature

warming and increasing the length of light period. According to investigations conducted on this type of fish(some of which were referred to in table 1) in higher latitudes, this type of fish reproduces in spring since early April to late June. Therefore, it can be concluded that reproduction takes place earlier in lower latitudes than higher latitudes. These is due to earlier water warming and increase in the length of light period. Variations in light periodmay be a decisive factor in the late maturity stage of Spermatozoaor the storage period. Therefore, it seems that water temperature variation is an environmental factor which controls annual reproduction cycle in this type of fish especially in female (Isdysulistio et al, 2000). Spawning time of this type of fish depends largely on water temperature. When water warms up immediately, spawning takes place in March. However, if warming takes place gradually from March to April, spawning may take place in early april. Temperature seems to be the main controlling factor in reproduction cycle and spawning (Miguad et al, 2003; 1979, Scott; 1993, Bromage).

Kjorsvik et al (1990) and Bromage and Roberts (1995) believed that other factors like nutrition, genetics, raising conditions and stress can affect this type of fish and influence quality of reproduction and quality.

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