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Fecundity and maturation of South Caspian spirlin, *Alburnoides* sp. (Actinopterygii: Cypriniade) from Iran

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Abstract

A total of 48 female spirlins *Alburnoides* sp. from the Kesselian stream (South Caspian Sea basin, northern Iran) were examined during a period from July 2008 to June 2009 to obtain data on fecundity, and to determine the spawning season of this carp. The mean absolute fecundity of females spirlin was 1722.92 (±653.88) eggs. The fecundity has positive correlation with the size of the female. The mean of the monthly GSI index and ova diameters shows that spirlin spawn once in a year during the months of June-July. GSI of 6.88 was considered as the indicator of sexual maturity for the females. The highest peak of relative condition factor (Kn) was 0.126 during the month of June. The stages of gonads maturation of Caspian spirlin have been classified as: I, immature; II, early developing; III, late developing; IV, mature; V, ripe (spawning)

Keywords: *Alburnoides*; spirlin, fecundity; GSI; spawning; Iran

1. Introduction

In order to obtain a comprehensive insight into the biology, population dynamics, applying monitoring, conservation and management programs of a particular species, its reproductive potentials should be investigated. Different reproductive parameters and indices such as number of produced eggs (fecundity), ova diameter, condition factor, stages of maturity, sex ratio, gonado-somatic index, modified gonado-somatic index (MGSI) and dobryial index (DI) have been used to determine reproductive status and spawning season of different fish species [1-8].

The number of produced eggs, including absolute and relative fecundity, defines reproductive strategy of fish species in terms of internal or external fertilizations, parental cares and continuity of gene flow in different generations and thus the species survival. The other above-mentioned parameters also reflect the reproductive potentials of fish species showing spawning season, frequency of reproduction, mating systems, fish health and energetic adaptations [9] and thus have to be considered.

South Caspian spirlin is a very abundant and widely distributed native cyprinid fish in the Caspian Sea drainage of Iran and its taxonomic position is currently under review [7, 10] and might be considered as a distinct species, at least in the center and east of the Southern Caspian Sea basin. Despite its abundance and importance in Iranian waters, the available information on the reproduction of south Caspian spirlin is quite scarce, therefore, the reproduction biology and life history of this fish including ova diameter, maturity stages, fecundity and spawning season have been investigated in the Kesselian stream (South Caspian Sea basin, Iran). The outcome of the present investigation shall be useful for the future management and conservation strategies of this native fish species.

2. Materials and methods

Sample collection and preparation

Female spirlins were collected at monthly intervals

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from July 2008 to June 2009 from the Kesselian stream (52° 59′E, 36° 13′N) in the south of the Caspian Sea basin, northern Iran using portable electro fishing gear. Some physical and chemical parameters of water were also determined. The average water temperature was 12.56°C, not exceeding 18 °C during May and 2 °C again in October. The average pH was found to be 6.5.

In the field the collected fish samples were fixed in 10% formaldehyde solution. The total length (TL) was measured using electronic digital caliper to the nearest 0.01 mm, and body weight (W) and gonad weight (GW) of the formalin fixed specimens were recorded by an electronic analytical balance having an accuracy of ±0.01 g. The fish were dissected laterally from the right side and the gonads were removed and finally fixed in 5% formalin for further examinations.

Ova diameter and maturity stages

To measure the ova diameter, 48 formalin fixed ovaries were used. The sub-sampling method was used to select ova randomly since it is not possible to measure all ova in the ovary. In the current study sub-samples were obtained from each ovary that had been divided into 3 sections (anterior, middle and posterior sections of each lobe) and this was followed by weighing a piece of each section [2]. All oocytes were measured and counted from the anterior, posterior and middle sections randomly. Diameters of 60 ova were measured randomly using a dissection microscope equipped with an optical micrometer. Usually, only one diameter of an ovum is measured, but in this case two different diameters (maximum width and length) were measured because the ova shapes were somewhat distorted [4].

Maturity of the ovary was performed based on morphological characteristics of the ovary including: colour, transparency, texture, size, shape and degree of occupation in the body cavity, length and weight of ovary, ova diameter and also based on the microscopic examination of ovary [1, 2]. The following stages of maturity were ascertained in female spirlin from the Kesselian stream: Stage I, immature ovaries, II, early maturing, III, maturing, IV, Ripe, V, (running), or (Spawning).

Spawning season

The spawning period of south Caspian spirlin was determined from the analysis of six variables:
(I) Percentage frequency of the maturity stages [11].
(II) Gonado-somatic index, GSI=(weight of gonads / weight of fish) ×100 [1, 8].
(III) Modified gonado-somatic index (MGSI), MGSI=(gonad weight/fish weight-gonad weight)×100 [1]
(IV) Dobriyal index (DI), DI= $\sqrt[3]{GW}$ [3] where *GW* is the average gonad weight
(V) Condition factor (Kn) Kn=$(W/L^3)\times100000$ where W and L are total weight and total length respectively [1, 2]
(VI) Oocyte diameter (OD) [11].

Fecundity

As knowledge of the fecundity of a species is one of the pre-requisites in fish management and in determining the racial differences in a population, the studies on the fecundity of south Caspian spirlin have been carried out for the first time using conventional method. The gonads were examined and absolute fecundity was measured in terms of the total number of oocytes presented in both the ovaries using Bagenal’s method [12]. For this purpose, the ovaries were accurately weighed after removing excess water on filter paper and then 3 subsamples were prepared from the anterior, middle and posterior parts of the ovaries. The number of eggs in the subsamples was counted and then the total number was calculated using the following formula:

$$F = Fs \text{ X } \frac{GW}{GWs}$$

where F is the absolute fecundity, Fs the number of eggs in the sub-samples, GW is the weight of the ovary and GW_S the weight of the sub-sample.

Relative fecundity (Fa) is obtained by the ration of the number of eggs per one gram fish body weight [2]. The quantitative data of the spirlin reproductive biology (ova diameter variations, GSI, MGSI, DI, Kn, F and Fa) were analyzed in Excel and SPSS softwares using standard statistical tests.

3. Results

Ovarian maturity stages

The maturity stages of Caspian spirlin were classified based on the morphological characteristics of ovary and ova diameter. There was no significant difference ($p > 0.05$) in the oocyte diameters among portions (anterior, middle, posterior) for an ovary but significant differences ($p < 0.05$) were found for the same section among individuals at various stages of development. Each gonad was classified according to the most advanced type of oocyte present. The diameter of oocytes of spirlin ranged between 0.02-1.67 mm. Mean egg length (horizontal x ventricle) was 0.08 mm x 0.11 mm for stage I eggs, 0.10 mm x 0.14

mm for stage II eggs and 0.19 mm x 0.23mm for stage III eggs, 0.35 mm x 0.40 mm for stage IV eggs and 0.42 mm x 0.53mm for stage V eggs (Table 1).

Table 1. Egg size (mm) of different stages of female spirlin from South Caspian Sea basin Iran

		Stage I 289	Stage II N=375	Stage III N=206	Stage IV N=72	Stage V N=73
Mean	Long Axis	0.11	0.14	0.236	0.409	0.536
	Short Axis	0.086	0.109	0.194	0.354	0.42
S.D.	Long Axis	0.038	0.066	0.194	0.354	0.50
	Short Axis	0.033	0.055	0.177	0.32	0.42
Minimum	Long Axis	0.03	0.04	0.04	0.05	0.04
	Short Axis	0.021	0.019	0.028	0.031	0.024
Maximum	Long Axis	0.29	0.64	1.38	1.46	1.67
	Short Axis	0.25	0.427	1.21	1.431	1.31

Spawning season

The spawning season of south Caspian spirlin was determined on the basis of monthly variations in the mean gonado-somatic index, modified gonado-somatic index, Dobriyal index, the mean diameter of the most advanced stage of oocytes, and the proportion of the developmental stages of the ovaries. The gonado-somatic index of females showed a slightly increasing pattern from August (0.47) to May (3.71) reaching maximum in the month of June (6.88), indicating that most of the females were found in ripe and running stages in June and early maturing stage (II) or spent in August, hence indicating the spawning season of this fish during this period. According to the present studies a GSI of 6.88 was considered a reliable indicator of reproductive maturity for female (Fig. 1). Monthly mean ova diameters showed the same trend to that of the mean monthly GSI index.

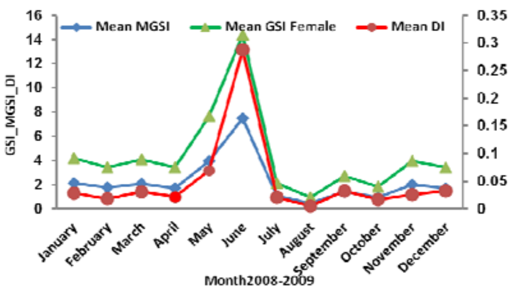

Fig. 1. Monthly variations of GSI-MGSI and DI indices in female specimens of spirlin from South Caspian Sea basin of Iran

The present observations also indicate that there was no significant difference between GSI and MGSI (Anova, p>0.05) in the south Caspian spirlin specimens showing the same trend. The values of mean of the MGSI are presented in Fig. 1. The DI index showed the same trends as GSI and MGSI (Fig. 1).

The relative condition factor (K_n) of spirlin in the south of the Caspian Sea basin from the north of Iran was calculated for different months for males and females. The highest peak of condition factor in spirlin was in June (0.126) when more ripe eggs were present and the lowest peak 0.098 in February. This shows the effect of gonad weight on the k_n value (Fig. 2).

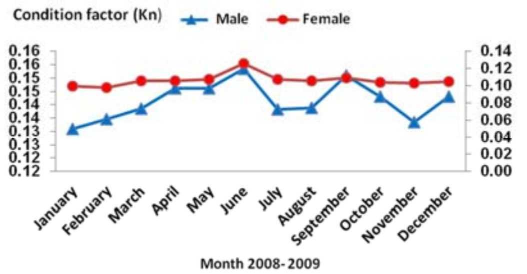

Fig. 2. Monthly variation of condition factor (K_n) formale and female spirlin from South Caspian Sea basin Iran

In order to determine the spawning season on the basis of ova diameter, percentage frequencies of ova diameter for graphs in different months have been plotted (Fig. 3). The Figure reveals the dominance of mature ova in the months of May and June, while in the other months the fish remain in the inactive stages of seasonal sexual maturity, indicating that one spawning season can be considered for this fish (Table 2). The high percentage of stages IV and V in the months of May and June also reveals the active reproductive status of this fish (Fig. 3).

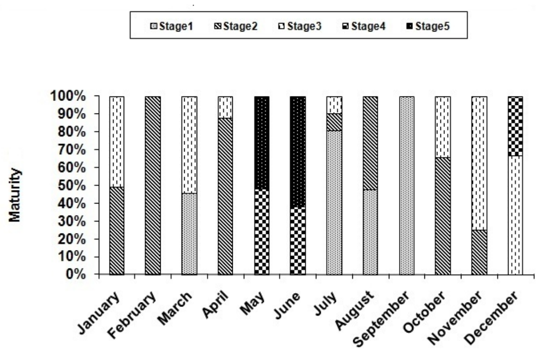

Fig. 3. Monthly changes in percentage occurrence of each ovarian maturity stage of female spirlins from South Caspian Sea basin of Iran

Fecundity

A total of 32 females with mature ovaries was used to estimate fecundity. In each ovary, oocytes > 0.1 mm in diameter (corresponding to the yolk vesicle stage) in the ovary subsample were counted and then the total number was calculated. Based on the observations, the mean fecundity of the 32 female spirlin was estimated to be 1722.92 (± 653.88) eggs. Individual fecundity ranged from 668 to 3042, in females it ranged from 82.18-110.47 mm total length (Table 3).

Table 2. Percentage occurrence of each ovarian maturity stage against months in female spirlin from South Caspian Sea basin of Iran

Stage	M 1	2	3	4	5	6	7	8`	9	10	11	12
1			45.7				80.7	47.4	100			
2	48.8	100		87.5			9.7	52.6		65.6	25	
3	51.2		54.3	12.5			9.7			34.4	75	66.7
4					47.9	37.7						33.3
5					52.1	62.3						

Table 3. Fecundity of female spirlin from South Caspian Sea basin of Iran (n = 32)

	Total length (mm)	Total weight (mg)	Gonad weight (mg)	Fecundity
Mean	98.72	12.27	0.92	1722.92
SD	7.08	2.55	0.42	653.88
Maximum	110.47	16.72	1.86	3042
Minimum	82.18	5.65	0.30	668

The relationship between fecundity and fork length was used. A linear regression provided positive and significant correlation between them. Y = -3025.4 + 52.506X (R^2 = 0.27, n = 32, $P < 0.05$) (Fig. 4).

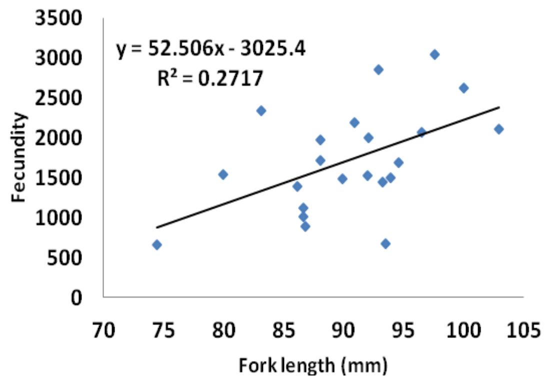

Fig. 4. Linear relationship between fecundity and fork length for female specimens of spirlin from South Caspian Sea basin of Iran

Analysis of the relationship between absolute fecundity (F) and body weight (BW) also revealed a linear regression model with a positive and significant relationship (Fig. 5): Y = -335.58 + 167.74X (R^2 = 0.4146, n = 32, $P < 0.01$). A linear regression between fecundity (F) and gonad weight (GW) was also found to be positive and significant, Y = 417.92 + 1405.6X (R^2 = 0.8389, n = 32, $P < 0.01$) (Fig. 6). The same significant relationship was estimated between fecundity (F) and GSI, Y = 972.69 + 242.72X (R^2 = 0.51, n = 32, $P < 0.01$) as is shown in (Fig. 7).

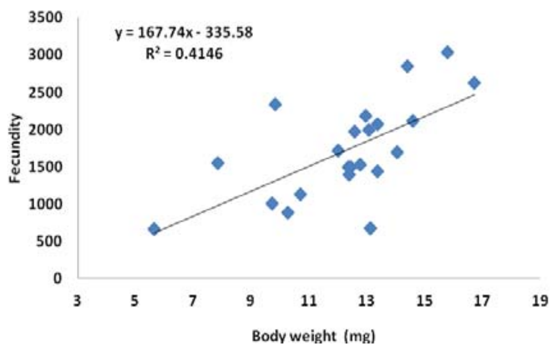

Fig. 5. Linear relationship between fecundity (F) and body weight (BW) for female specimens of spirlin from South Caspian Sea basin of Iran

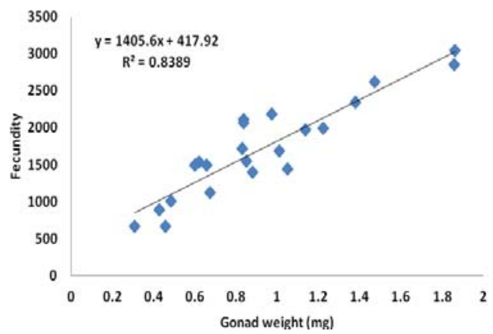

Fig. 6. Linear regression between fecundity and gonad weights for female spirlin from South Caspian Sea basin Iran

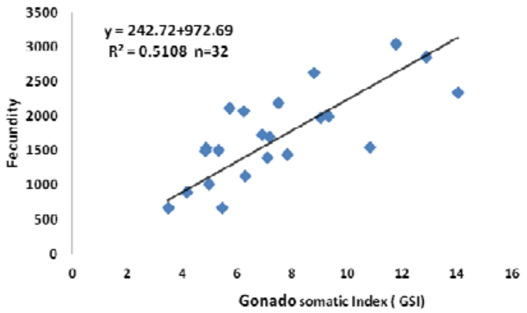

Fig. 7. Linear regression between fecundity and gonadosomatic index for female speciments of spirlin from South Caspian Sea basin of Iran

4. Discussion

There can be no doubt that the key to the effective conservation of species is the knowledge of its life history. Determination of the maturity stages, reproductive indices and fecundity are just a few of the important parameters in population dynamics and management models of fish species and to assess the status of fish stock. These aspects for spirlin from the south of the Caspian Sea basin in Iran have been discussed in this paper.

Study of ovarian maturity of south Caspian spirlin revealed that this organ could be classified into 5 different maturity stages. These stages were found in close agreement with the other fishes [1, 13]. Ovaries were evaluated to measure the reproductive indices such as GSI, MGSI and DI. The monthly variations in GSI were highly associated with the seasonality of the maturity stages assigned macroscopically [14]. Gonadosomatic index can be used to determine spawning season because GSI increases with the maturation of fish, being maximum during the period of the peak of maturity and declining abruptly thereafter, when the fish become spent [15]. The other reproductive indices could also be used for this purpose [6, 16]. In the present investigations high value of the GSI in June and its sharp decrease in the month of August shows that the fish spawn during June–July. The same results were observed using MGSI, DI data. It has been found that the reproductive indices for females were always higher than for males. The same is found in other fish species [6, 16]. This is due to the large amount of yolk materials being reserved in oocytes.

According to the present findings, it is clear that the gonad mass gives a better correlation with reproductive capacity (in estimating DI) than the body mass. Hence, this could be used for determination of spawning season, sexual maturity and frequency of spawning. This requires only the data related to sexual organs; it is easy for interpretation and calculation and provides a narrow range of index if the gonad mass is very low or very high. It has also been used to access the reproductive condition of other fishes [6,16]. Similarly, from the mean oocyte diameter in each month and presence of large oocytes in the months of April and May and small oocytes in the month of August it can be concluded that the breeding season of spirlin falls during June–July.

This result is generally consistent with the observations of Zhukov, [17] who revealed spawning occurs during May and June. Skóra [18] mentions "the character of spawning" of *A. bipunctatus*. The results given on multiple spawning during a potential breeding period ranging from the beginning of April to July explain the variety of these statements.Papadopoli and Cristofori (1980) [19] reported a large reproductive potential of spirlin in Romania, where it reproduces four to five times in a season. During the first episode of spawning more than a quarter of the eggs are released and the rest of the gametes are removed three to four times at intervals of approximately 15 days. Therefore, gametes are found at different stages of maturity in the ovaries and testes of two other cyprinids [20].

Bless [21] revealed that the potential spawning period in *A. bipunctatus* is very prolonged. At least some females spawn several times during one season. The spirlin therefore has to be considered as a multiple spawner. According to experiments done by Bless [21], females spawn more than once during different weeks. Observations of the spawning behaviour and controls of the substrates revealed that the eggs were placed deeply in the interstices. Spawning started at the end of April and ended after two weeks. In a period of two days females shed their eggs totally. Spawning occurs during the entire day. The eggs are positioned in portions at equal depth in the interstices of the substrate. Only a few eggs could be observed adhering to the surface of the sediment at the spawning place, very shortly after they were invariably eaten by the adult fishes. There is no evidence for a multiple spawning within the year [22]. The *A. bipunctatus* showed a peak in June, as reflected by both monthly changes in the percentage occurrence of each ovarian maturity stage and gonadosomatic index of female. Based on the different reproductive indices and ova diameter of south Caspian Sea spirlin, it is evident that this fish is not a multiple spawner and the present observations are in agreement with those of Bless [22].

The term condition was applied to analyse the variation from the expected weight for length of individual fish or relevant group of individuals as indications of fatness, general "well being," gonad development, etc. [23]. The relative condition factor (K_n) in *A*.cf *bipunctatus* in the south of the Caspian Sea Basin from the north of Iran for different months for males and females showed almost the same pattern with gonado-somatic index. The maximum and minimum of the condition factor in female was in June and February respectively. It shows the effect of gonad weight on the k_n value. In fact, releasing the oocytes in females and sperm in males decreased the weight of the fish so, they became thin. The change of condition factor (k_n) value in months may be due to spawning, stress, changes in temperature, pH or pollution of water, availability of food and the ratio between weight of food ingested and body weight. This has been pointed out for other fish species too [24, 25].

In this study the mean fecundity of the 32 female Spirlin was 1722.92 (±653.88) eggs. The estimated maximum numbers of ova in females are 3042.42 and the minimum numbers of ova are 667.80, which range between 82.18–110.47 mm total length.

Research in various countries of Europe showed a great dispersion of the absolute fecundity of the female during Spirlin reproduction. The average number of eggs per season varies between 800 and 3000 [26, 27, 19, 28]. Absolute fecundity of spirlin from the south of the Caspian Sea Basin from the north of Iran was similar to that reported by Polacikand Kovac [29]. The mean absolute fecundity of female spirlin *Alburnoides bipunctatus* from the Rudava stream in pre-spawning phase was 3020 eggs and mean relative fecundity was 430 eggs, but varied within a range to that of the samples from the river Radimna in Romania, where this fish had 1581-6110 eggs [19]. In contrast, spirlin from the Oltu stream have been reported to attain mean absolute fecundity 13 135 eggs [30]. The difference between mean absolute fecundity of spirlin from two populations of Turkey and Iran may be due to the bigger mean size of the Turkish females (mean fork length 10.3 cm, mean weight 18.9 g). Fecundity increases with body size because the amount of energy available for egg production and the body cavity accommodating the eggs increases with the fish size.

In the present research work a wide variation in fecundity among individual specimens of the south Caspian spirlin was found. In different teleosts, this variation has been attributed to differential abundance of food [31], effects of age, egg size and genetic factors [32] and also the possibility of inter-relations between fecundity, egg size and spawning periodicity [33].

It is well known that fecundity is affected by age, size, species, feeding of fish, season and environmental conditions [34-36]. Additionally, it differs between populations of the same species and does not remain constant from year to year. A major feature of the fecundity is its increase (within certain limits) during the growth of fish. A large fish lays more eggs than a small one, and the correlation of fecundity with weight in most fish is higher than that with length, which in turn is higher than that with age [37].

In conclusion, south Caspian Sea spirlin spawn during June-July. This finding is strongly supported by the pattern of monthly variations in GSI, MGSI, DI, oocyte diameter and frequency of maturity stages.

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