BIHAREAN BIOLOGIST 10 (2): 93-97
Article No.: e151309

http://biozoojournals.ro/bihbiol/index.html

Age, sex ratio, spawning season and fecundity of *Alburnoides samiii* (Pisces: Cyprinidae), from Sefidroud River (the southwestern Caspian Sea basin)

Mohammad MANSOURI-CHOREHI[1], Hamed MOUSAVI-SABET[1,2,*], Masoud SATTARI[1,2], Akbar NASROLLAHZADEH[1] and Aliakbar HEDAYATI[3]

1. Department of Fisheries, Faculty of Natural Resources, University of Guilan, P.O. Box 1144, Sowmeh Sara, Guilan, Iran.
2. The Caspian Sea Basin Research Center, University of Guilan, Rasht, Guilan, Iran.
3. Department of Fisheries, Faculty of Fisheries and Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
*Corresponding author, M. Mousavi-Sabet, Phone: +98 (13) 44320895, Fax: +98 (13) 44320895, E-mail: mousavi-sabet@guilan.ac.ir, mosavii.h@gmail.com

Received: 01. June 2015 / Accepted: 10. September 2015 / Available online: 28. October 2016 / Printed: December 2016

Abstract. The objective of this study was to determine reproductive biology of *Alburnoides samiii* including age structure, maturity, sex ratio, spawning season and fecundity, which are barely known. A total of 312 specimens were caught monthly from the Sefidroud River in the southwestern Caspian Sea basin, including 152 males, 128 females and 32 immature. Age, sex ratio, fecundity, oocytes diameter, gonadosomatic, modified gonadosomatic and Dobriyal indices were estimated. Regression analyses were used to find relations between fecundity and fish size (length and weight), gonad weight and age. Sex ratio differed significantly from unity, and the percentage of males was greater than that of females. This fish reached sexual maturity at a total length of 45 and 49 mm (1+ in age) for females and males, respectively. The mean of oocytes diameter was calculated as 0.732 mm. Spawning was started from mid spring and latest to late summer. The gonad indices (GSI, MGSI and DI) increase from late April, peaking at early May, and decrease until late September. The averages of absolute and relative fecundities were calculated 1571 per individual and 202 per body weight in gram, respectively. The absolute fecundity was significantly related to body length and gonad weight. Based on the pattern of gonad indices, it was concluded that this fish has a prolonged active reproductive period.

Key words: Gonadosomatic index, Dobriyal index, Oocyte diameter, Spirlin, Iran.

Introduction

The genus *Alburnoides*, distributed in Europe and the Middle East from France north of the Alps eastwards to the Black, Caspian and Aral Sea basins (Berg 1949, Coad & Bogutskaya 2009). In Iran eleven *Alburnoides* species are found including; *A. samiii* De Filippii, 1863 *A. holciki* Coad and Bogutskaya, 2012, *A. idignensis* Bogutskaya and Coad, 2009, *A. namaki* Bogutskaya and Coad, 2009, *A. nicolausi* Bogutskaya and Coad, 2009, *A. petrubanarescui* Bogutskaya and Coad, 2009, *A. qanati* Coad and Bogutskaya, 2009, *A. tabarestanensis* Mousavi-Sabet, AnvariFar & Azizi, 2015, *A. coadi* Mousavi-Sabet, Vatandoust & Doadrio, 2015, *A. parhami* Mousavi-Sabet, Vatandoust & Doadrio, 2015, and *A. samiii* Mousavi-Sabet, Vatandoust & Doadrio, 2015 (Bogutskaya & Coad 2009, Coad 2015, Azizi et al. 2015, Mousavi-Sabet et al. 2015a,b). The members of the genus *Alburnoides* have small body size, which generally prefers shallow, high oxygen, fast-flowing waters, rocky and mountain streams to live, and spawns on gravel in swift currents (Penaz 1995, Freyhof 2014).

Since the reproduction parameters are particularly important in the management of resources, conserve and restore fish populations and asthere is no comprehensive data on biological aspects of *A. samiii*. Therefore, the main aim of the present study was to provide data on the reproductive biology of this species, including age structure, maturity, sex ratio, gonadosomatic index, fecundity, oocyte diameter, and spawning season, time and duration which are necessary for conservation programs of this fish.

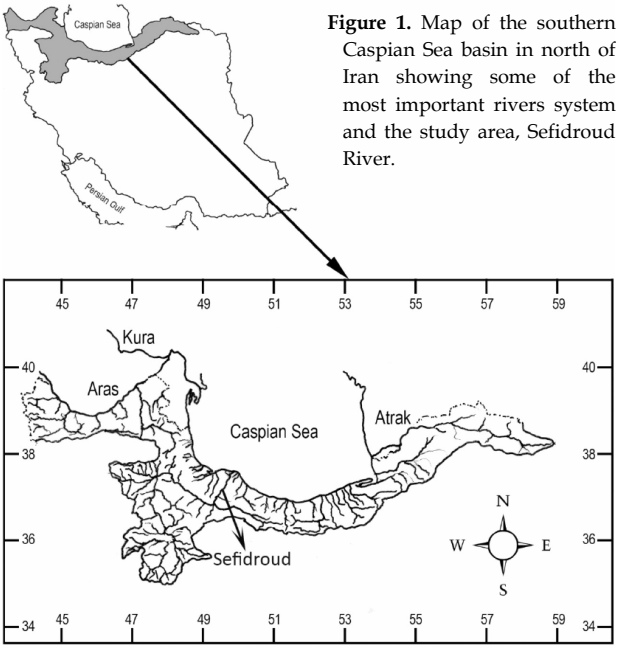

Figure 1. Map of the southern Caspian Sea basin in north of Iran showing some of the most important rivers system and the study area, Sefidroud River.

Materials and methods

Study area, sampling, and habitat.

The study was carried out in downstream of the River Sefidroud in the southwestern Caspian Sea basin (Fig. 1), which is situated in Guilan prov., at the north of Iran (latitude: from 37°01'16.82" N to 36°50'44.71" N; longitude:from 49°37'56.86" E to 49°35'01.23" E). The bottom of the sampling site was commonly sandy with incidental silt and pebbles with 5-30 cm in diameter and the water was generally clear with slow running. The depths of river at the sampling sites ranged from 20 to 70 cm. Fish samples were obtained from the river monthly intervals from November 2013 to October 2014 by electrofishing (200-300 V), with mesh size of 0.5 cm. Each sampling covered about 100 meters of river. Two times sampling were done in May 2014, because of the higher reproductive activity. After anesthesia, the caught specimens were preserved in 10% formaldehyde. The water temperature, dissolved oxygen, pH and water hardness of the sample site were measured simultaneously. The parameters given above ranged 9.4- 26.7 °C, 7.29-8.04, 5-10 mg L^{-1} and 175-199.5 mg L^{-1}, respectively.

Biometry and data analysis

Total length (TL), standard length (SL) and fork length (FL) to the nearest 0.1 mm, body weight (*Wb*) to the nearest 0.01 g and gonad weight (*Wg*) to the nearest 0.001 g were measured. The fish size at first maturity was estimated by observation of ripe gonads. To examine the monthly changes in gonads as a mean for estimating the spawning season, gonad indices including: gonadosomatic (GSI), modified gonadosomatic (MGSI) and Dobriyal (DI) were calculated following the formulas: GSI= ($Wg \times Wb^{-1}$) × 100, MGSI = ($Wg \times Wb^{-1}$) – Wg × 100 (Nikolsky 1963) and DI= $\sqrt[3]{Wg}$ (Dobriyal et al. 1999). The absolute fecundity (F_a) was estimated in 26 ovaries by calculating the number of oocytes with a diameter greater than 0.2 mm. The sex of each specimen was determined using a Zeiss SV 6 dissecting microscope. Six to eight scales were removed from the left side of the body between the lateral line and dorsal fin and mounted under binocular stereoscope for age determination (Ambrose, 1989). The relationships between fecundity and body length, body weight, ovary weight, and age were determined by regression analysis. The Chi square test was used to assess sex ratio deviation from a 50:50 (Wootton, 1998). Comparison of significant differences in the GSI index between samples taken on different months was done using analysis of variance (ANOVA I). The data were analyzed by SPSS (version 16.0) software and Microsoft Excel 2010 spreadsheet.

Results

Size, sex ratio, and age.

A total of 312 specimens of *Alburnoides samiii* were caught, including 152 males, 128 females and 32 immature. The range of total length was 45-112 mm, standard length was 35-95 mm, fork length was 40-102 mm, and body weight was 0.8-17.3 g. The longest captured fish for male and female were 98 and 112 mm, respectively. The age of the fish ranges from 1+ to 3+ years (Table 1). The majority of the caught fish were 2+ years old (56.09%), some were 1+ (30.77%) and small percentage was 3+ (13.14%). *Alburnoides samiii* reached sexual maturity at total length of 45 and 49 mm (1+ in age) for female and male, respectively. The overall ratio of females to males was 1: 1.19 and χ^2 analysis showed this to be significant (χ^2= 1.029; P< 0.05).

Gonadosomatic, modified gonadosomatic and Dobriyal indices

In order to determine the reproduction period, GSI, MGSI and DI were evaluated by monthly intervals. Significant differences were observed in female and male for GSI, MGSI and DI in different months (P<0.05). There was no significant difference between GSI and MGSI trend for each sex (P>0.05). The gonad indices increased from late April, peaked in early May, and decreased in late September (Figs 2-3). In females, DI was estimated as 0.37 in early May and fall down in late September (0.02) for females. According to GSI, MGSI and DI the spawning period was started in May and continue to until late September in this region.

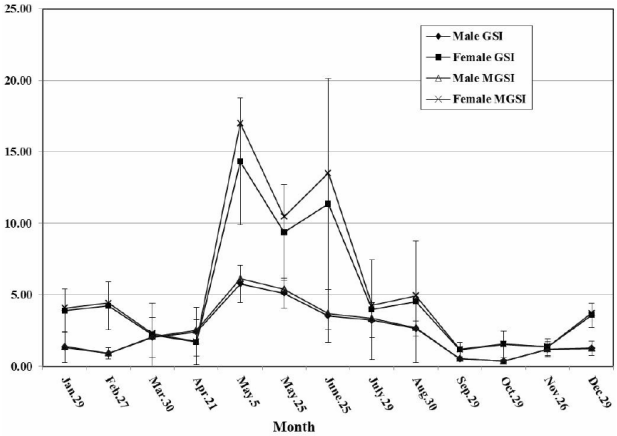

Figure 2. Variation of mean (±SE) gonadosomatic index (GSI) and modified gonadosomatic index (MGSI) of female (F) and male (M) for *A. samiii* from the southwestern Caspian Sea basin in different months, from November 2013 to October 2014.

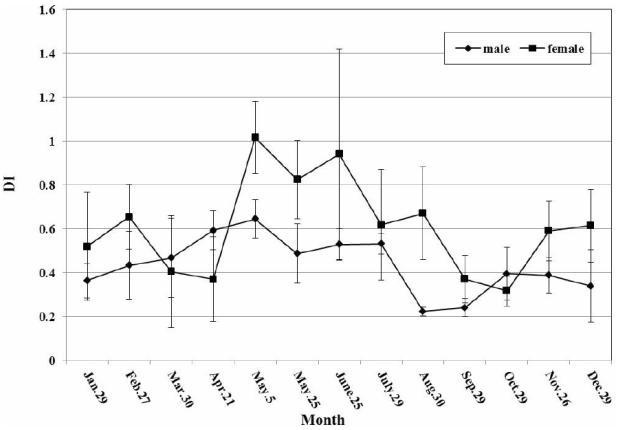

Figure 3. Variation of Dobriyal Index (D.I.) and of female (F) and male (M) *A. samiii* from the southwestern Caspian Sea basin in different months, from November 2013 to October 2014.

Oocyte diameter and fecundity

The size of oocytes ranged from 0.1 to 1.7 mm with a mean of 0.732 (SD ±0.384). They were highest in April (1.1 mm) and May (1.14 mm) and lowest in November (0.51 mm). There was a peak in the number of yellowish-yolk oocytes (1-1.7 mm diameter) in May (Fig. 4).

The absolute fecundity varied in a wide range from 763 to 2901 eggs per specimen with an average of 1571.1 (SD ± 586.7). The relative fecundity was 88 to 412 with a mean of 202.7 (SD ± 79.8) per gram body weight (Table 2).

Significant correlations were observed between absolute fecundity and fish body length and also gonad weight (Table 3). The regression coefficient values were calculated as 0.3976 and 0.3318 for gonad weight and total body weight, respectively.

Table 1. Length and weight (mean ± SD) in different age groups of males and females of *A. samiii* from the southwestern Caspian Sea basin (SL = standard length; TL = total length; *Wb* = body weight; *n* = number of specimens; SD = standard deviation; M = male; F = female).

Age	*n*		SL (mm)		TL (mm)		*Wb* (g)	
	M	F	M	F	M	F	M	F
1+	46	30	54.04±7.08	51.04±6.24	66.14±7.99	62.25±7.51	3.21±1.40	2.57±0.92
2+	97	66	62.38±8.18	66.26±9.46	74.99±13.64	81.55±11.07	5.04±1.83	6.31±2.34
3+	9	32	71.54±5.19	76.05±7.27	88.07±6.52	93.12±7.58	7.51±1.73	9.31±2.79

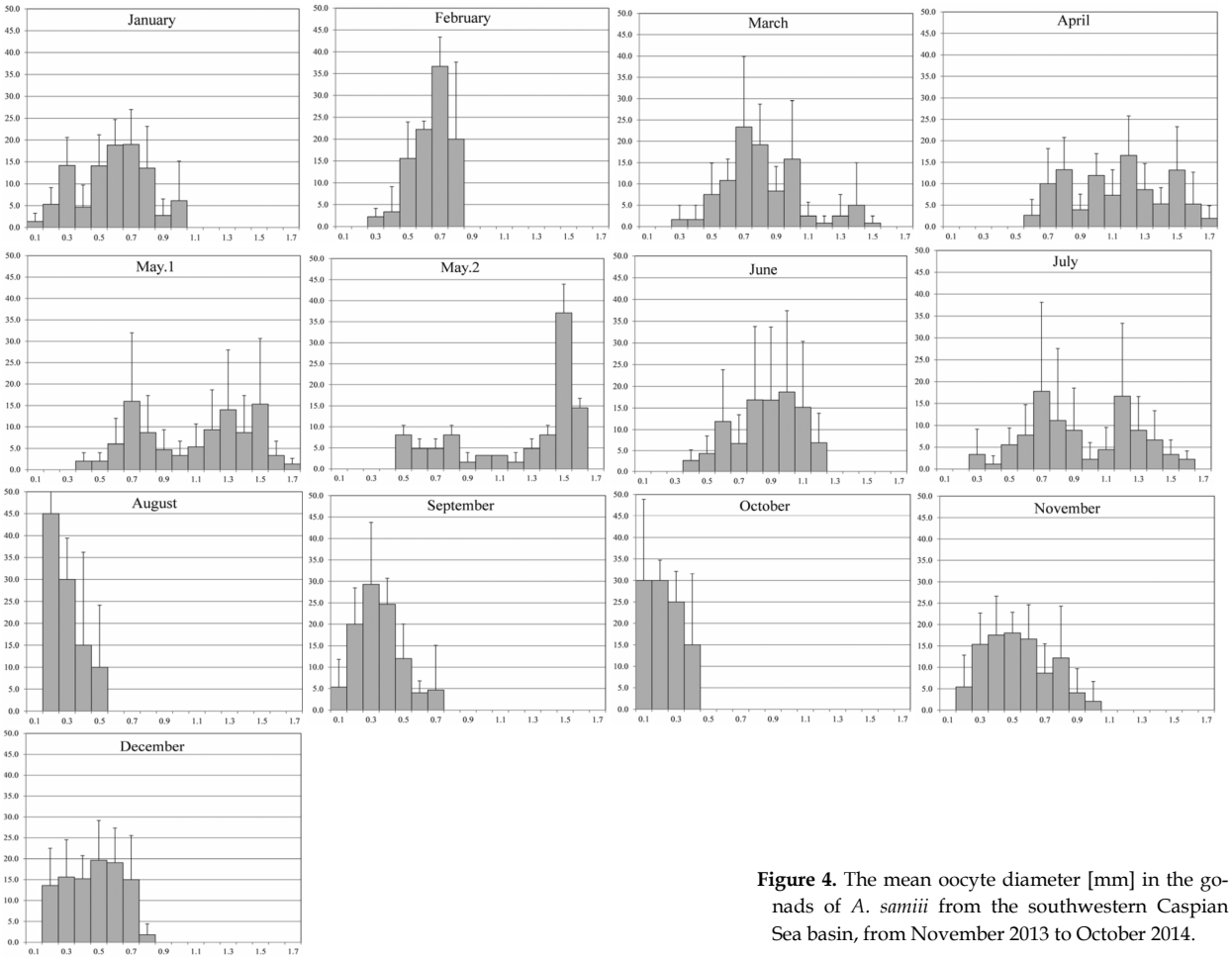

Figure 4. The mean oocyte diameter [mm] in the gonads of *A. samiii* from the southwestern Caspian Sea basin, from November 2013 to October 2014.

Table 2. Absolute (*Fa*) and relative (*Fr*) fecundity in particular ranges of body length (SL), body weight (*Wb*), and age of *A. samiii* females from the southwestern Caspian Sea basin.

Parameter		*n*	F_a		F_r	
			Range	Mean ± SD	Range	Mean ± SD
SL [mm]	45.1-60	3	823-1081	914±145	157-332	216±100
	60.1-75	15	863-2901	1616±185	88-412	220±19
	75.1≤	8	1619-2161	1734±306	99-226	165±35
Wb[g]	2.1-6	4	823-912	859±39	157-332	211±82
	6.1-10	16	763-2901	1696±421	88-412	219±85
	10.1≤	6	1140-2161	1714±468	99-208	154±45
Age	1+	2	837-912	875±53	197-332	264±96
	2+	10	823-2901	1594±704	109-412	227±89
	3+	14	762-2236	1654±486	88-318	183±69

Table 3. Correlation coefficients *r* and regression equations for the relation between absolute fecundity (*Fa*) and: body length (TL), body weight (*Wb*), weight of ovary (*Wg*), and age of *A. samiii* females from the southwestern Caspian Sea basin.

Relation	*n*	Linear regression	r^2	F-value	P-value
F_a - SL	26	y = 29.874x - 559.69	0.2343	7.345	0.012
F_a - *Wb*	26	y = 81.862x + 903.97	0.1101	2.968	0.098
F_a - *Wg*	26	y = 460.31x + 1164.80	0.1581	4.507	0.044
F_a - Age	26	y = 234.77x + 1011.30	0.0650	1.669	0.209

Discussion

Age and size of fish

Most of the examined fish were 2+ years old, some were 1+ and small percentage was 3+ (13.14%). Matthews (1998) stated that a proportion of oldest specimens is low indicates that very few individuals survive to maximum age, as is typical of most fishes. The examined *A. samiii*, were mature at 1+ in age for both sexes. In Azerbaijan, maturity in *A. bipunctatus* is attained at 1-2 years and life span is 3 years

(Abdurakhmanov, 1962). In the studied specimens, body length and weight of females were larger than males also males were darker than females in body colour (especially in the reproductive period). In this regard and by comparison between female and male spirlin, Bura et al. (2008) stated that average body length and weight, and maximum body height in females is bigger than males.

Reproduction

The gonad indices (GSI, MGSI and DI) were low in winter and then increased at the beginning of spring, peaked at the mid of spring and then decreased until late summer. The process of accumulating reserve substances in the ovaries of the females can be obtained partly by tracing the changes in the gonad indices. In species which spawn in late spring and in summer such as spirlin, these indices remain low in winter and then increase sharply just before the spawn (Wootton 1979, Marconato & Rasotto 1989, Rinchard & Kestemont 1996). A rapid increase in the weight of ovaries takes place when the temperature rises and increasing amounts of food are consumed (Wootton 1979).

Spawning of *A. samiii* was started from beginning of May and latest to late summer, which at the late September the ovaries of most of the spirlin under study indicated the end of the reproductive period. While a shorter spawning period is reported for *Alburnoides* sp. from Rudava stream, in western Slovakia (Polačik & Kováč 2006), and from the Keselian stream in the southeastern Caspian Sea basin (Seifali et al. 2012). It has been found that the reproductive indices for females were always higher than for males. Papadopol and Cristofori (1980) reported a large reproductive potential of spirlin in Romania, where it reproduces four to five times in a season. It can be concluded that the potential spawning period in these fishes (*Alburnoides* sp.) is very prolonged (Bless, 1994).

Our obtained results showed that the spawning season of *A. samiii* started from the mid spring to late summer (19.7-22.8 °C), while Abdurakhmanov (1962) reported that spawning of *A. bipunctatus* takes place in spring (April-June) at 13-15.6°C and Yildirim (1999) concluded that *A. bipunctatus fasciatis* (Nordman, 1840) began to spawn at the end of May when water temperature was 16 °C and the spawning continued to the end of the July when water temperature was 25°C.

Fecundity and oocyte diameter

In this study, the average absolute fecundity of female *A. samiii* from the southwestern Caspian Sea basin was 1571 (±586.70) eggs per individual, ranging from 763 to 2901. In a related study, Seifali et al. (2012) stated that the mean absolute fecundity of female *A. bipunctatus* was 1723 (±653.88) eggs per individual. *Alburnoides bipunctatus fasciatis*, from the Oltu Stream (Turkey) has been reported to attain mean absolute fecundity 13135 eggs (Yildirim et al., 1999). The wide and significant difference between mean absolute fecundity of spirlin from Turkey (Yildirim et al. 1999; 13135 eggs) and Iran (the present study and Seifali et al. 2012, 1571-1723 eggs) may be due to the bigger mean size of the Turkish females (mean fork length 103 mm vs. 72 mm, mean weight 18.9 g vs. 6.18 g). It is well known that fecundity is affected by species, age, size, feeding, season and environmental conditions (Thorpe 1984, Fleming & Gross 1990).

Oocyte diameter distribution analysis shows that the reproductive season was absolutely protracted (from late May to late September), a common feature for batch spawner fishes. Spirlin is reported to belong to batch spawners (fractional, portional, heterochronal; Papadopol and Cristofor 1980, Bless 1996, 2001, Breitenstein and Kirchhofer 2000). Statistically significant relationships were found between the absolute fecundity and body length and gonad weight in *A. samiii*. 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 (Nikolsky 1969).

The results of this study provide the first data on reproductive biology of *A. samiii*, including age structure, maturity, sex ratio, gonadosomatic index, fecundity, oocyte diameter, and spawning time and duration. From the point of view of management, the obtained information is useful for conservation programs of this fish. Upstream of Sefidroud River is more preferred for living by *A. samiii*, since there are more individuals. Spawning in this species takes place between early May and late September, although in some cases a later starting and shorter period is reported, the reproductive cycle starting earlier and its duration being longer in lower latitudes. This population of spirlin had a narrow age range (1+ to 3+ years), which most of the examined fish were 2+ years old. Based on our observation during the sampling and the obtained results, it can be concluded the population of *A. samiii* (and probably other fish species in the river) is under pressure by habitat degradation and illegal sport fishing. So, an urgent habitat protection and a limited fishing activity are suggested.

Acknowledgement. The authors are grateful to Hanieh Mojdakanlou for her help in laboratory works, and Abouzar Habibi, Adeleh Heidari, Mitra Mohammadi and Kobra Ghasemzadeh for their help in fish collections.

References

Abdurakhmanov, Y.A. (1962): Ryby Presnykhvod Azerbaidzhana [*Freshwater Fishes of Azerbaidzhan*]. Akademii Nauk Azerbaidzhanskoi SSR, Institute Zoologii, Baku.

Ambrose, J.J.R. (1989): Age determination. pp. 301-325 In: Nielson, L.A., Johnson, D.A. (eds.), Southern Printing Company, Virgina, SA.

Azizi, F., Anvarifar, H., Mousavi-Sabet, H. (2015): Morphological differentiation between isolated populations of Caspian spirlin (*Alburnoides eichwaldii*) (Pisces: Cyprinidae) affected by dam. International Journal of Animal Biology 1(2): 28-37.

Berg, L.S. (1949): Freshwater fishes of the USSR and adjacent countries. Israel Program for Scientific Translations, Jerusalem (1962-1965).

Bless, R. (1994): Contributions to the spawning behaviour of the spirlin-*Alburnoides bipunctatus* (Bloch) under laboratory conditions. Fischokologie Koln 7: 1-4.

Bless, R. (1996): Reproduction and habitat preference of the threatened spirlin (*Alburnoides bipunctatus* Bloch) and soufie (*Leuciscus souffia* Risso) under laboratory conditions (Teleostei: Cyprinidae). pp.249–258. In: Kirchhofer A., Heffi, D. (eds.), Conservation of endangered freshwater fish in Europe. Birkhauser Verlag.

Bless, R. (2001): Spawning and niche shift of some threatened riverine fishes of Europe. Large Rivers 12(2–4): 293–305.

Bogutskaya, N.G., Coad, B.W. (2009): A review of vertebral and fin-ray counts in the genus *Alburnoides* (Teleostei: Cyprinidae) with a description of six new species. Zoosystematica Rossica 18: 126-173.

Breitenstein, M.E., Kirchhofer, A. (2000): Growth, age structure and species association of the cyprinid *Alburnoides bipunctatus* in the River Aare, Switzerland. Folia Zoologica 49(1): 59–68.

Bura, M., Banatean-Dunea, I., Muscalu, R. (2008): Researches on somatic measurements to spirlin (*Alburnoides bipunctatus*) from Nadrag river basin. Zootehnieşi Biotehnologii 41: 23-26.

Coad, B.W., Bogutskaya, N.G. (2009): *Alburnoides qanati*, a new species of cyprinid fish from southern Iran (Actinopterygii, Cyprinidae). ZooKeys 13: 67-77.

Coad, B.W., Bogutskaya, N.G. (2012): A new species of riffle minnow, *Alburnoides holciki*, from the Hari River basin in Afghanistan and Iran (Actinopterygii: Cyprinidae). Zootaxa 3453: 43-55.

Coad, B.W. (2014): Freshwater Fishes of Iran. < www.briancoad.com>, accessed at: 2015.08.22.

Dobriyal, A.K., Rautela, K., Rautela, A. (1999): Invention of a new index for the determination of sexual maturity in fishes. Uttar Pradesh Journal of Zoology 19(3): 207-210.

Fleming, I.A., Gross, M.R. (1990): Latitudinal clines: a trade-off between egg number and size in Pacific salmon. Ecology 71: 1-11.

Freyhof, J. (2014): "*Alburnoides eichwaldii*". IUCN Red List of Threatened Species.version 2014.2. International Union for Conservation of Nature. <www.iucnredlist.org/>, accessed at: 2014.08.29.

Marconato, A., Rasotto, M.B. (1989): The biology of a population of spined loach, *Cobitis taenia* L. Italian Journal of Zoology 56(1): 73–80.

Matthews, W.J. (1998): Patterns in freshwater fish ecology. Kluwer Academic Publishers, Norwell, MA.

Mousavi-Sabet, H., AnvariFar, H., Azizi, F. (2015a): *Alburnoides tabarestanensis*, a new species of riffle minnow from the southern Caspian Sea basin in Iran (Actinopterygii: Cyprinidae). Aqua 21(3): 144-152.

Mousavi-Sabet, H., Vatandoust, S., Doadrio, I. (2015b): Review of the genus *Alburnoides* Jeitteles, 1861 (Actinopterygii, Cyprinidae) from Iran with description of three new species from the Caspian Sea and Kavir basins. Caspian Journal of Environmental Science 13(4): 293-331.

Nikolsky, G.V. (1963): The ecology of fishes. AcademicPress, London.

Nikolsky, G.V. (1969): Theory of Fish Population Dynamics. Oliver & Bloyd, Edinburg.

Papadopol, M., Cristofor, S. (1980): Recherchessurl'ecologie de deux populations de spirlin, *Alburnoides bipunctatus*, des eaux de la Romanie. Travaux Du Museum D'Histoire Naturelle Grigore Antipa 22: 483–493. [in French]

Penaz, M. (1995): *Alburnoides bipunctatus*. pp. 151-154.In: Baruš, V., Oliva, O. (eds,), Fauna ČR a SR/ Mihulovci a ryby (2) Fauna ČR a SR/Mihulovci a ryby II. [*Fauna of the Czech Republic and Slovakia/Lampreys and fishes II*], Academia, Prague.

Polačik, M., Kováč, V. (2006): Fecundity and annual course of maturation in spirlin, *Alburnoides bipunctatus*. Folia Zoologica 55(4): 399–410.

Rinchard, J., Kestemont, P. (1996): Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish. I. Morphological and histological features. Journal of Fish Biology 49(5): 883–894.

Seifali, M., Arshad, A., Esmaeili, H.R., Kiabi, B.H., Yazdani Moghaddam, F., Fardad, N. (2012): Fecundity and maturation of South Caspian spirlin, *Alburnoides* sp. (Actinopterygii: Cypriniade) from Iran. Iranian Journal of Science and Technology A2: 181-187.

Thorpe, J.E., Miles, M.S., Keay, D.S. (1984): Developmental rate, fecundity and egg size in Atlantic salmon. *Salmo salar* L. Aquaculture 43: 289-305.

Wootton, R.J. (1979): Energy costs of egg production and environmental determinants of fecundity in teleost fishes. Symposia of the Zoological Society of London 44: 133–159.

Wootton, R.J. (1998): Ecology of Teleost Fishes, second edition. Kluwer, Dordrecht.

Yildirim, A., Erdogan, O., Turkmen, M., Demir, B.C. (1999): Coruh Havzasi Oltu Cayı'nda Yaşayan *Alburnoides bipunctatus fasciatis* (Nordman, 1840)`in bazı üreme özelliklerinin araştılırması. [*The investigation of some reproduction characteristics of the Alburnoides bipunctatus fasciatis (Nordman, 1840) living in Oltu Stream, Çoruh Basin*]. Turkish Journal of Veterinary and Animal Sciences 23: 679-686. [in Turkish with English summary]