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Reproductive biology of Mossul bleak (*Alburnus mossulensis*) in Bibi-Sayyedan River of Tigris basin in Iran

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ABSTRACT

Reproductive characteristics of Mossul bleak, *Alburnus mossulensis*, a native species of Cyprinidae family in Bibi-Sayyedan River of Semirom, Isfahan, were investigated by monthly sampling during December 2010 to December 2011. Five hundred and fourty three specimens (256 females, 159 males and 31 immature) were caught by a sein net (5 mm mesh size). The collected sampeles were anesthesized in 1% clove oil solution and transported to the laboratory on ice and subjected to dissection and biometric measurments. Age of males and females ranged from 0^+ to 5^+ years. The sex ratio was 1M: 2.2F ($p < 0.05$). The females outnumbered the males in all age classes. The largest female was 16.8cm in total length and 49.12g in weight and the largest male was 14.1cm in total length and 28.19g in weight. The age and total length at first maturity of females and males were estimated as 1 year and 5.3cm total length, and 2 years and 4.4cm, respectively. The minimum, maximum, and avarge absolute fecundity were 2064, 10316, and 5505 ± 2686, respectively and also the relative fecundity was 203 ± 58 egg/g body weight. Macroscopic analysis of gonads and gonadostomatic index values suggested that spawning of *A. mossulensis* occurs from March to June with a peak in April. Thus, *A. mosullensis* is considered a group-synchronous type species with a capacity for multiple ovulations within a reproductive season (multiple spawner).

Key words: Cyprinidae, Fecundity, Age, Gonad development, Gonadosomatic index.

INTRODUCTION

The Mossul bleak (Shah-kuli Jonobi, in Farsi) is a cyprinid fish widely distributed in Iran and adjacent countries (Fig. 1). This species is found in Tigris (Karun and Karkheh river basins), Fars, Bushehr and Hormuz basins in Iran (Keivany *et al.* 2016a; Coad, 2017; Esmaeili *et al.* 2017), however, the biology of this species is not well documented in Iran and other countries, partly because this fish is not of commercial importance due to its slow growth rate and small size (Keivany & Ghorbani 2017). Nevertheless, it is used as an edible fish at a local scale and hence, there is no information on its landings. Success of reproduction depends upon normal gonadal development stimulated by favorable environmental conditions such as photoperiod and temperature (Lam 1983; Hontela & Stacey 1990). The ovarian development is described according to the division of ovarian tissues into seven or eight (Crim & Glebe 1990) or five (Brown-Peterson *et al.* 2011) stages of maturity based upon the presence of dominant gametogenic cell types.

Ergene (1993), Turkmen & Akyurt (2000) Başusta & Çiçek (2006), and Yildirim *et al.* (2007) studied the reproduction and growth of this species in Euphrates basin of Turkey.

Alhabiab (1981), Epler *et al.* (2001) and Younis *et al.* (2001) studied this species in Iraq. Esmaeili *et al.* (2014) and Keivany *et al.* (2016b) studied the length-weight relationships in this species in Iran. However, despite its importance in food chains and as a genetic resourse of a native species, there is little information on its reproduction in Iran. The aim of this study was to investigate the reproductive biology of *A. mossulensis* in Bibi-Sayyedan River of Semirom, a tributary of Karun River system in Tigris basin.The results of this study could be used for conservation and management purposes of this species.

Fig. 1. Photo of *A. mossulensis* from Bibi–Sayyedan River.

MATERIALS AND METHODS

Bibi-Sayyedan River is located approximately 18 km south of the town of Semirom. This river is flowing south-westward from the high central part of Zagros Mountain, joining Marbor River and terminating in Khersan River a tributary of Karun River (Tigris basin). For this study, 543 specimens were (256 females, 159 males and 31 immature) captured by monthly collection from December 2010 to December 2011 at 31°11'0.8"N, 51°26'59"E (Fig. 2). Specimens were caught by a seine net (5 mm mesh size), anesthetized in 1% clove oil solution and transported to the laboratory on ice and subjected to dissection and biometric measurements (length to the nearest 0.1 cm, and weight to the nearest 0.01g).

For each specimen, 10-15 scales were removed from above the lateral line below the anterior extent of the dorsal fin on the left side of the fish, washed in water and dried between two slides for microscopic studies (Lagler, 1956). Scales, mounted dry between glass slides, were used for age estimation and were read by three people. Sex was determined by examination of the gonads. The sex ratio deviation from 1:1 was tested statistically by chi-squared analysis (Sokal & Rohlf 2012).

The relative gonad weight or gonadosomatic index (GSI) of females and males were calculated as the proportion of the weight of the gonad to the weight of eviscerated body in percent (GSI= gonad weight / body weight × 100). Also, water temperature was measured to determine the relationship between temperature and gonad growth by a digital thermometer. The hepatosomatic index (HIS) was calculated as a proportion of liver weight to body weight in percent (HSI= liver weight / body weight × 100). The absolute fecundity was determined by the gravimetric method. While relative fecundity was determined as the proportion of absolute fecundity to the eviscerated body weight (number of mature eggs per gram of the body weight F= (n×G)/g). Egg diameter was measured with a scaled ocular micrometer. Maturity stages were determined following Brown-Peterson *et al.* (2011). After identifying the various stages of sexual maturation, diameter of sex cells were measured during different growth phases under magnifications of ×100 by a scaled ocular micrometer. Reproductive seasonality was determined by examination of the monthly changes in the gonadosomatic index. One-way

ANOVA followed by Duncan analysis was used to analyze differences in means of GSI and egg diameter of fish at 5% probability level in SPSS 19 computer software.

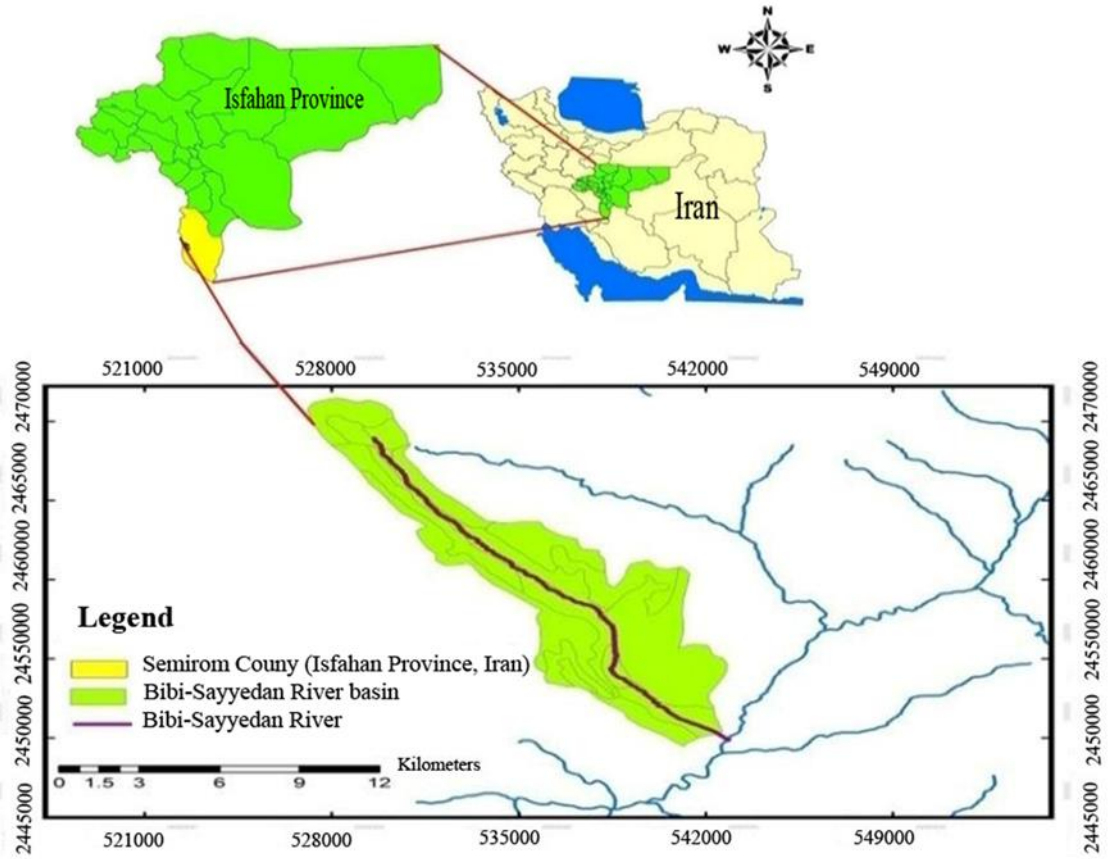

Fig. 2. Map of Bibi–Sayyedan River, Bibi–Sayyedan River basin, Semirom County, Isfahan Province, Iran.

RESULTS

The water temperature varied between 10.5-21.4°C during the sampling period in Bibi-Sayyedan River. The lowest temperature was in March and then began to increase in April along with daytime and a sudden increase in river flooding.

The length and weight of females ranged from 4.5 to 16.8 cm (10.8 ± 2.38) and 0.72 to 55.67 g (13.79 ± 9.03) and those of males from 4.6 to 14.1 cm (10.19 ± 1.98) and 0.91 to 30.91 g (10.81 ± 5.94), respectively. The specimens were composed of 31% males and 69% females. The ratio of male to female was 1M: 2.2F which was significantly different from the expected 1:1 ratio ($X^2 = 13.31$, $p < 0.05$). Sexual maturity was obtained in the first year in males at 4.6-9.1 cm and in the second year of life in females at 5.3-9.9 cm. The sex ratio was in favour of the females throughout the yearand in all age groups (Table 1).

Assessment of the main spawning period of *Alburnus mossulensis* in Bibi-Sayyedan River was based on the GSI (Fig. 3), analysis of seasonal development in mean egg diameter (Table 2) and direct observation of the gonads. Significant differences were found in the males and females GSI and in egg diameters ($p < 0.05$) in different months. The highest GSI value for both sexes occurred in March-May.

Thus, the maximum reproductive activity of this species in this particular environment occurs from March to May when GSI is considerably higher and water temperature is between 17.2-19.9°C.

Some unspent individuals were observed among the population during this period, the ova were reabserved in June. The highest HSI value for males was in March-April and for females in March-May (Fig. 3).

Table 1. Number and sex ratios of Alburnus mossulensis specimens in different age groups.

Age group	Sex	Number	M:F ratio
0^+	M	3	1:2.1
	F	7	
1^+	M	16	1:2.3
	F	36	
2^+	M	88	1:1.7
	F	151	
3^+	M	24	1:2.5
	F	60	
4^+	M	17	1:3.5
	F	60	
5^+	M	11	1:3
	F	29	

Table 2. Mean egg diameter variations in *A. mossulensis* from December 2010-December 2011. It could have singnifacnt variation with letters a, b, c.

Months	Mean TL ± SD (cm)	Mean TW ± SD (g)	Egg diameter range	Mean egg diameter ± SD
December 2010	11.03 ± 2.98	14.72 ± 8.99	0.1-1.0	0.572 ± 0.222^{c}
January 2011	12.42 ± 1.90	20.10 ± 9.86	0.3-1.1	0.651 ± 0.142^{c}
February	11.45 ± 1.74	16.18 ± 7.96	0.2-0.9	0.650 ± 0.165^{c}
March	11.40 ± 1.28	16.77 ± 6.04	0.2-1.0	0.679 ± 0.205^{bc}
April	10.67 ± 2.45	15.20 ± 10.31	0.3-1.3	0.880 ± 0.219^{a}
May	11.91 ± 1.40	18.59 ± 8.77	0.4-1.3	0.952 ± 0.228^{a}
June	11.86 ± 1.55	16.96 ± 6.32	0.3-2.0	0.906 ± 0.345^{a}
July	11.58 ± 0.57	15.67 ± 2.95	0.1-1.3	0.720 ± 0.342^{b}
August	13.10 ± 0.14	23.73 ± 0.37	0.2-0.7	0.379 ± 0.159^{d}
September	15.90 ± 1.27	44.20 ± 6.95	0.2-0.7	0.421 ± 0.143^{d}
October	16.20 ± 0.28	37.69 ± 0.43	0.3-0.6	0.519 ± 0.610^{d}
November	14.10 ± 2.27	31.83 ± 20.65	0.3-0.8	0.622 ± 0.156^{c}
December 2011	12.96 ± 2.01	19.51 ± 9.85	0.3-1.0	0.629 ± 0.153^{c}

The Mean egg diameter ranged between 0.1-2 mm and was significantly different during the year ($p < 0.05$). Obviously, the highest mean egg diameter (0.952 ± 0.228mm) was observed in May and the lowest in August (0.379 ± 0.159mm) (Table 2). The mean ± SE of the absolute fecundity of 38 females determined during the spawning period was 2000-10300 (5500 ± 2700) egg and mean relative fecundity was 200 ± 60 egg/g body weight.

The linear relationships between fecundity-Total length, fecundity-weight and fecundity-Age were direct and high (Fig. 4).

The gonad development stages of *A. mossulensis* are described in five stages following Brown-Peterson *et al.* (2011) which has been accepted by many researchers as the standard procedure (e.g., Tomkiewicz *et al.* 2011, Abaszadeh *et al.* 2013, Dopeikar *et al.* 2015).

Stage I (never spawned)

Testes and ovaries were very small and near the spinal column, often clear and threadlike and transparent grayish in color, blood vessels indistinct. Eggs were not visible to the naked eye and sex could not be distinguished. The mean diameter of oocytes was 4.714 ± 0.521(SE) µm. This stage was observed throughout the year.

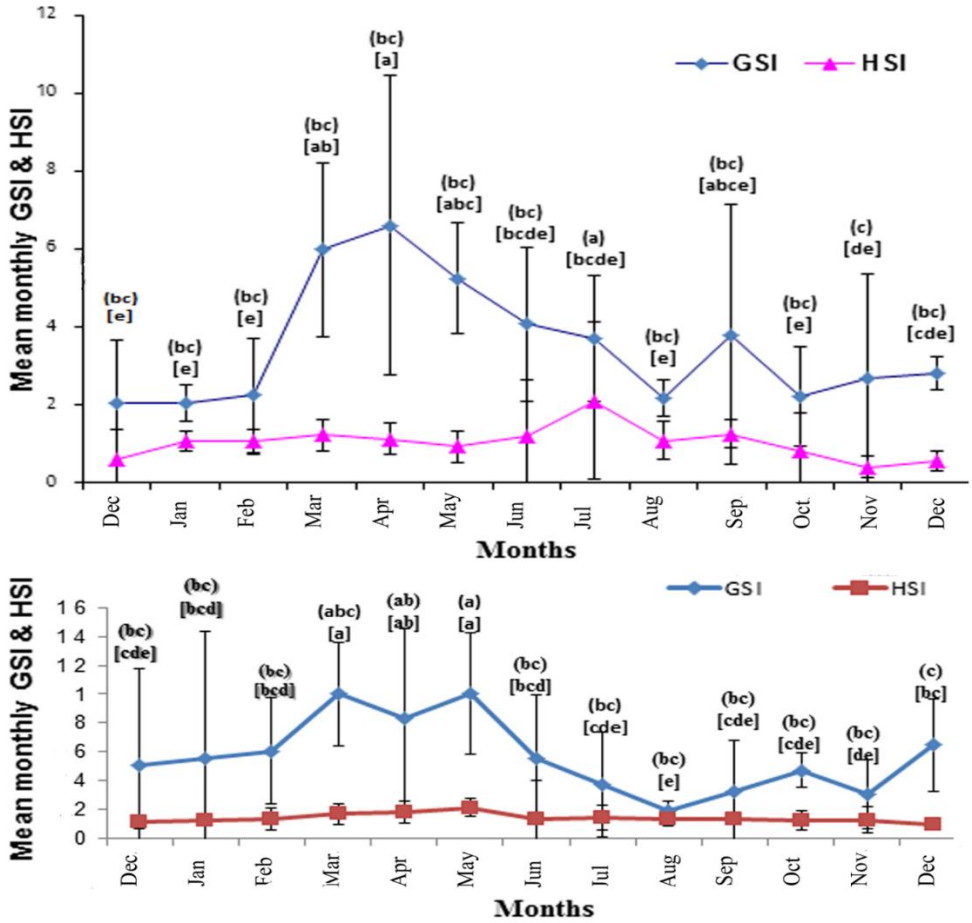

Fig. 3. GSI and HIS variations in males (Top) and females (Bottom) *of A. mossulensis* in Bibi–Sayyedan River from December 2010-December 2011.

Stage II (Early developing; developing; ovaries and testes beginning to develop, but not ready to spawn)

Ovaries and testes like an opaque reddish tube occupying almost half the body cavity. Blood capillaries were evident on the surface. Immature eggs which resemble white spots were visible to the naked eye.

This stage was observed in August-November.

Stage III (Spawning capable; fish were developmentally and physiologically able to spawn)

Gonads filled the body cavity.

Ovaries were orange and testes were white. Some sperm was released after applying hand pressure. Migratory nucleus and hydration stages were distinguished. In migratory nucleus stage, nucleus began to leave central position and migrate towards periphery. Yolk globules filled more than two third of the cytoplasm.

Oocyte size remained relatively stable. In hydration stage, yolk globules filled the entire cytoplasm. Nucleus was observed at animal pole. Mean diameter of oocytes was 96.00 ± 9.66 µm. This stage was seen from April to August, and the highest frequency of these oocytes was in April and May. Testes were large and firm. Actively spawning subphase (macroscopic): milt released with gentle pressure on abdomen. This stage was observed in March-May.

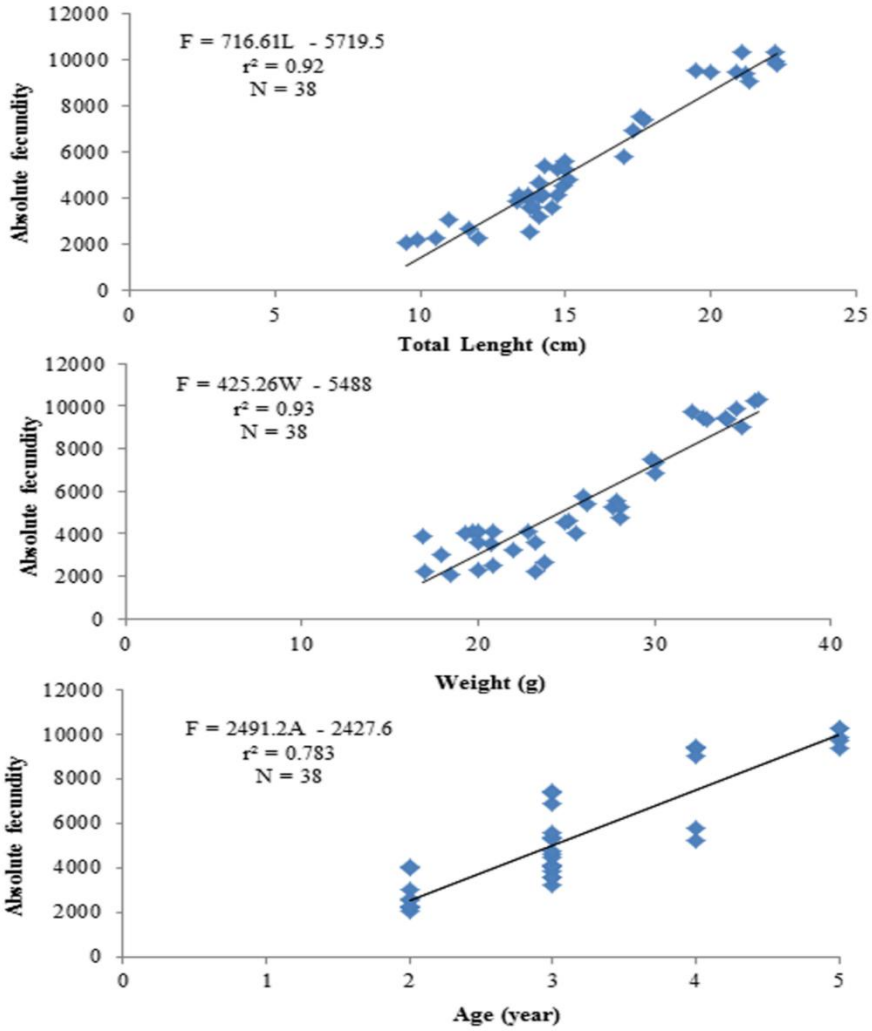

Fig. 4. Fecundity-total length, fecundity-weight and fecundity-age relationships in *Alburnus mossulensis* in Bibi–Sayyedan River from December 2010-December 2011.

Stage IV (Regressing; cessation of spawning)
Ovaries were flaccid and blood vessels prominent.
Testes were small and flaccid, no milt released with pressure. This stage was observed in March-May.

Stage V (Regenerating; sexually mature, reproductively inactive)
Ovaries and testes were empty, loose and red. Few remains of eggs in resorption process. This stage was observed in June-July.
In the final two stages or after ovulation, the spent ovaries were small, bloodshot and granular with scattered residual oocytes.
These ovaries composed of postovulatory follicles, immature oocytes and mature eggs left unspawned.

DISCUSSION

The length and weight of females were higher and females were more abundant than the males (1M: 2.2F). Unlike what was found in this study, Turkmen & Akyurt (2000) found it as 1M: 0.9F and Turkmen & Erdogan (2003) as 1M: 1.1F in Karasu River of Turkey, however, in younger fish the ratio was in favour of males. This might be due to the shorter life and earlier maturation of the males. Also, it could be due to differential occurrence of the males and females in various water columns or females live in deeper areas which make them less available and/or naturally, the abundance of females is less than that of males in this population. Dominance of one sex relative to the other can be due to different behaviors leading to an easier catch of one sex and

differences in mortality of sexes (Rajaura 1992, Sandovy *et al.* 1994, Wirtz & Morato 2001; Siami *et al*. 2017).

In this study, the maturity age was estimated as 1 year for males (4.6 cm) and 2 years for females (5.3 cm). Yildirim *et al.* (2007) reported the age composition of this species between 1-5 years and 2 years old fish were dominant. Age at first spawning in the Karaso River (Turkey) was 1.3 years in males (9.2 cm) and 1.8 years in females (9.7 cm). The first spawning age in fish is affected by species, size of fish and environmental factors such as temperature, quality of food and feeding (Yildirim *et al.* 2003).

The GSI in males and females was coincided, increasing the reproducion success (Bromage *et al.* 1992), however, it was lower in males, probably due to less energy investment by males (Buxton & Garratt 1990). Parsa and Bahramian (2010) found similar figures in this species. From the variation and maximum recorded GSI values, it is evident that the reproductive season of *A. mossulensis* extends from March to June but most extensively in April. Since this species has a relatively long spawning period, the fish in stage I of maturity were found throught the year, in stage II in August-November, Stages III and IV in March-May and stage V in June-July. Egg diameter also approved this observation. Yildrim *et al.* (2007) in Karasu River reported the maturity of this fish in June-August (at 15°C). Nikolsky (1963) pointed out that the spawning characteristics of a fish vary in respect to their species and ecological characteristics of water systems in which it lives. The largest size was in March-May when the spawning occurs. The egg size could vary from population to another or even from year to year depending on temperature, available food etc. The long period of spawning and the wide range size of eggs, indicate a multiple bach group synchronous spawner (Nagahama 1983). This might be due to unfavorable condition which in this way, the small size of the eggs coupled with hhigh fecundity and long spawning season ensures the survuival of the population (Gonçalves & Erzini 2000). The hepatosomatic index as seen in other fish was higher before the spawning and decreased therafter, due to consumption of faty resources during the oogenesis (Haddy & Pankhurst 1998).

The wide fecundity range indicates individual differences (2000-10000) in this species. Absolute fecundity was slightly lower than that reported by Yildirim *et al.* (2007) in Karasu River (3000-11000 eggs in specimens ranging 112-18 cm). Fecundity in females was correlated with total length and more with total weight. It is well known that fecundity is affected by age, size, species, feeding, season and environmental conditions. Additionally, it differs among populations of same species and does not remain constant from year to year (Unlu & Balci 1993). The results of observation on the gonad development stages, GSI and egg diameter indicate that the spawning season of bleak in Bibi-Sayyedan River starts in March and continues until June. Spawning may occur 2-3 times in a season varying with locality, once temperature reaches 15°C (Yildrim *et al.* 2007), ceasing if the temperature exceeds 20°C.

Macroscopic observations of larger fish gonads in winter (coinciding with the period of vitellogenesis) showed that ovaries were yellowish to non-transparent, with appearant capillaries. During maturation period in April ovaries were orange, lobular, and with visible capillaries in surface as seen in other fish species (Keivany & Soofiani 2004, Soofiani *et al.* 2006, Asadollah *et al.* 2011, Keivany *et al.* 2012, Abaszadeh *et al.* 2013, Dopeikar *et al.* 2015). But owing to the GSI peak, the maximum diameter of the eggs and highest number of hydrated oocytes were observed in April, therefore, spawning peak of *A. mossulensis* occurred in April.

Macroscopic development of the testes followed a trend similar to those of the ovaries. Testes volume increased along with the maturity stages, but it was always smaller than the ovaries. In the immature stage, testes were inactive and transparent threadlike.

Therefore, the rhythm of gonadal development depend on various external factors such as

temperature, photoperiod, and social and behavioral factors such as visual, olfactory and auditory stimuli (Keivany & Soofiani 2004, Soofiani *et al.* 2006, Asadollah *et al.* 2011, Keivany *et al.* 2012, Abaszadeh *et al.* 2013, Dopeikar *et al.* 2015; Keivany & Daneshvar 2015; Kiani *et al*. 2016).

It could be concluded that *Alburnus mossulensis* is a group synchronous multiple bach spawner with a relatively high fecundity and a long period of reproduction which lasts from March to June and peaks in April.

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بیولوژی تولیدمثل شاه‌کولی جنوبی (*Alburnus mossulensis*) در رودخانه بی‌بی‌سیدان حوضه تیگریس در ایران

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چکیده

ویژگی‌های تولیدمثلی شاه‌کولی جنوبی، *Alburnus mossulensis*، یکی از کپورماهیان بومی در رودخانه بی‌بی‌سیدان سمیرم اصفهان، از آذرماه ۱۳۸۹ تا آذرماه ۱۳۹۰ به صورت ماهیانه مورد بررسی قرار گرفت. پانصد و چهل و سه قطعه ماهی (۲۵۶ ماده، ۱۵۹ نر و ۳۱ نابالغ) با استفاده از تور پره (با چشمه ۵ میلی‌متر) صید گردید. نمونه‌های صید شده در محلول روغن گل‌میخک ۱٪ بی‌هوش و روی یخ به آزمایشگاه منتقل و مورد کالبدشکافی و اندازه‌گیری قرار گرفتند. سن ماهی‌های نر و ماده از صفر تا ۵ سال بود. نسبت جنسی مشاهده شده برابر ۱نر:۲/۲ ماده بود ($p < 0.05$). در همه سنین تعداد ماده‌ها بیشتر از نرها بود. بزرگترین ماده ۱۶/۸ سانتی‌متر طول کل و ۴۹/۱۲ گرم وزن داشت و بزرگترین نر ۱۴/۱ سانتی‌متر طول کل و ۲۸/۱۹ گرم وزن داشت. سن و طول کل در اولین بلوغ در ماده‌ها و نرها به ترتیب ۱ سال و ۵/۳ سانتی‌متر و ۲ سال و ۴/۴ سانتی‌متر برآورد شد. حداقل، حداکثر و میانگین هماوری مطلق به ترتیب ۲۰۶۴، ۱۰۳۱۶ و ۲۶۸۶ ± ۵۵۰۵ و هماوری نسبی ۵۸ ± ۲۰۳ عدد به ازای هر گرم وزن بدن بود. بررسی ماکروسکوپی و شاخص جنسی گنادها نشان داد که زمان تولیدمثل آنها اسفند ماه تا خرداد ماه بوده و حداکثر آن در اردیبهشت ماه است. بنابراین، شاه‌کولی جنوبی یک گونه همزمان گروهی با توانایی تخم‌ریزی مکرر در یک فصل تولیدمثلی هستند.

* مولف مسئول