Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2024.131751 2024, 23(5) 819-831

Research Article

Biological and ecological aspects of South Caspian Spirlin (*Alburnoides eichwaldii*, De Filippi, 1863) from Tajan River, Northern Iran

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Keywords

Population parameters,
Fecundity,
Age determination,
Gonadosomatic index

Article info

Received: January 2024
Accepted: April 2024
Published: September 2024

Abstract

Age, growth, and reproductive traits of the South Caspian Spirlin in the southern part of the Caspian Sea basin, Tajan, River, were investigated. A comprehensive study was conducted on 663 specimens including 226 females, 297 males, and 140 unsexed individuals with a total length ranging from 25.88 to 121.66 mm. The specimens were collected using an electrofisher at monthly intervals. The female-to-male sex ratio was calculated as 1: 1.29. The growth rings on the scale were employed for age determination in this study. Both sexes exhibited only four age groups (0+ to 3+), with the 2+ age group dominating the population. Females consistently displayed larger sizes compared to males. The von Bertalanffy growth function yielded $L_t = 123.90\,(1 - e^{-0.25(t+0.87)})$ and $L_t = 128.10\,\left(1 - e^{-0.29(t+0.69)}\right)$ for males and females, respectively. Furthermore, the Fulton condition factor value was significantly higher in females than males. Notably, the maximum gonadosomatic index for both males and females coincided with the spawning seasons (May to August). Absolute fecundity varied from 290 to 1710 oocytes, with a mean of 793 oocytes. This study presents comprehensive data on the biological characteristics of the South Caspian Spirlin, providing valuable insights for fish stock assessment studies.

Introduction

The life-history characteristics of fish, including age, growth, and reproduction, play a crucial role in determining their ecological niches and are closely linked to their adaptive capacity (Claudet *et al.*, 2010). Consequently, understanding age and growth parameters is essential for the development of strategies to ensure sustainable management (Kim *et al.*, 2016). Knowledge of growth rate is necessary for studies in population dynamics, and accordingly, small changes in this value show growth rates can have significant effects on population analysis (Erzini *et al.*, 2001; Megalofonou *et al.*, 2003). Furthermore, investigating aspects of reproduction, such as sexual differentiation and spawning seasons, is vital for the evaluation and management of fish populations (Velasco *et al.*, 2011).

The Caspian Sea is the world's largest enclosed body of water. After discovering large oil and gas fields, some issues such as political, economic and environmental events, made the Caspian Sea important. The ecology of the Sea is being endangered due to several issues such as petroleum extraction, river and sea pollution, biological damage, decline in the Caspian species and lack of legal regime among the neighbors. Activities around the Caspian Sea endangered the balance of this very important and fragile ecosystem (Nasrollahzadeh, 2010).

Alburnoides eichwaldii widely known as the South Caspian Spirlin, a member of the family Cyprinidae, is a small-sized fish that inhabits fast-flowing rivers and has a wide distribution from North to West Asia (Mousavi-Sabet *et al.*, 2015). In recent decades, human activities and organic pollution, particularly in European waters, have led to a decline in the population of *Alburnoides* sp. (Lusk *et al*., 1995; Marszał *et al.*, 2018). Consequently, the relatively high abundance of Spirlin in the rivers of the southern Caspian Sea basin is at risk due to the entry of pollutants and habitat destruction (Zarkami *et al.*, 2019).

In the rivers of the Caspian Sea basin, the Spirlin attains sexual maturity at the age of 1 to 2 years, with its spawning season occurring in spring, with a peak in May. During this period, females deposit their eggs on sandy and gravel beds at a temperature ranging from 13 to 16℃ (Patimar *et al.*, 2012; Seifali *et al.*, 2012; Tabatabaei *et al.*, 2014). The species primarily feeds on benthic diatoms, insect larvae, and green algae (Treer *et al.*, 2006; Abbasi *et al.*, 2013).

Numerous studies have been devoted to the age, growth, and reproductive traits of game fishes (Paiva *et al.*, 2018; Freitas *et al.*, 2019; Kyritsi and Kokkinakis, 2020), leaving a noticeable gap in our understanding of these aspects for many non-game fishes (Robinson *et al.*, 2010). Furthermore, existing research on Caspian Spirlin has predominantly focused on age, growth, and mortality, with studies conducted in the Caspian basin (Tabatabaei *et al.*, 2014) and the Aras basin in Turkey (Cicek *et al.*, 2016), as well as exploration of morphological characteristics in Shahid Rajaei Dam and the Tajan River (Azizi *et al.*, 2015). Despite these efforts, inadequate information exists regarding the life history traits of the South Caspian Spirlin in the southern basin of the Caspian Sea.

The primary objective of this study is to elucidate the age, growth, reproductive traits, and various biological features of the South Caspian Spirlin captured in the Caspian Sea basin in Iran. The obtained information holds significant potential for enhancing current fish stock management practices and proposing constructive approaches for decision-makers.

Material and methods

Study area and sample collection

The present study was conducted in the Tajan River (36^0 to 36^0 $22'$ N and 53^0 $3'$ to 53^0 $27'$ E), located in Mazandaran Province, Iran. This river holds significant importance in the southern part of the Caspian Sea, spanning approximately 160 km in length, with a watershed area of around 2,000 square kilometers. To ensure representative sampling, three specific sites with the highest occurrence of Caspian Spirlin were chosen based on previous records (Fig. 1).

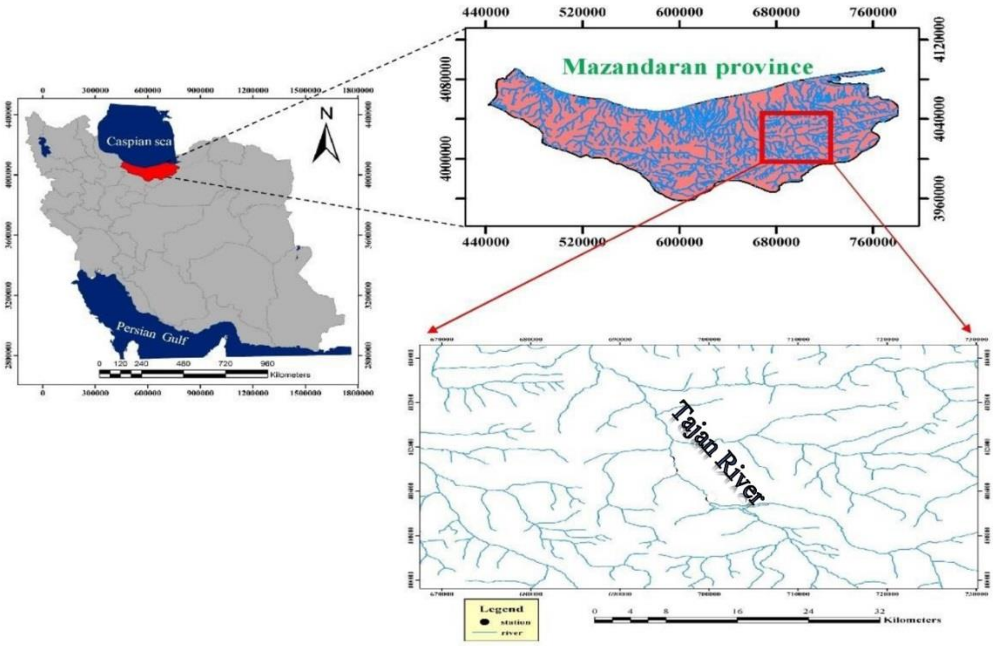

Figure 1: Sampling area of *Alburnoides eichwaldii*. Tajan River, Southern Caspian Sea.

Sampling was conducted monthly from May 2012 to March 2013 at the three designated stations, utilizing an electrofisher with a voltage range of 100-200 V. Stations were selected based on previous experience as well as better access to this species. All three stations were located in the downstream part of Shahid Rajaei Dam. It's worth noting that due to the exceptionally high and turbulent flows, sampling in April was not successful.

Following sample collection, individuals were promptly measured for total length (to the nearest 0.01 mm) and weighed for body weight (to the nearest 0.01 g) before being released back into the river. Each month, only 20 South Caspian Spirlin samples were transferred to the Aquatic Ecology Laboratory at Sari

Agricultural Sciences and Natural Resources University for subsequent laboratory studies.

Age determination and growth

The growth rings on the scale were utilized for age determination. The scales were taken from the area posterior to the pectoral fin, the scales were cleaned of mucus, placed in a paper envelope, and allowed to dry before growth rings were studied under a microfiche reader. The identification of age relied on counting the number of annuli present on each scale. To ensure accuracy and minimize bias, three individuals with varying skill levels were engaged in estimating the age of the fish. Each reader independently assessed age without knowledge of the size or sex of the individual fish, or awareness of the age assignments made by the other readers (Vandergoot, 2008).

To estimate size at first maturity the following equation was used:

$P = {}^{1}\!/_{(1 + Exp[-rm(L - Lm50)])}$

Where, P is the proportion of reproductive females/males for each size class; rm is the curve asymptote; L is the total length and L50 is the size at first maturity.

The relationship between total length and body weight was specified for each sex using the equation: $W = a\mathrm{L}^{\mathrm{b}}$. The parameters a and b were estimated through the logarithmic form: $Log\ (W) = Log\ (a) + b\ Log\ (L)$. Fulton's condition factor (CF) for each individual was calculated with the formula: $CF = \mathrm{W}/\mathrm{L}^3$ (Le Cren, 1951). To examine significant differences in length-weight relationships between sexes, an analysis of covariance (ANCOVA) was conducted using SPSS 23. The von Bertalanffy growth model (VBGF) was applied to the observed length-at-age data for each sex through a nonlinear least-squares function. The VBGF was fitted to the data using the equation: $\mathrm{L_t} = (\mathrm{L}_\infty (\mathrm{e}^{-\mathrm{k(t-t_0)}})$. The VBGF parameters were estimated utilizing FiSATII. The growth performance index was calculated using the equation: $\varphi' = \ln K + 2 \ln L_\infty$(Pauly and Munro, 1984).

Reproduction

Gonads were extracted and sex determination was performed through visual observation of gonads or using a stereomicroscope. The Gonadosomatic Index (GSI) was calculated by dividing the gonad weight (W_g), which includes the testicle mass and its accessories in males and oocytes and ovaries in females (Taborsky, 1998), by the body weight of the fish (W_b), $GSI = W_g/W_b \times 100$.

According to their appearance features, gonads were classified into six maturity stages (I–VI) (Bazzoli and Godinho, 1991). We used stageIV ripe gonads collected from May to June for estimating absolute and relative fecundity. After weighing the eggs, they were placed in Gilson's fluid for 3-4 weeks to harden the eggs and dissolve the ovarian membranes. Absolute fecundity of females during the spawning season was calculated using the Gravimetric method $F = n \times {}^{G}\!/_{g}$ where n is the egg number of the ovarian sample, g is the weight of the ovarian sample, and G is the weight of the whole gonads), based on three pieces removed from the ovary. Relative fecundity

(RF) was determined by dividing absolute fecundity (F) by the total body weight (W_b), $RF = {}^{F}/_{W_b}$ (Bagenal, 1978).

Furthermore, to determine spawning performance, the diameter of oocytes in ripened gonads, after fixation in 10% formaldehyde, was measured during the breeding season using an optical microscope. Simultaneously, the sex ratio was analyzed using the chi-square test. In order to compare length, weight and condition factor between male and female, T-test and confidence level of *p*<0.05 was used. Also, one-way ANOVA test and then Duncan test were used to compare between seasons.

Results

The present study involved the examination of 663 *Alburnoides eichwaldii* specimens, ranging in total length from 25.88 to 121.66 mm as well as total weight from 1.9 to 24.1 g. Among these samples, 226 (34.1%) were identified as females, 297 (44.8%) as males, and 140 (21.1%) as juveniles whose gender could not be recognized (Table 1). The female-to-male sex ratio was calculated as 1:1.29 ($x^2 = 8{,}6$, *p*<0.05). In this study, sexual maturity was observed in males and females at a total length of 63.6 mm and 68.1 mm, respectively, both occurring at age 1+.

Table 1: Observed length (mm) and weight (g) of South Caspian Spirlin in the Tajan River collected from April 2012 to March 2013

Sex	n	Length		Weight	
		(Min-Max)	(mean±S.D)	(Min-max)	(mean±S.D)
Male	297	54.3-116.1	83.1±0.77	1.9-24.1	7.6±0.21
Female	226	57.4-121.6	84.8±0.86	2.2-24.1	8.8±0.29
Juvenile	140	25.8-62.1	46 ±0.76	0.03-2.71	1.12±0.06

Age and growth

All removed scales were examined thoroughly. Scale analysis revealed a maximum age of 3 years for *A. eichwaldii* in the Tajan River. Four distinct age groups were identified, consisting of 0+ (32.28%), 1+ (19.15%), 2+ (45.85%), and 3+ (2.72%). Notably, the 2+ age group represent the dominant part of the population. Figure 2 illustrates the age class composition of male, female, and juvenile South Caspian Spirlin in the Tajan River.

The length- weight relationship (TL-TW) was calculated as follows: $TW = 0{,}00001L^{3{,}0541}$ for males, $TW = 0{,}000003L^{3{,}3233}$ for females, and $TW = 0{,}000003L^{3{,}3251}$ for juveniles.

A statistical comparison of the total length-weight relationship in juveniles, males, and females revealed significant differences. The b-values of the relationships and t-test indicated positive allometric growth. The relationship between TL and TW for each sex is depicted in Figure 3.

Fulton condition factor (CF) values for females were notably higher than those for males (*p*<0.05). Furthermore, with increasing age, the CF index tended to increase, particularly in females. This factor exhibited significant variation among seasons and age groups in both sexes (*p*<0.05). Figure 4 illustrates the variations of this factor across different seasons and age groups.

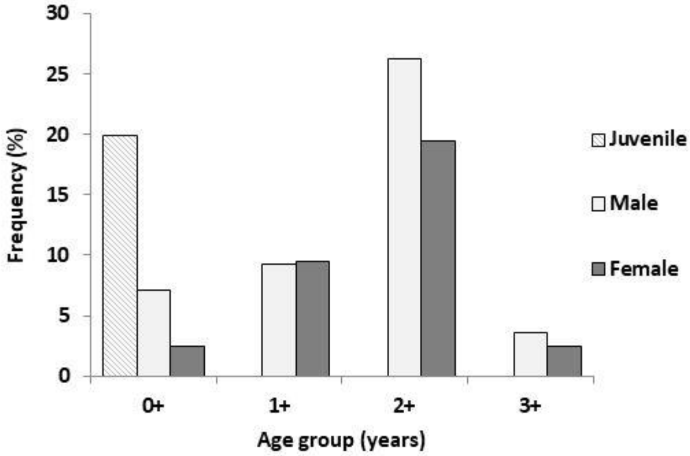

Figure 2: Age class composition of males, females and juveniles *Alburnoides eichwaldii* in the Tajan River collected from April 2012 to March 2013.

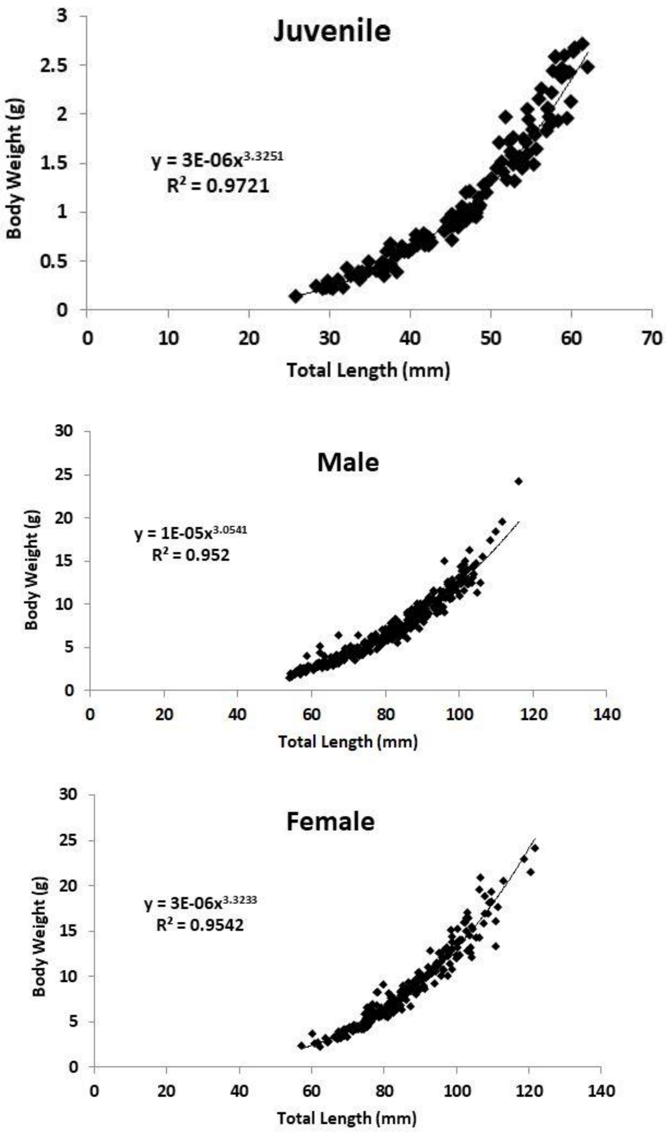

Figure 3: Relative growth curves (total length-body weight) for juveniles, males and females of South Caspian Spirlin in the Tajan River, Caspian Sea basin, North of Iran.

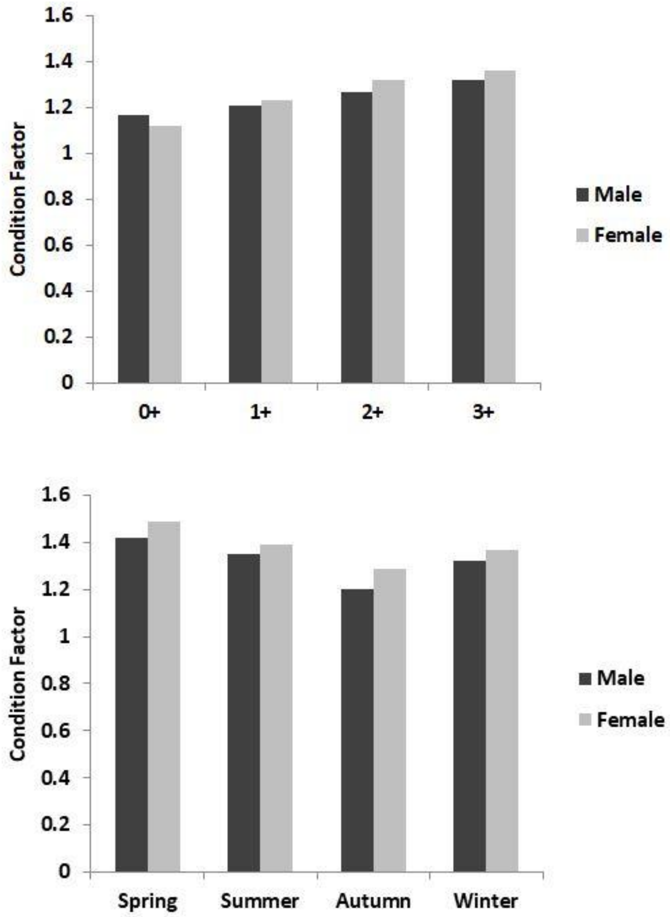

Figure 4: Fulton's condition factor of males and females South Caspian Spirlin in different seasons and different age groups.

The von Bertalanffy growth function (VBGF) was applied to the observed length-at-age data for each sex using the following equations: $L_t = 123{,}90\ (1 - e^{-0{,}25(t+0{,}87)})$ for males and $L_t = 128{,}10\ (1 - e^{-0{,}29(t+0{,}69)})$ for females. The growth performance index (φ') for males and females in the Tajan River was 3.64 and 3.86, respectively. Notably, φ' showed significant differences between males and females (p<0.05).

Reproduction

The gonadosomatic index (GSI) of South Caspian Spirlin exhibited a similar variation pattern for both sexes, with the highest mean value calculated in May. The GSI Values for males were significantly lower than those for females (p<0.05). The mean GSI for females and males was 6.35 and 2.55, respectively. According to the GSI, the reproductive period for this species in the Tajan River extended from May to August, with peaks observed in May, June, and July for both females and males (Fig. 5). Subsequently, there was a decrease in September, indicating the onset of the gonad resting period. These results suggest that the breeding period of Caspian Spirlin spans from May to August.

Absolute fecundity demonstrated an increasing trend with age in South Caspian Spirlin.

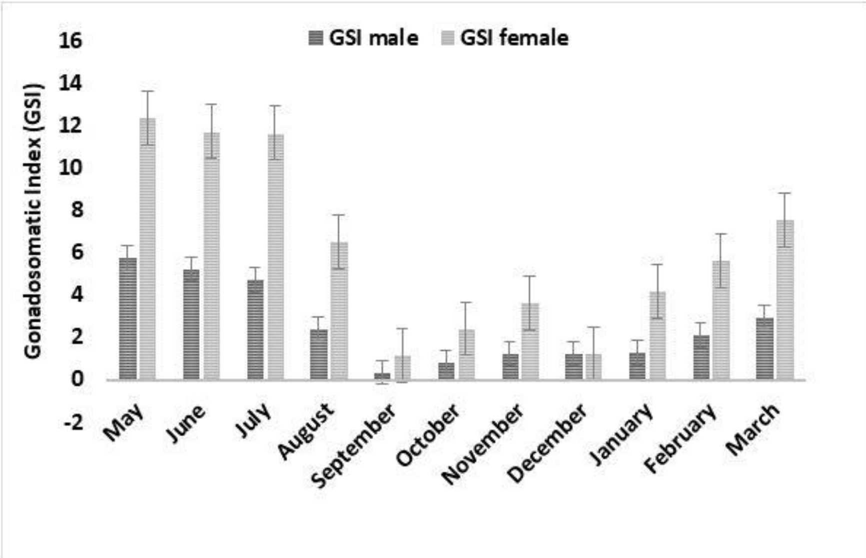

Figure 5: Monthly variations of mean±SE gonadosomatic index (GSI) values of *Alburnoides eichwaldii* in the Tajan River, Caspian Sea basin, Iran.

The absolute fecundity ranged from 290 ova (1+ age group samples) to 1710 ova (3+ age group samples). The mean relative fecundity was calculated as 64.67 eggs/g. The regression relationship between relative fecundity and fish size (total weight and total length) was statistically significant ($p<0.05$). Concurrently, the diameter of the oocytes varied from 0.85 to 2.75 mm during the spawning period (Fig. 6). The highest oocyte diameter was observed in the 1+ age group (Table 2).

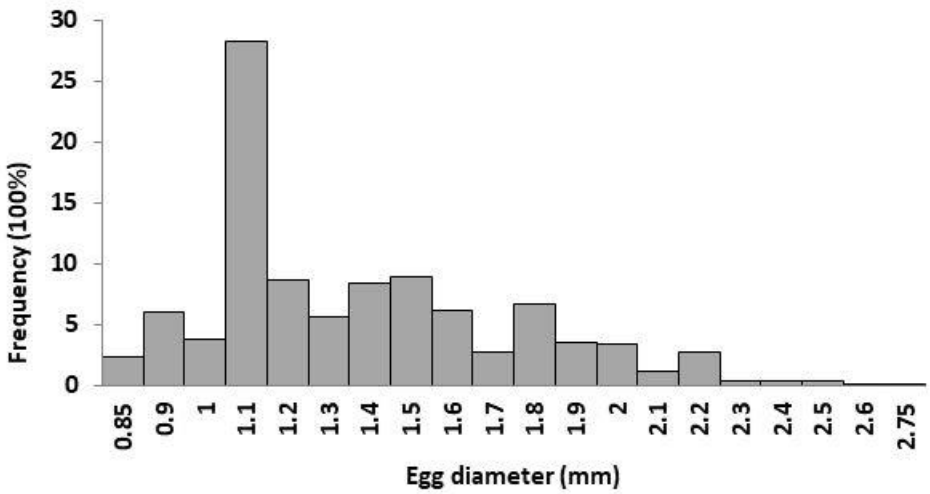

Figure 6: Size frequency distribution of oocyte diameters of female South Caspian Spirlin in the Tajan River, Iran.

Table 2: The minimum, maximum, and mean of absolute and relative fecundity and oocyte diameter of South Caspian Spirlin in the Tajan River, Iran

	Min.	Max.	Mean	Standard Error
Absolute Fecundity (gr)	290	1710	793.84	396.32
Relative Fecundity (eggs/gr)	34.29	112.51	64.67	21.98
Oocyte Diameter (mm)	0.85	2.75	1.38	0.21

Discussion

In the present study, key population parameters of South Caspian Spirlin were estimated in the southern part of the Caspian Sea. The initial step in studying population dynamics and assessing fish stocks involves determining the length-weight relationship (Yigin and Ismen 2012; Lim *et al.,* 2014). A comparison of the length-weight relationship of slopes and intercepts in this research with previous studies in European waters and other Iranian basins (Table 3) revealed significant differences in these parameters among regions. These variations can be attributed to inter-population patterns associated with diverse habitat quality, growth rates, and natural selection (Patimar *et al.,* 2012). Additionally, the maximum determined age in this study was 3+ years, indicating a shorter lifespan of this species in the Tajan River compared to other areas. For example, Cicek *et al*. (2016) reported a maximum age of 5+ years for this species from the Aras River basin.

Table 3: Length-weight relationship Parameters of *Alburnoides sp.* in different regions

Author (year) / Location	sex	a	b	r^2
Tabatabaei *et al*. (2014)/ Iran	F	0.01	3.26	0.98
	M	0.01	3.30	0.88
Patimar *et al.* (2012) / Qanat of Uzineh, Iran	F	0.0068	3.2559	0.93
	M	0.0079	3.2067	0.94
Seifali *et al*. (2012) / Kesselian Stream, Iran	-	0.000006	3.1221	0.958
Cicek *et al*. (2016) / Aras River, Turkey	-	0.00644	3.2221	0.98
Treer *et al*. (2006) / Sava River, Croatia	-	0.0083	3.025	0.885

The Von Bertalanffy growth model for Caspian Spirlin revealed that the $L_\infty$was lower in males than females. Infinite length is considered a measure of growth capacity (Bagenal and Tesch, 1978). Additionally, the growth coefficient (k) was higher in females than males, indicating a faster growth rate males in reaching L_∞. In the present study, individuals belonging to the age group 3+ were predominantly females. Short-lived species often reach their L_∞ in a shorter period and exhibit a higher k (Hart and Chute, 2009). Different Von Bertalanffy growth models can suggest variations in resource allocation between growth and reproduction. Therefore, differences in growth parameters between the sexes reflect disparities in fish reproduction efforts (Patimar *et al.,* 2012). The growth performance index (φ') in the Tajan River (male: 3.64 and female: 3.86) was lower than those obtained from the Sava River (φ'= 4.44) (Treer *et al.,* 2006) and River Aare (φ'= 4.70) (Breitenstein and Kirchhofer, 2000). This difference is likely due to the lower growth coefficient (or growth rate) of Caspian Spirlin in the Tajan River.

The condition factor is a vital biological parameter offering insights into the status of fish populations, playing a crucial role in fish stock conservation and management (Ndiaye *et al.,* 2015). Condition factor tends to increase during feeding, reproduction, and aging in both males and females, and its fluctuation pattern is comparable in both sexes (Turkmen *et al.,* 2000), aligning with the findings of our study. In current research, the pattern of condition factor increase and decrease followed a consistent trend. Previous studies (Treer *et al.,* 2006; Tabatabaei *et al.,* 2014; Ghojoghi *et al.,* 2017) have also noted a similar fluctuation pattern in condition factor, although the actual values may differ in various habitats.

The gonadosomatic index served as a reliable indicator of the reproductive status of South Caspian Spirlin. The maximum and minimum GSI values for both adult females and males exhibited a gradual decrease throughout the year until the spring season. In this study, the spawning season of South Caspian Spirlin extended from May to August, signifying a lengthy spawning period. The prolonged spawning period in these fish may be attributed to the higher environmental variability in river environments compared to marine environments, prompting South Caspian Spirlin to increase their reproductive effort (Humphries *et al.,* 2013). Given the challenges and sometimes impossibility of calculating the number of fish eggs and larvae in the natural environment, determining fecundity remains one of the most common methods to estimate fish stocks (Pitcher and Hart, 1996). The mean absolute fecundity of females in this study was found to be 793 eggs. Seifali *et al.* (2012) reported a mean absolute fecundity of 1723 eggs for this species in the Kesselian Stream. Several studies have indicated that factors such as age, size, feeding habits, season, population density, and environmental conditions influence fecundity (Unlu and Blaci, 1993; Mansouri-Chorehi *et al.,* 2016). Additionally, the egg diameter of South Caspian Spirlin at different age groups revealed that as age and size increase, the diameter of oocytes decreases, leading to an increase in fecundity.

Conclusions

This study furnishes comprehensive insights into the age, growth, and reproductive traits of South Caspian Spirlin, offering valuable information for stock management and enhancing our understanding of the life history of this species. The limited age range observed in this fish within the Tajan River suggests that the population of South Caspian Spirlin is potentially facing pressure, likely attributed to habitat degradation. Consequently, it is imperative to implement conservation measures to safeguard this species and other fish inhabiting the Tajan River.

Acknowledgments

We extend our gratitude to Dr. Khosrow Jani-Khalili and Fatemeh Esfahani for their invaluable assistance with sample collection.

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