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New data on the biology of *Cobitis* cf. *satunini* from the southern Caspian basin (northern Iran)

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Abstract. Little is known of the life history of *Cobitis* cf. *satunini*, a loach species native to the Caucasus and southern Caspian Basin. Life history characteristics of this species were examined in 568 specimens collected from the Siahroud stream (northern Iran) between February and June 2010. The specimens ranged in size from 27 to 103 mm total length and weighed from 0.13 to 8.93 g total weight. The overall sex ratio was unbalanced as females dominated, with 342 females and 226 males being caught (sex ratio 1.5 : 1). Length-weight relationships implied that growth was positively allometric for both sexes. The von Bertalanffy growth function was estimated to be $L_t = 94.95\ (1 - e^{-0.49(t + 0.34)})$ for males and $L_t = 121.65\ (1 - e^{-0.39(t + 0.43)})$ for females. Egg diameter ranged from 0.44 to 1.02 mm, with a mean value of 0.77 mm. Absolute fecundity varied from 212 to 4666 eggs. Fecundity relative to total weight fluctuated from 383 to 268 eggs g^{-1}. These growth and reproduction traits may be interpreted as species life history capacity and/or adaptation to environmental conditions caused by the southern Caspian climate.

Key words: age, growth, egg diameter, fecundity, reproduction, *Cobitis satunini*, northern Iran

Introduction

Cobitis cf. *satunini* is considered native to Georgia and Iran. Its distribution encompasses much of northern Iran (southern Caspian basin), being found throughout the upper parts of rivers flowing into the Caspian Sea. Previous studies have described this loach as *C. taenia* (Abdoli 2000, Naderi & Abdoli 2004), however, *C. taenia* is recognised as a northern species (Bohlen & Ráb 2001) and the species in the southern Caspian basin is most likely *C.* cf. *satunini*. *C. satunini* is listed as "data deficient" under the IUCN classification scheme. Based on a limited data set provided by Abdoli (2000) on the morphology and biology of this species, *C. satunini* reaches 115 mm total length and spawns between March-May in the upper parts of streams.

In recent years, habitat degradation, caused by a range of anthropogenic activities, has occurred in many of the northern rivers of Iran that are home to loaches (Kiabi et al. 1999). Within these rivers, loach populations are subject to a variety of different habitat conditions that influence the life history parameters of the species. A lack of knowledge about the biology of the species in these rivers, however, prevents an evaluation of the impact of the ongoing changes. In the case of *C. satunini*, accurate information on growth and reproduction of this species would be a useful guideline in conservation and management of both fish populations and the river ecosystem.

The aim of this study, therefore, was to describe the age structure, growth and reproduction of a population of *C. satunini* in the Siahroud stream.

Material and Methods

The present study was carried out in the Siahroud stream, a small stream in the Sefidroud basin, situated in the western Elburz Mountains (Guilan province in northern Iran). With an overall length of 52 km, the river is characterised by a typical Caspian-Elburz mountain climate (Afshin 1994). Water depth at point of sampling was about 30-50 cm. The stream has clear and colourless water, a temperature of 12-16 °C, pH of 7.5-7.9, salinity of 1.21-1.30 ‰ and the stream bed is sandy. The most frequent co-existing fish species include *Alburnoides bipunctatus*, *Squalius cephalus* and *Neogobius fluviatilis*.

Fish were collected monthly from February to July 2010 using a surber-type fish sampler. In the field, all fish specimens were immediately preserved in 10 % formaldehyde solution. In the laboratory, the total length was measured to the nearest 1 mm for all fish sampled. Total weight, weight of gonads of both sexes and weight of ovary sub-samples taken for determination of fecundity and egg diameter in females were recorded with an electronic analytical balance to the nearest 0.01 g. Age was determined from banding patterns in both operculae using a 10-30× binocular microscope under reflected light. Growth annuli from each operculum were counted three times, each time by a different person. The relationship between total length and total weight was determined by fitting the data to a potential relationship in the form of: $W = aL^b$, where W is the weight in g, L the total length in cm, and a and b are the parameters to be estimated, with b being the coefficient of allometry based on the Pauly test (Pauly 1984). The growth model adopted was the specialised von Bertalanffy growth function (VBGF), expressed as: $L_t = L_\infty (1 - e^{-k(t - t_0)})$, where L_∞ (mm) is the predicted asymptoic length, L_t (mm) is the size at age t, k ($year^{-1}$) is the instantaneous growth coefficient, and t_o (year) is the point at which the von Bertalanffy curve intersects the age axis. The parameters were estimated using the Ford-Walford method (Everhart & Youngs 1975). Sex was determined by examination of the gonad tissue using a 25-40× binocular microscope. Gonadosomatic index (*GSI*) was calculated as *GSI = gonad weight/total body weight x 100.*

To estimate fecundity, ovaries of 210 ripe females caught in April-May, at maturity stage IV based on the scale given by Nikolsky (1963), were removed, weighed, and then placed in Gilson's fluid for 3-4 days to harden the eggs and dissolve the ovarian membranes. The number of eggs was estimated by the gravimetric method, using three pieces of approximately 0.02 g each from the anterior, medial and posterior positions of both ovarian lobes. The relative fecundity index was calculated as *RF = F/TW*, where *F* is absolute fecundity and *TW* total weight (Bagenal & Tesch 1978).

Average egg diameter was examined by measuring 30 eggs chosen randomly from each ovary used for estimation of fecundity, 10 oocytes being taken from each of the three positions of the ovary and the mean measurement taken. Measurements were made to the nearest 0.05 mm with an ocular micrometer microscope. Analysis of co-variance (ANCOVA) was performed to test for significant differences in length-at-age and weight-length relationships between sexes (Zar 1984). The degree to which *b* differed from three was tested by using the equation given by Pauly (1984). Comparison of *GSI* values during the reproductive period, condition factor between sexes and temporal variation for each sex was carried out by analysis of variance (ANOVA). Any significant difference in the overall sex ratio was assessed using the Chi square test (Zar 1984). All statistical analyses were performed with a significance level of $p < 0.05$ using the SPSS 17 software package.

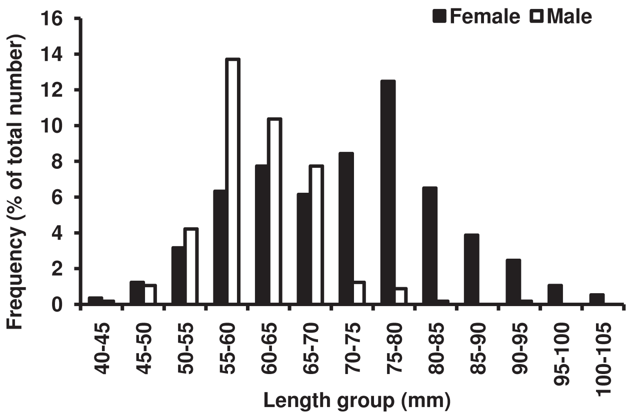

Fig. 1. *Size frequency distribution (%) of males and females of* Cobitis *cf.* satunini *sampled in 2010 from the Siahroud stream, southern Caspian Sea (northern Iran).*

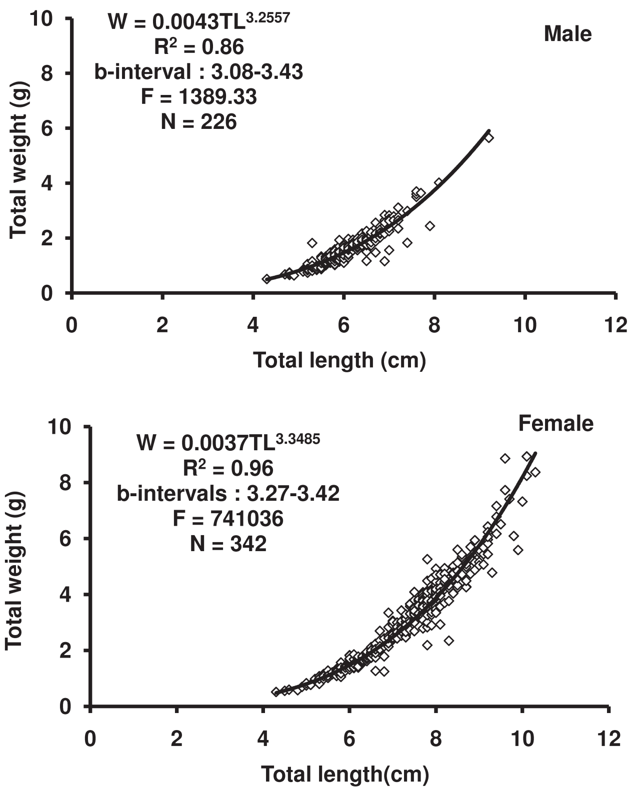

Fig. 2. *Relative growth curves (total length – total weight) for* Cobitis *cf.* satunini *from the Siahroud stream, southern Caspian Sea (northern Iran).*

Table 1. Mean total length (TL) and weight (WT) at age (± S.D.) for Cobitis *cf.* satunini *from the Siahroud stream, southern Caspian Sea (northern Iran).*

Age (year)	1+	2+	3+	4+
Female				
Number of specimens	63	198	78	3
Mean observed TL (mm)	55.51 ± 4.30	71.93 ± 6.07	87.06 ± 5.01	101.67 ± 1.15
Mean observed WT (g)	1.16 ± 0.31	2.88 ± 0.90	5.05 ± 1.06	8.51 ± 0.37
Male				
Number of specimens	30	187	7	2
Mean observed TL (mm)	52.03 ± 2.91	62.36 ± 4.45	76.00 ± 1.73	86.50 ± 7.78
Mean observed WT (g)	0.92 ± 0.25	1.74 ± 0.49	3.10 ± 0.72	4.83 ± 1.15

Results

Age, growth and population structure

A total of 568 specimens of *C. satunini* were caught during the sampling period. Males ranged from 43 to 92 mm and 0.51 to 5.65 g, while females ranged from 43 to 103 mm and 0.13 to 8.93 g. Opercula readings revealed that the majority of specimens were of age group 2+, with 4+ being the oldest age recorded for both sexes (Table 1). Observed length-at-age in the population was different between sexes, females being longer and heavier than males (ANCOVA, $F_{TL} = 104.05$, $F_{TW} = 31.41$, $p < 0.05$).

Length frequency distribution of the fish (Fig. 1) indicated that the most frequent size classes in the samples were 56-60 mm for males and 76-80 mm for females. Males were rare in length classes greater than 80-85 mm.

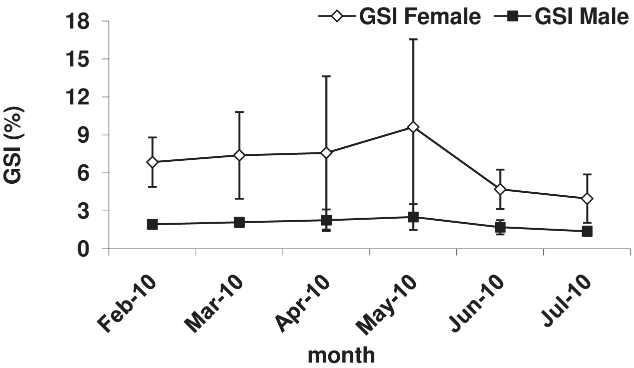

Fig. 3. Monthly change of GSI in Cobitis *cf.* satunini *from the Siahroud stream, southern Caspian Sea (northern Iran).*

Length-weight relationships for both sexes had high regression coefficients (Fig. 2) and were significantly different from each other (ANCOVA, $F = 11.03$, $p < 0.05$). For both males and females, *b*-values were significantly different from 3 (*t*-test, $t_{male} = 14.15$, $t_{female} = 13.71$, $p < 0.05$) and displayed positive allometric form.

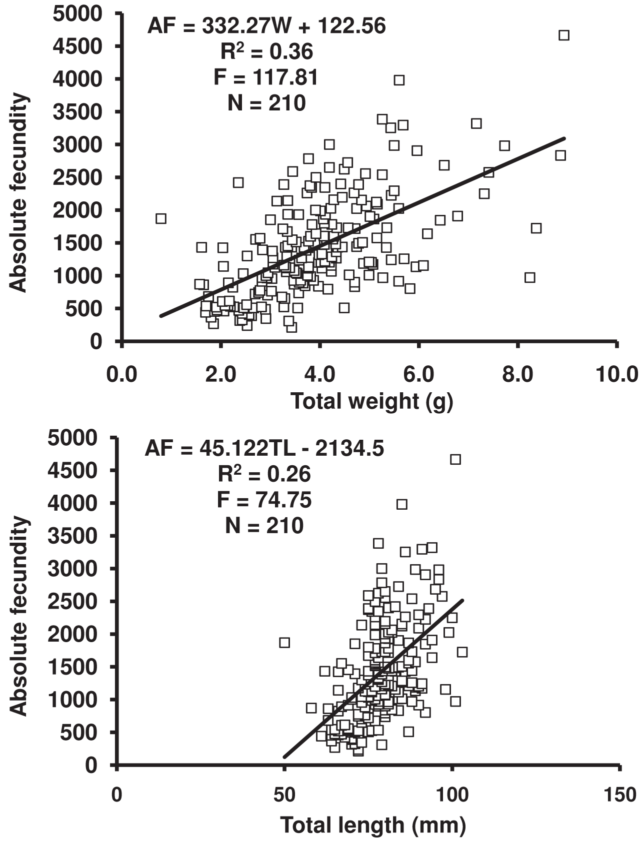

Fig. 4. Relationship between absolute fecundity and fish size total length (mm) and total weight (g) of female Cobitis *cf.* satunini *from the Siahroud stream, southern Caspian Sea (northern Iran).*

The von Betalanffy growth model (male: $L_t = 94.95 (1 - e^{-0.49(t+0.34)})$, female: $L_t = 121.65 (1 - e^{-0.39(t+0.43)})$ suggests that males grow faster than females ($k_{female} < k_{male}$), whereas asymptotic total length is greater in females than in males.

Sex ratio

The overall ratio of males to females was 1 : 1.5 and Chi-square analysis indicated a significant difference from an expected ratio of 1 : 1 ($\chi^2 = 24.43$, $p < 0.05$). An

Table 2. *Mean absolute fecundity at age (± S.D.) for* Cobitis *cf.* satunini *from the Siahroud stream, southern Caspian Sea (northern Iran).*

Age (year)	1+	2+	3+	4+
Number of specimens	15	114	78	3
Mean absolute fecundity	778.50 ± 510.50	1176.03 ± 660.45	1660.49 ± 649.25	2390.92 ± 891.87

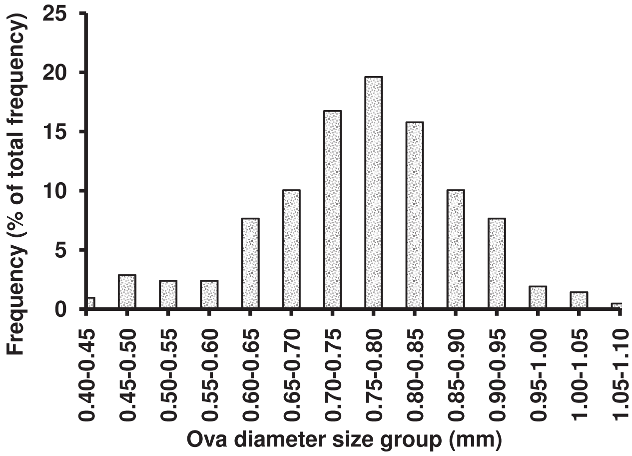

Fig. 5. *Oocyte diameter size frequency distribution for* Cobitis *cf.* satunini *from the Siahroud stream, southern Caspian Sea (northern Iran).*

unequal sex ratio was observed among length classes (Fig. 1), females dominating in the older size classes (> 70 mm) and males in the younger (< 70 mm).

Gonadosomatic index

Significant changes were observed in the temporal variation of gonad activity (ANOVA, F_{female} = 8.29, F_{male} = 6.04, $p < 0.05$). The highest recorded mean *GSI* value (± standard deviation, SD) was 2.51 ± 1.02 for males and 9.62 ± 6.94 for females in May (Fig. 3). Following the seasonal cycle of the gonadosomatic index, the reproductive period for this species in the Sefidroud stream is estimated to range from April to June, when *GSI* is considerably higher.

Fecundity

Analysis showed a significant positive effect of age on absolute fecundity (*AF*), the relationship being $AF = 171.05 + 532.17$ age ($F = 97.76$, $r^2 = 0.97$; Table 2). Minimum and maximum values of 212 and 4666 eggs were from a 2+ and 4+ female, respectively. The mean value (± SD) of absolute fecundity was 1423 ± 766 eggs/female. The linear function was adequate for expressing fecundity-total weight and fecundity-total length relationships (Fig. 4). All correlation coefficients calculated between fecundity and each of the independent variables, while moderate, were statistically significant ($p < 0.05$). Fecundity relative to total weight fluctuated from 62 to 1029 eggs/g, with a mean value (± SD) of 361 ± 157, and relative to total length from 3 to 47 eggs/mm, with a mean value of 18 ± 9. The relationship of relative fecundity (*RF*; = fecundity per gram) with total weight was not found to be statistically significant ($p > 0.05$), while the relative fecundity-total length relationship was significant though with a low correlation coefficient ($RF = 0.394TL - 13.497$, $r^2 = 0.15$, $p < 0.05$).

Egg diameter

Egg diameter ranged from 0.40 to 1.10 mm, with a mean value (± SD) of 0.76 ± 0.12. Size distribution

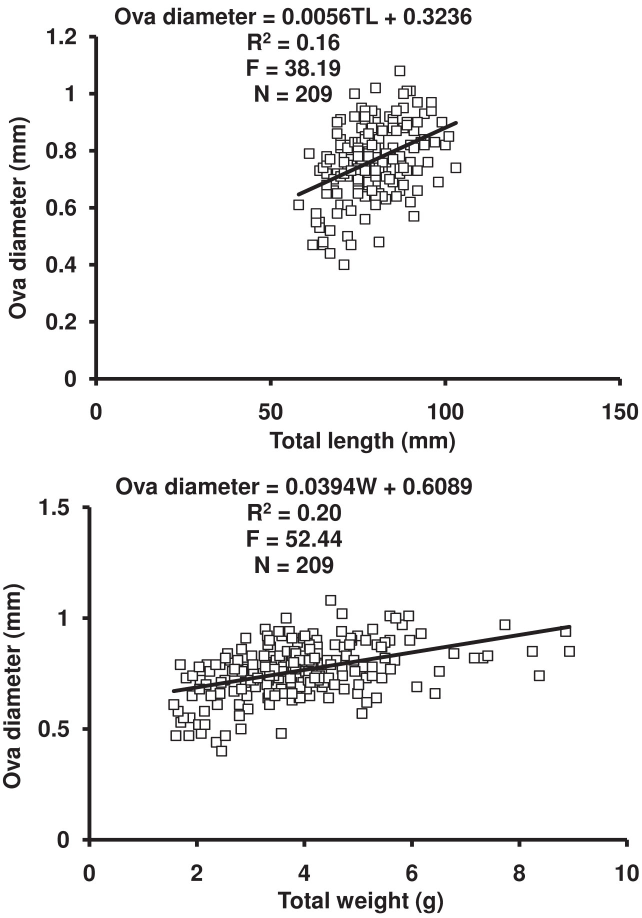

Fig. 6. *Relationship between ova diameter and total length (mm) and total weight (g) of female* Cobitis *cf.* satunini *from the Siahroud stream, southern Caspian Sea (northern Iran).*

Table 3. *Maximum age observed in different populations of* Cobitis *species.*

Species	Sex	Maximum age	Reference
C. taenia	Female	4+	Slavík & Ráb (1996)
	Male	3+	
	Female	4+	Marconato & Rasotto (1989)
	Male	3+	
C. paludica	Female	5+	Soriguer et al. (2000)
	Male	4+	
	Female	5+	Przybylski & Valladolid (2000)
	Male	3+	
	Female	4+	Oliva-paterna et al. (2002)
	Male	3+	
	Female	5+	Kottelat & Freyhof (2007)
	Male	4+	
C. elongatoides	Female	5+	Kottelat & Freyhof (2007)
	Male	3+	
C. narentana	Female	5+	Kottelat & Freyhof (2007)
	Male	3+	
C. calderoni	Female	3+	Kottelat & Freyhof (2007)
	Male	2+	
C. bilineata	un-sexed	4+	Kottelat & Freyhof (2007)
C. satunini	Female	4+	Present study
	Male	4+	

of eggs indicated that the majority of oocytes ranged from 0.70 to 0.90 mm (Fig. 5). Egg diameter was significantly correlated with female total length and weight (Fig. 6).

Discussion

To our knowledge, there is no other published information on the maximum age of this species for comparison. A comparison of *Cobitis* species (Table 3) shows that maximum age varies considerably among species. Compared to the other species, *C. satunini* has a medium life span, not-exceeding four years, and females exhibit a much wider range in length and a higher maximum length than males, a trend common to the loaches.

Weight-length relationships produced good fits and biologically sound results and could be used for comparison proposes. The total length-somatic weight relationship showed that growth was positively allometric for both sexes and for the population as a whole. Different *b*-values between the sexes of *C. satunini*, as well as for those of some other loaches (Slávik & Ráb 1996, Przybylski & Valladolid 2000, Soriguer et al. 2000), suggest an apparent growth model between sexes and species. Furthermore, this reflects a slight change in body form with sex and species, itself probably an effect of different environmental habitat conditions and species characteristics.

Estimated maximum length (L_∞) values appear to be reasonable, given that the highest values observed for the species are lower than L_∞. A trade-off between growth rate (k) and maximum theoretical size (L_∞) is often found and this is usually explained by local environmental factors. The higher coefficient 'k' in males suggests that they undergo rapid early growth and approach their asymptotic length (L_∞) earlier in life. This 'front loading' may explain in part the slight dominance of females at the study site, with higher survival rates amongst older females. Fish species would usually be expected to have a sex ratio that does not differ significantly from unity (i.e. 1 : 1). For *C. satunini*, the highly female dominated sex ratio could be due to a higher survival rate or a longer life span in females. Our data show that longevity was the same for both sexes, however, and the most likely hypothesis, therefore, is that survival rate is different between the sexes. In most *Cobitis* populations, the sex ratio is slightly biased toward females and Bohlen & Ritterbusch (2000) have proposed that males are more vulnerable to predation due to their smaller size. The dominance of a polyploid sperm parasite observed in many *Cobitis* populations in Central and Eastern Europe (Bohlen & Ráb 2001) is unlikely to have contributed to the sex ratio in the studied population of *C. saturnini*.

The spawning period for *C. satunini* in the southern Caspian basin (from April to June) is similar to that described for *C. elongatoides*, *C. trichonica*, *C. vettonica* and *C. meridionalis* in some rivers of Europe. Other European *Cobitis* species have different spawning periods, e.g. *C. bilineata* and *C. narentana* in April-August, *C. paludica* and *C. taenia* in April-July, and *C. tanaitica* in May. In the southern Caspian basin, spawning occurred over a period when the highest environmental food supply has been reported for fish species inhabiting the rivers and streams of the basin (Abdoli & Naderi 2009).

The single peak in *GSI* and oocyte size-frequency distribution during the spawning season indicates that *C. satunini* is not a multiple spawner in the southern Caspian basin. The production of multiple batches of eggs provides certain advantages (Burt et al. 1988), especially for those species living in fluctuating environments (Nikolsky 1963), and has been suggested for *C. taenia*, *C. bilineata* and *C. paludica* (Marconato & Rasotto 1989, Bohlen 2000a, b, Oliva-Paterna et al. 2002), but could not be confirmed for *C. satunini* in the study area.

In the present study, absolute fecundity was positively correlated with fish size (length and weight). Biologically, it might be deduced that total energetic investment in reproduction tends to increase with fish size, while the relationship between relative fecundity and fish weight is not significant. This implies that proportional energetic investment in reproduction, as energy allocation per unit of fish size, is variable and not significant for this species. It was revealed from the study that absolute fecundity and egg size in *C. satunini* increase linearly with an increase in fish size.

The positive relationships observed in the present study correspond well with earlier reports on *C. paludica* (Oliva-Paterna et al. 2002). The maximum absolute fecundity value of 4666 eggs from a 4+ years old *C. satunini* female is higher than the 1400 eggs (Lobon-Cervia & Zabala 1984), 1235 eggs (Soriguer et al. 2000) and 1986 eggs (Oliva-Paterna et al. 2002) observed for *C. paludica*, and the 4282 eggs for *C. taenia* (Bohlen 1999). Compared with these *Cobitis* species, therefore, the low-latitude population of *C. satunini* in this study is characterised by high fecundity. The variation in *Cobitis* fecundity is believed to be not only due to species characteristics but also due to nutrition, food availability and supply, and ecological conditions in the water bodies. It is possible that the higher number of eggs in *C. satunini* also correlates with an increased mortality rate of fry and fingerlings, i.e. a lower survival rate during the juvenile period.

To summarise, the life history traits described for *C. satunini* from the southern Caspian basin indicate a moderate life span (4+ years for both sexes), a high body weight (weight-length relationship: $b > 3$), a short duration of spawning season, relatively high heterogeneity in egg size (0.40 to 1.10 mm), and intermediate egg number (ranging from 212 to 4666). These findings provide important new data with respect to the life history of this native species. In following the future status of *C. satunini*, scientists should endeavour to expand the database on growth and reproduction and to assess the potential impacts of habitat degradation on populations of this species.

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