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Biological characteristics of autumn and spring runs of *Caspiomyzon wagneri* into the Shirood River, Iran

F. Shirood Mirzaie*[1], C.B. Renaud[2], R. Ghorbani[1]
[1]Fisheries Department, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
[2]Research and Collections Division, Canadian Museum of Nature, Station D, Ottawa, Ontario, Canada K1P 6P4.

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Abstract
Various biological characteristics were compared between autumn and spring runs of *Caspiomyzon wagneri* migrating into the Shirood River, in an attempt to determine whether these were reproductively isolated. Two spawning runs of the same lamprey species in a single river are apparently unique to the Shirood. The autumn sample collected between 21 October and 1 November 2009 comprised 15 adults and the spring sample collected between 22 March and 18 April 2009 comprised 38 adults. While spawning was not witnessed, the significantly shorter mean total length of at least 40 cm in both males and females, and the significantly higher gonadosomatic index in females of the autumn run versus the spring run suggest that the former is closer to spawning than the latter. If one assumes a single spawning population per year in the river, the report by Nazari and Abdoli (2010) of recently spent individuals of Caspian lamprey collected in the Shirood River during April, would therefore imply that the spawning period is remarkably protracted. In order to test this hypothesis, the spawning sites need to be searched for higher upstream over the entire year, particularly in areas where suitable spawning substrate of gravel and sand occurs.

Keywords: Upstream migration, *Caspiomyzon wagneri*, Shirood River, Caspian Sea

*Corresponding author; f.shmirzaie@yahoo.com

Introduction

Lampreys give us a glimpse of the form and possibly the mode of life of some of the early vertebrates. Along with hagfishes, they are the only living representatives of the most primitive craniates, the jawless fishes (Hubbs and Potter, 1971). There are 40 recognised lamprey species worldwide, 36 of which are present in the northern hemisphere (Renaud, 2011). In Eurasia and North America lampreys are represented by the genera *Caspiomyzon*, *Entosphenus*, *Eudontomyzon*, *Ichthyomyzon*, *Lampetra*, *Lethenteron*, *Petromyzon* and *Tetrapleurodon*. Lampreys have experienced declines in abundance throughout the world due to human disturbances. About half of the lamprey species in the northern hemisphere are considered to be endangered or vulnerable in at least a portion of their range (Renaud, 1997). According to the International Union for Conservation of Nature, (IUCN) (2012), the Caspian lamprey, *Caspiomyzon wagneri* (Kessler, 1870), is a Near Threatened species. It is vulnerable in Europe generally (Lelek, 1987; Maitland, 1991), sharply declining in numbers in Russia (Pavlov *et al*., 1985) and extirpated from the Sefidrud River and rare in Anzali Lagoon and its tributaries in Iran (Renaud, 1997). The decreases in abundance were in large part due to diminished habitat and profound alterations to its breeding areas caused by hydroelectric installations.

Caspiomyzon wagneri, the unique representative of its genus, is endemic to the Caspian Sea basin. It occurs in the Caspian Sea and its tributaries in Europe and Asia. In the southern Caspian basin, in Iran, *C. wagneri* occurs in the following rivers: Shirood (also spelled Shirud and Shiroud), Talar, Babolrud, Gorganrud, Tajan, Haraz, Sardabrud, Aras, Tonekabon, Polrud, Sefidrud (the latter historically), as well as Anzali Lagoon (Kiabi *et al*., 1999; Nazari, 2007).

During their upstream spawning migration, anadromous lampreys stop feeding. Their gut undergoes atrophy, while the growing gonads gradually occupy the entire abdominal cavity, and somatic energy reserves fuel sexual maturation resulting in shrinkage in body size (Hardisty and Potter, 1971a; Hardisty, 1979, 1986; Beamish *et al*., 1979; Larsen, 1980). Therefore, the body length and mass will decrease while the egg size will increase. Lampreys usually mate with individuals of similar size; homogamy is supposed to be the rule (Hardisty and Potter, 1971b; Beamish and Neville, 1992).

Malmqvist (1986) showed that parasitic, anadromous lamprey species exhibited the largest egg sizes in absolute terms as well as the greatest body sizes at maturity, although nonparasitic, fluvial species had larger eggs relative to body size. Absolute fecundity in both parasitic and nonparasitic lamprey species was positively correlated with female size (Malmqvist, 1986).

Caspiomyzon wagneri is an anadromous lamprey that migrates in spring and autumn from the sea into estuaries for spawning. Females release all their eggs but males may spawn again with other females, ammocoetes hatch after 8–10 days at 17–23°C and metamorphosis of ammocoetes occurs at lengths of 80–110 mm in October in Iran (Coad, 2013). The duration of the larval stage is estimated to be 3 years in the Volga River, Russia (Ginzburg, 1970) and 2 to 4 years in the Kura River basin, Azerbaijan (Holčík, 1986). The adult life is at least 1 year and 5 months. Lampreys are semelparous; all adult lampreys dye after a single spawning event (Renaud, 2011).

In Iran, there is no commercial fishing for the species. Studies on *C. wagneri* are few and many of the bio-ecological characteristics of this species remain poorly known. The purpose of this study is to determine some of its biological characteristics, namely the sex ratio, total length, body and gonadal masses, length-mass correlation, absolute and relative fecundities, egg size, gonadosomatic index and condition factor and compare these among the two spawning runs in the Shirood River to establish whether the apparent reproductive isolation between the two runs is genuine. The gaining of better knowledge of this species will help in more efficient conservation efforts.

Materials and methods

Study area

All specimens were collected in the Shirood River located between 34º 44′ N and 36º 51′ N and between 50º 48′ E and 50º 49′ E (Figure 1). The length of this river is about 36 km, the width at its estuary 50-80 m, and the depth 1.5-2.5 m (Nazari and Abdoli, 2010).

Caspiomyzon wagneri migrates into the Shirood River in the spring and autumn for spawning purposes and initiates its spring migration from the middle of March to late April and for the fall migration from early October to early November (F. Shirood Mirzaie, personal observations). The specimens were collected in 2009 under the Shirood Bridge, using either a cast net (mesh size 8 mm) or by hand during the two time periods (i.e., 22 March-18 April and 21 October-1 November; no lampreys were seen between 18 April and 21 October in 5 sampling attempts). In total, 53 specimens were collected, 38 in the spring and 15 in the autumn. All specimens were fixed in 10% formalin.

Morphometrics and counts

Total length of the individuals was measured to the nearest 0.5 mm. The body including the gonads and without them were weighed for each specimen to the nearest 0.01 g. The sex was determined through dissection. A sample of 40 eggs per female was weighed to the nearest 1 mg and divided by 40 to obtain the mass of an individual egg. The long and short axes of 40 eggs from the anterior, medial and posterior parts of the ovaries of each female were measured to the nearest 0.01 mm.

The number of parasites associated with the intestine was determined internally in the anterior, medial and posterior sections, as well as externally.

Data analysis

A Chi-Square test was used to test for significant differences in sex ratio. The significance level used was $\alpha \leq 0.05$. The length-mass correlation was calculated using the formula $W = a L^b$, where W is total body mass (g), L is total length (mm) and a, b are constants (Biswas, 1993).

The gonadosomatic index (GSI) was calculated as follows: GSI = gonadal mass / body mass ×100 (Fukayama and Takahashi, 1985). Spawning occurs when the GSI reaches its highest level (Biswas, 1993).

The absolute fecundity (i.e., the number of eggs in the ovaries) was determined by the counting-weighing method (Kucheryavyi *et al.,* 2007). The egg samples were taken from the anterior, medial and posterior parts of the ovaries and the number of eggs in 0.25 g from each section was counted. This combined number was then multiplied by the appropriate factor to account for the entire mass of the ovaries using the formula $N = nW / w$, where N and n are the total and partial number of eggs, respectively and W and w are the total and partial mass of eggs, respectively (Yamazaki *et al.,* 2001). The relative fecundity was calculated in relation to the body mass and total length (Kucheryavyi *et al.,* 2007). The largest (d_1) and shortest (d_2) diameter of eggs were measured and then used to calculate the radius (r), mean diameter (d), surface (S) and volume (V) using the following formulae: $d = (d_1 + d_2)/2$, $r = d/2$, $S= 4\pi r^2$, $V = 4/3\ \pi r^3$ (Imanpoor *et al.,* 2009). The condition factor was calculated using Fulton's formula $K' = TW/TL^b \times 100$, where TW and TL are the observed total mass (g) and total length (cm) of a lamprey, respectively and b is the slope of the lamprey length-mass relationship.

Excel (2010) and SPSS (16.0) were used to perform the statistical analyses and draw figures. Total length, gonadosomatic index, absolute and relative fecundities and egg mass and egg dimensions were compared using the Mann-Whitney U-test. The significance level used was $\alpha \leq 0.05$. Correlations between short and long egg axes length and total mass were analysed using the Spearman rank correlation coefficient test and the significance level used was $\alpha \leq 0.05$.

Results

The mean lengths of the females and males of the spring run were significantly greater ($p<0.05$) than those of the autumn run (Table 1). The mean total masses and gonadal masses of the females and males of the spring run were also greater than those of the autumn run (Table 1). However, the mean egg mass showed no significant difference ($p>0.05$) in the two runs (Table 1). While the sex ratio in the spring run was not significantly different ($p=0.52$), the autumn run showed a significant difference ($p = 0.02$), but the latter may be an artefact of the low number of males collected (Table 1). The condition factor in females and males in both the spring and autumn runs was very similar (Table 1).

The length-mass relationships generally showed highly positive correlations in both females and males of both runs ($r^2 > 0.7$, Figure 2), except in females of the autumn run where the positive correlation was rather weak ($r^2 = 0.52$, Figure 2b).

In the case of spring run individuals, the gonadosomatic index reached its maximum on 14 April (10.5±0.3) for females and on 31 March (6.9±1.6) for males, while in autumn run individuals the gonadosomatic index reached its maximum on 21 October (13.2±1.8) for females as well as for males (5.0±1.8), the only date that the latter were collected (Figure 3). The mean gonadosomatic index was significantly higher ($p<0.05$) in autumn run females compared to spring run females (Table 1).

Table 1. Biological characteristics of spring and autumn run *Caspiomyzon wagneri* of the Shirood River. Data presented are the mean ± standard deviation and the range is in parentheses.

Character	Spring run		Autumn run	
	♀	♂	♀	♂
Number of specimens	17	21	12	3
Total length (mm)	407.5±20.1 (380-439)	381.6±29.5 (331-447.5)	364.5±32.4 (321-427)	332.3±25.6 (304-354)
Total mass (g)	122±17.2 (92.2-148.4)	99.7±21.1 (71.1-154.1)	85.5±17.7 (66.8-120.4)	66.7±14.7 (51.2-80.7)
Gonadal mass (g)	12.2±1.9 (9.7-13.5)	6.2±1.7 (3.1-8.9)	10.5±1.7 (8.2-13.5)	3.2±1.2 (2.0-4.5)
Egg mass (mg)	0.3±0.1 (0.2-0.3)	-	0.3±0.1 (0.1-0.4)	-
Gonadosomatic Index (%)	10.1±1.1 (7.1-11.7)	6.3±1.5 (2.4-9.4)	12.6±2.8 (7.1-17.9)	5.0±1.8 (2.9-6.4)
Condition factor	0.16±0.01 (0.13-0.20)	0.16±0.01 (0.13-0.19)	0.15±0.01 (0.12-0.17)	0.17±0.00 (0.16-0.17)
Sex ratio (♂:♀)	1.2:1		0.25:1	

In the spring run specimens, the lowest number of eggs (30333) was recorded in a 388 mm long female, and the highest number (50211) in a 436 mm long female (Table 2). In the autumn run specimens, the lowest number of eggs (20435) was recorded in a 349 mm long female, and the highest (56202) in a 427 mm long female (Table 2). The absolute fecundity in spring and autumn run lampreys was not significantly different (Table 2; $p > 0.05$).

The relative fecundity was calculated based on total length as well as body mass (Table 2). In the spring run, the lowest value of relative fecundity based on total length was in a 388 mm female and the highest value was in a 399 mm female. The corresponding values in the autumn run were 349 mm and 427 mm, respectively. The relative fecundity based on total length was not significantly different between the spring and autumn runs (Table 2; $p>0.05$). In the spring run, the lowest and highest values of relative fecundity based on body mass were in females of 144.8 g and 92.3 g, respectively and in the autumn run in females of 75.6 g and 70.9 g, respectively. The relative fecundity based on body mass was significantly different between the spring and autumn runs (Table 2; $p<0.05$).

The correlations between absolute fecundity and total length in the spring and autumn run females were very similar and the data were therefore combined (Figure

4). The resulting correlation coefficient (r^2 = 0.20) shows that absolute fecundity is very weakly correlated with the total length of females.

Table 2. Absolute fecundity and relative fecundity based on body mass and total length in females of *Caspiomyzon wagneri* collected during the spring and autumn runs in the Shirood River. Data presented are the mean ± standard deviation and the range is in parentheses.

Run	Number of females	Absolute Fecundity	Relative Fecundity	
			based on body mass	based on total length
Spring	17	41076±6030 (30333-50211)	339.75±51.37 (276.42-472.61)	100.79±14.09 (78.17-125.54)
Autumn	12	36768±9267 (20435-56202)	436.31±102.14 (270.19-600.01)	100.91±23.36 (58.55±131.62)

In the spring run, the minimum and maximum values for the egg long axis length were recorded in females with total lengths of 383 mm and 418 mm, respectively, while the minimum and maximum values in the autumn run were observed in females with total lengths of 427 and 388 mm, respectively (Table 3). In the spring run, the minimum and maximum values for the egg short axis length were recorded in females with total lengths of 399 mm and 439 mm, respectively, while the minimum and maximum values in the autumn run were observed in females with total lengths of 427 mm and 388 mm, respectively (Table 3). The egg long axis length was not significantly different in the spring run versus autumn run females and nor was the egg short axis length (Table 3; $p > 0.05$).

In the spring run the length of both the short and the long egg axes showed weak positive correlations with total mass (Figure 5; $r^2 > 0.12$), while in the autumn run the length of both the short and the long egg axes showed weak negative correlations with total mass (Figure 5; $r^2 > 0.35$). However, none of these correlations were statistically significant (Figure 5; $p>0.05$).

Table 3. Egg dimensions in females of *Caspiomyzon wagneri* collected during the spring and autumn runs in the Shirood River. Data presented are the mean ± standard deviation and the range is in parentheses.

	Short axis (mm)	Long axis (mm)	Radius (mm)	Mean diameter (mm)	Volume (mm^3)	Surface (mm^2)
Spring run	0.94±0.05 (0.85-1.01)	1.10±0.05 (1.02-1.19)	0.51±0.03 (0.47-0.55)	1.03±0.05 (0.95-1.10)	0.57±0.09 (0.44-0.68)	3.31±0.33 (2.80-3.75)
Autumn run	0.93±0.07 (0.78-1.04)	1.09±0.07 (0.94-1.21)	0.50±0.03 (0.43±0.56)	1.02±0.07 (0.86-1.10)	0.56±0.11 (0.33-0.76)	3.27±0.46 (2.33±4.02)

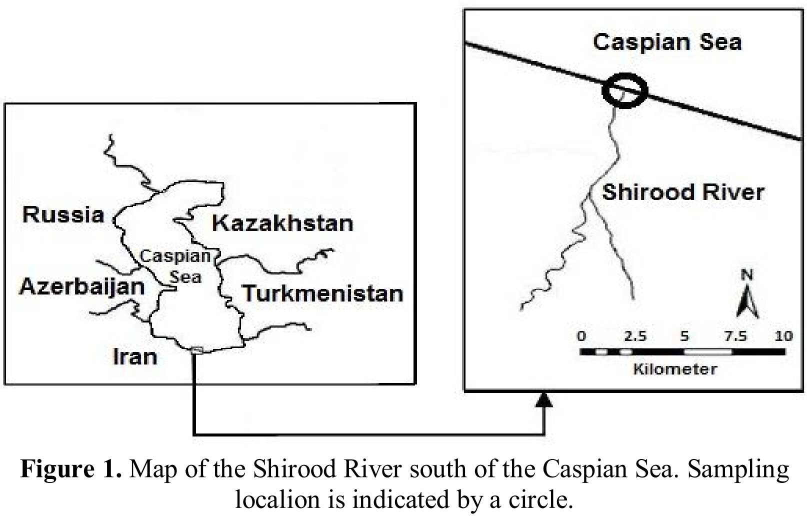
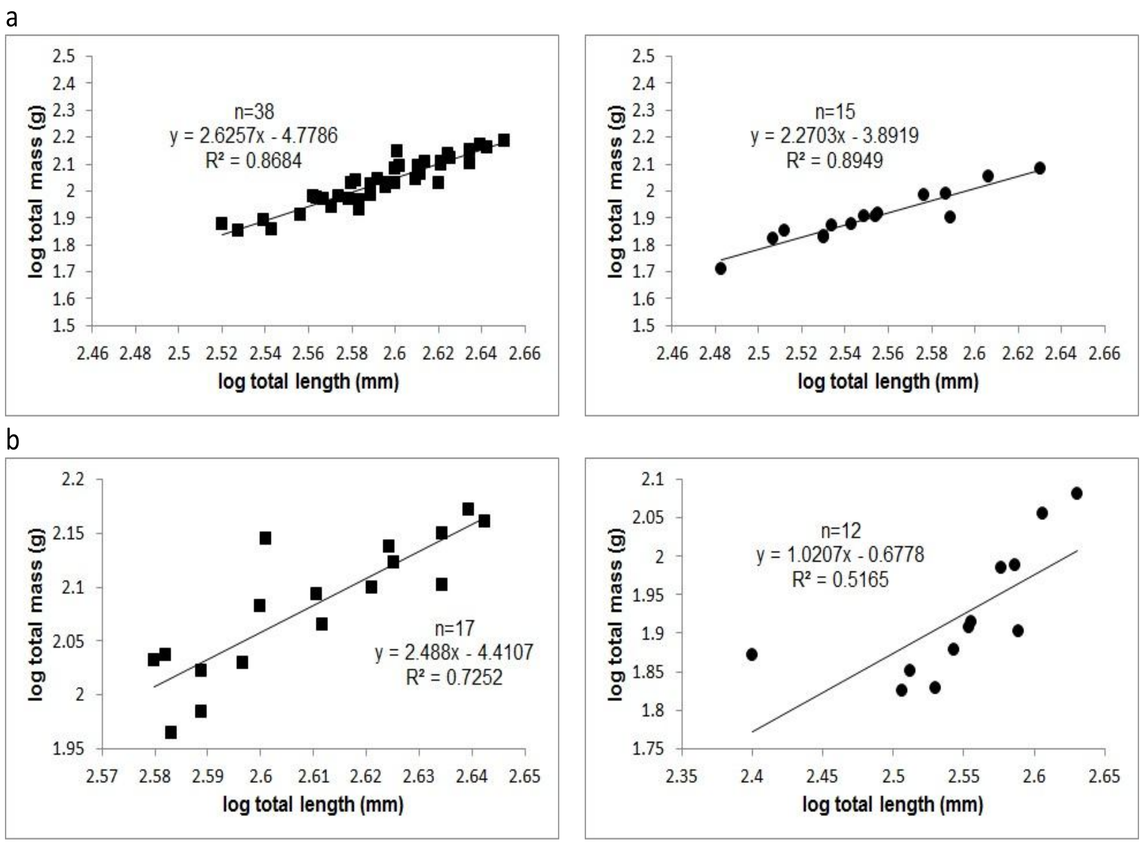


Figure 1. Map of the Shirood River south of the Caspian Sea. Sampling localion is indicated by a circle.

Other than parasites, the intestines of all specimens were empty except for a few individuals in which some digested algae were found in the posterior section of their intestine. Parasites were found attached to the inside, as well as to the outside of the intestine (Table 4). These were identified as *Corynosoma* sp. (phylum Acanthocephala). The spring run showed a much greater incidence of parasites than the autumn run. Additionally, females had a greater incidence of parasites than males in both runs.

Table 4. Number of *Corynosoma* sp. parasites associated with the intestine in *Caspiomyzon wagneri* collected during the spring and autumn runs in the Shirood River. Data presented are the mean ± standard deviation and the range is in parentheses.

		Spring run			Autumn run		
Sex (Number)		Total specimens (38)	♀ (17)	♂ (21)	Total specimens (15)	♀ (12)	♂ (3)
Number of parasites		20	10	10	1	1	0
Location	- Anterior	9	8	1	-	-	-
	- Medial	9	1	8	-	-	-
	- Posterior	1	1	-	-	-	-
	- External	1	-	1	1	1	-
Percentage Incidence		26.31	35.29	23.80	6.66	8.33	0
Infection Intensity		0.04±0.09 (0-5)	0.04±0.12 (0-3)	0.05±0.18 (0-5)	-(0-1)	-(0-1)	0



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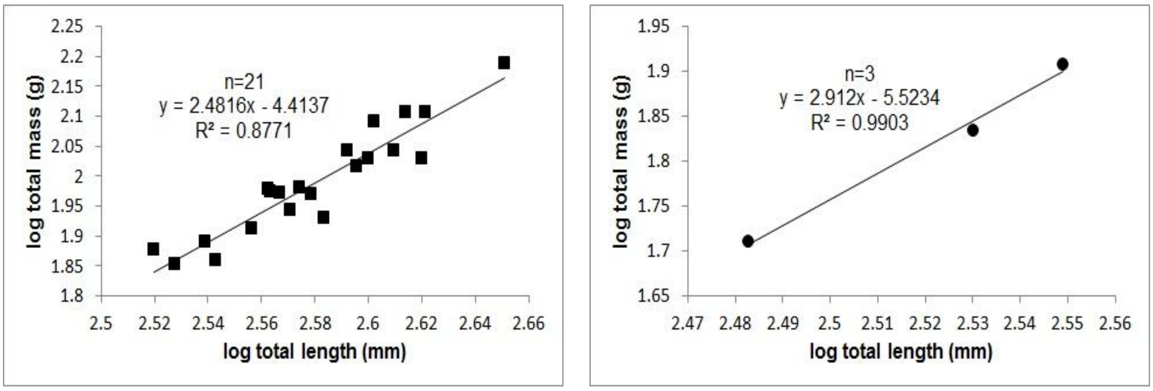

Figure 2. Relationship between total length (mm) and total mass (g) in Caspian lamprey collected during the spring (squares) and autumn (circles) spawning runs in Shirood River. a: Females and males combined, b: Females, c: Males.

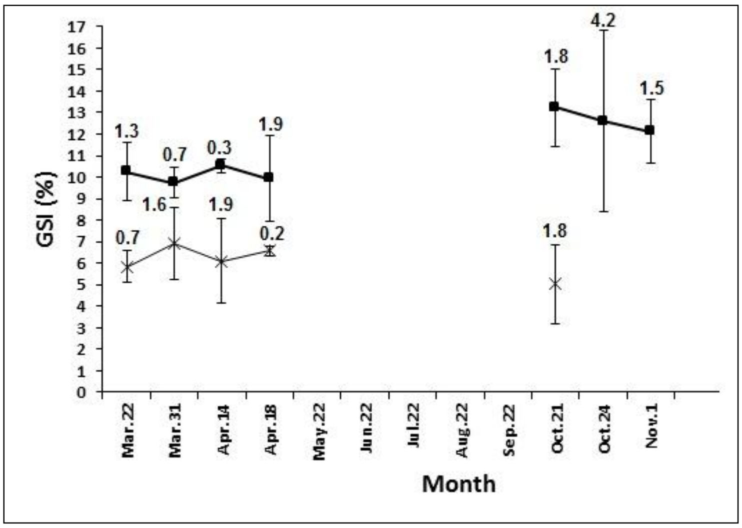

Figure 3. Gonadosomatic index of Caspian lamprey females (squares) and males (x) collected during the spring and autumn runs in Shirood River. The error bars represent ±1 standard deviation with the numerical value indicated above.

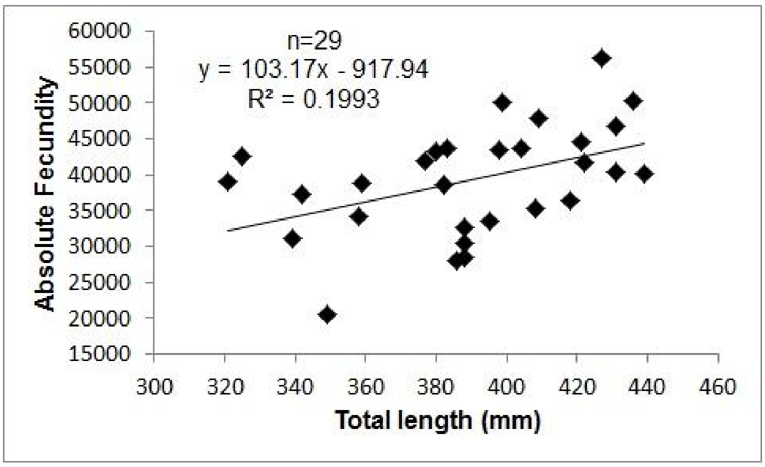

Figure 4. Relationship between absolute fecundity and total length (mm) in females of the Caspian lamprey collected during the spring and autumn runs in the Shirood River.

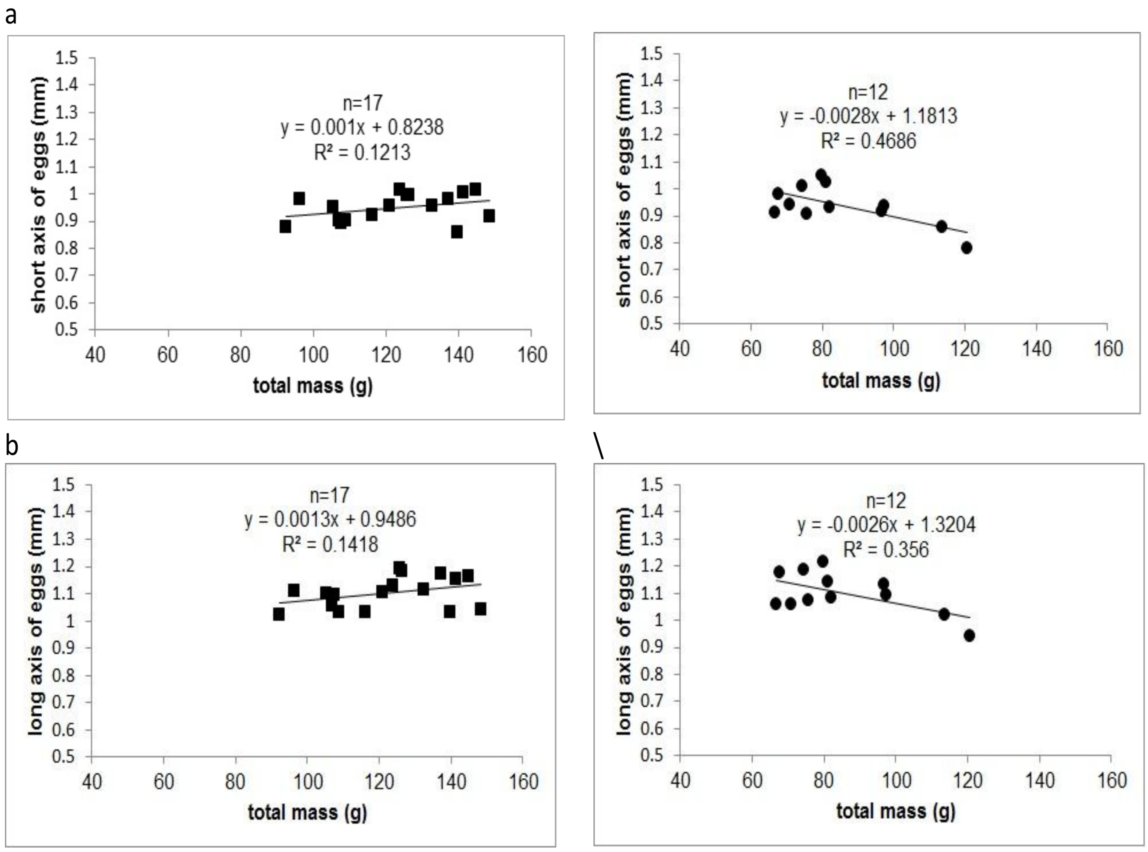

Figure 5. Relationships between the long and the short axes of the egg (mm) and the total mass (g) in females of Caspian lamprey collected during the spring (squares) and autumn (circles) spawning runs in the Shirood River. a: short axis, b: long axis.

Discussion

The occurrence in *Caspiomyzon wagneri* of two distinct upstream spawning migrations in a single river, one in spring and the other in autumn, has so far only been reported for the Shirood River in Iran (Ahmadi *et al.,* 2011; this study). Elsewhere in the Caspian Sea basin, specifically in the Volga River, Russia and in the Kura River, Azerbaijan, only one migration is reported (Holčík, 1986). Both these rivers are heavily dammed and, at least in the case of the Volga River at the Volgograd dam, this has resulted in a later shift in the peak spawning migration from late autumn (i.e., beginning of December) prior to the dam construction (Holčík, 1986) to mid-winter (i.e., mid-February) since the dam construction (Ginzburg, 1969). The timing of the peak migration up the Kura River is not known historically, but since its damming it occurs in December-January (Holčík, 1986). It is important to note that the Shirood River in not dammed.

According to this study, the sex ratio (♂:♀) in spring and autumn spawning runs of Caspian lamprey is 1.2:1 and 0.25:1, respectively, while Ahmadi *et al.* (2011) reported that the sex ratio is 1.07:1 in the spring run and 0.88:1 in the autumn run (re-calculated from the raw data presented in their paper as the ratios reported were erroneous). Nazari and Abdoli (2010), who recorded the sex ratio of only the spring run of *C. wagneri* in the Shirood River, did so weekly over a five-week period and showed that it varied from a preponderance of males in the first week (1.41:1) to a preponderance of females in the second week (0.62:1), and over the entire period the ratio was 1.07:1. The highly skewed sex ratio of 0.25:1 in the autumn run in this study is undoubtedly an artefact of low sample size, as only three males were collected. Otherwise, it appears that the sex ratio generally approaches 1:1 over the course of the migration, whether in spring or in autumn. It is interesting to note that in both our study and in Ahmadi *et al.* (2011), the number of specimens collected in the spring, 38 and 89, respectively, was much larger than in the autumn, 15 in both studies. The reason for this is not known, but it is not for lack of fishing effort in our study at least.

The overall range in total length of 304-447.5 mm for the 53 specimens in this study (spring and autumn runs combined) more closely conforms to the large form of the species, rather than the small (forma praecox) as proposed by Berg (1931, 1948). Indeed, Berg (1931, 1948) gave mean lengths of 370-410 mm and an upper limit of 553 mm for the large form and an overall range of 191-310 mm for the small form. The mean total lengths of the spring and autumn runs in the Shirood River reported in Ahmadi *et al.* (2011) were respectively 384.9±22.9 mm and 339.2±8.7 mm for males, and 384.3±28.2 mm and 334.0±19.4 mm for females. The mean shrinkage in total length of 45.7 mm in males and 50.3 mm in females between the spring and autumn runs reported in Ahmadi *et al.* (2011) is very similar to the ones reported in this study (i.e., 49.3 mm in males and 43.0 in females).

The mean total body masses of the spring and autumn runs reported by Ahmadi *et al.* (2011) were respectively 96.20±15.33 g and 68.38±13.04 g for males and 97.40±16.29 g and 69.9±12.58 g for females. The mean loss in mass of 27.82 g in males and 27.50 g in females between the spring and autumn runs reported in Ahmadi *et al.* (2011) is less, but still quite comparable to the ones reported in this study (i.e., 33.0 g in males and 36.5 g in females).

The mean gonadosomatic index of pre-spawning female Caspian lamprey in the Kura River, Azerbaijan increases progressively from 3.4 in December to 11.7 in May and in spawning female from 20.0 in June to 28.0 in July (Holčík, 1986). Nazari and Abdoli (2010) reported a range of 5.83-31.44 in the gonadosomatic index of female *C. wagneri* in the Shirood River, Iran collected between 26 March and 31 April. These values roughly encompass the range given for spawning lampreys in the Kura River. While spawning was not witnessed by Nazari and Abdoli (2010), two spent females were collected by them on 21 April indicating that spawning had occurred recently. Ahmadi *et al.* (2011) reported that the mean gonadosomatic index of females in the spring and autumn runs of Caspian lamprey in the Shirood River was 12.05 and 15.31, respectively, compared with 10.1 and 12.6 in the present study. Although the means are slightly lower in this study compared to those in Ahmadi *et al.* (2011), both studies show a significant increase in the gonadosomatic index in the autumn run relative to the spring run.

The absolute fecundity in the Caspian lamprey varies from 14000 to 60000 eggs (Berg, 1948; Ginzburg, 1969; Holčík, 1986). Nazari and Abdoli (2010) reported the range in absolute fecundity for the spring run of *C. wagneri* in the Shirood River as 31758-51198 eggs, with mean 41924. Ahmadi *et al.* (2011) reported mean absolute fecundities for spring and autumn runs of Caspian lamprey in the same river of 17778 and 20247 eggs, respectively, while this study reported considerably higher means of 41076 and 36768 eggs, respectively, with an overall range of 20435-56202 eggs. The mean value reported for the spring run in Nazari and Abdoli (2010) is very similar to the one reported in this study (i.e., 41924 and 41076 eggs, respectively), while the mean value reported in Ahmadi *et al.* (2011) is about 2.3 times less. While this difference is substantial, the values fall within the range of absolute fecundity reported for the species.

The presence of algae in the posterior section of the intestine in some of the individuals in this study confirms the earlier report by Berg (1948) and the presence of acanthocephalans of the genus *Corynosoma*, associated with the intestine of the upstream-migrating adults in this study, corroborates the findings of Ginzburg (1969) and Renaud *et al.* (2009).

Berg (1948) suggested that the Caspian lamprey in the Volga River basin consisted of a winter race and a spring race because the species spawns in the delta as well as very far upstream (circa 1500 km). Those that spawn far upstream begin as early as September their upstream migration as immature individuals and progressively mature as they approach their spawning grounds and those that spawn in the delta enter the river only in May, but are already fully mature. The situation in the Shirood River is drastically different as it only measures 36 km in length. The presence of maturing individuals in this river between March and November is therefore perplexing.

Ahmadi *et al.* (2011) examined sex steroid levels, gonadal histology and various biological indices in spring and autumn run Caspian lamprey in the Shirood River, and although they concluded that both runs were in spawning readiness, the spawning period remained unknown.

Conclusion

This study and that of Ahmadi *et al.* (2011) suggest that the autumn run is closer to spawning than the spring run is. The evidence for this is twofold: 1) males and females of the autumn run had significantly shorter total lengths than those of the spring run (mean decrease > 40 mm) and progressive shrinkage in length is a well-known and generalized phenomenon in lampreys as they approach spawning, and 2) the gonadosomatic index of females was significantly higher in the former, also indicating that spawning was more imminent. However, the evidence presented by Nazari and Abdoli (2010) suggests that the Caspian lamprey spawns in the Shirood River during the month of April, since the gonadosomatic index of females reaches its maximum on the 13 April and recently spent individuals were collected on the 21st April. If one parsimoniously assumes that a single spawning population of Caspian lamprey occurs in the Shirood River per year, then the studies of Nazari and Abdoli (2010), Ahmadi *et al.* (2011) and this study, taken in combination, suggest that its spawning period is remarkably protracted. It is important to note that spawning of Caspian lamprey has not yet been witnessed in the Shirood River. Elsewhere in the Caspian Sea basin, specifically in the Volga, Kama, and Sura rivers (Volga River basin) and in the Sakmara River (Ural River basin), all present in the northern part of the Caspian Sea basin, the reported spawning period is from mid-March to mid-July (Berg, 1931, 1948). If we assume that the spawning period in the Shirood River is likewise from early spring to early summer, then the implication is that the adult lampreys in the river remain in reproductive stasis from late October to mid-April. In order to test the hypothesis that spawning occurs over a protracted period of time in the Shirood River, spawning sites need to be searched for higher upstream in this river over the entire year, particularly in areas where suitable spawning substrate of gravel and sand occurs.

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