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Sex steroids, gonadal histology and biological indices of fall and spring Caspian lamprey (*Caspiomyzon wagneri*) spawning migrants in the Shirud River, Southern Caspian Sea

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Abstract This study investigates sex steroids, gonadal histology and some biological indices of fall and spring spawning migrants of Caspian lamprey *Caspiomyzon wagneri* (Kessler, 1870). Blood and gonad samples were collected from 15 migrants during fall and spring. Serum sex steroid levels including testosterone (T), 17β-estradiol (E_2) and progesterone (P) were determined with ELISA and gonadal development was studied using conventional histological methods. Our results showed serum E_2 level in females were similar in the two seasons but in males, fall migrants had higher serum E_2 concentrations. No differences were found in T levels in fall and spring migrants. Serum P concentration in fall migrant males were significantly higher than spring migrant males while spring migrant females had higher serum P levels compare to fall migrant females. Gonads in both fall and spring migrants were in the final stage of maturity. Fall and spring migrant males had similar HSI and GSI; fall migratory female had significantly higher HSI and GSI. Sex ratios were close to 1:1 in both seasons. There was a significant relationship between fecundity and length in both seasons. Comparison of fecundity and egg diameter between fall and spring seasons showed no significant difference. These results indicate that both fall and spring migrants Caspian lamprey were ready for spawning. Further studies are needed to clarify when spawning occurs in Caspian lamprey.

Keywords Caspian lamprey · Sex steroids · Gonads histology · Migration

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Introduction

The Caspian lamprey, *Caspiomyzon wagneri* (Petromyzontidae), is endemic to the Caspian Sea and rivers in its northern, western, and southern watersheds (Holčík 1986). Larval Caspian lamprey inhabit freshwater rivers and migrate to the Caspian Sea for feeding after a radical metamorphosis to the juvenile phase. When sexual maturation approaches, adults migrate back to the rivers for reproduction (Holčík 1986). The Caspian lamprey migrates to the Volga, Ural, Terek, and Kura rivers (Coad 2008) and in the southern Caspian basin (Iran) migrates to rivers such as the Shirud, Talar,

Babolrud, Gorganrud, Tajan, Haraz, Sardabrud, Aras, Tonekabon, Polrud, Sefidrud and Anzali Lagoon (Nazari and Abdoli 2010).

Migration strategies are different among lamprey species. *Lampetra tridentata* and *Geotria australis* have a long migration (15–16 months). Those species migrate into fresh water and move upstream to spawn from May to September, overwinter, and spawn in early spring the following year (Beamish and Levings 1991; Kelso and Glova 1993). But sea lamprey (*Petromyzon marinus*), river lamprey (*Lampetra fluviatilis*) and Caspian lamprey have a short freshwater upstream migration phase (3–4 months) (Potter and Beamish 1977; Holčík 1986; Lucas et al. 2001; Nazari and Abdoli 2010). Optimum environmental conditions, a long migration to reach spawning sites, and higher metabolic status (Holčík 1986; Lucas et al. 2001) cause variations in lampreys migration time. Holčík (1986) suggested that barriers changed migration time (from early December to February) in the Caspian lamprey.

Upstream migration of Caspian lamprey in the Shirud River begins about the middle of March and finishes late-April in spring and also takes place from the middle of September to late-October in the fall season (Nazari and Abdoli 2010, pers. obs.). The autumn migrants represent an initial period of activity that is halted by low winter temperatures (Lucas et al. 2001). *C. wagneri* spawns from late-March to the beginning of July depending on the geographical location of the river and the distance of the spawning sites from the estuary (Koblitskaya 1981; Holčík 1986).

There is a lack of information about the migration and reproductive biology of Caspian lamprey in the southern Caspian Sea rivers, and few studies have been conducted there. There is no detailed information available about differences in reproductive physiology of Caspian Sea spring and fall migrants. Although we generally know that lamprey gonads are morphologically similar to those of teleost fishes and that they undergo the same developmental processes, including vitellogenesis and oocyte final maturation in females, and spermatogenesis and spermiation in males (Bryan et al. 2008), no published information is available about Caspian lamprey in this regards. Concentrations of certain steroids, such as 17β-estradiol (E_2) and progesterone (P), are correlated with the reproductive stage (e.g., ovulation and spermiation) and behavior in lampreys (reviewed by Sower 2003). Information on sex steroid levels during spawning migration may increase our understanding of the reproductive and migratory behavior of lampreys (Mesa et al. 2010). Thus, the goal of this study was to investigate the variation of reproductive indices such as gonadal stages and also serum sex steroid levels in Caspian Sea fall and spring migrants.

Materials and methods

We sampled lampreys from late-March to the middle of May for the spring season, and from late-September to early-November for the fall season in 2008. Sampling was done biweekly. Lampreys were caught by hand, under Shirud Bridge (~200 m upstream from the river mouth, 34° 44 ′–34° 51′ N, 50° 48′–50° 49′ E; Fig. 1). Adult lampreys migrated upstream exclusively at night (Nazari and Abdoli 2010).

Blood was collected from the caudal veins of spring migrants (n=16, 7 females 9 males) and fall migrants (n=15, 8 females 7 males) using heparinized vacutainer. Plasma was separated by centrifugation (3,000 rpm for 20 min) and stored at −20°C until steroids (T, E_2 and P) were extracted and analyzed by

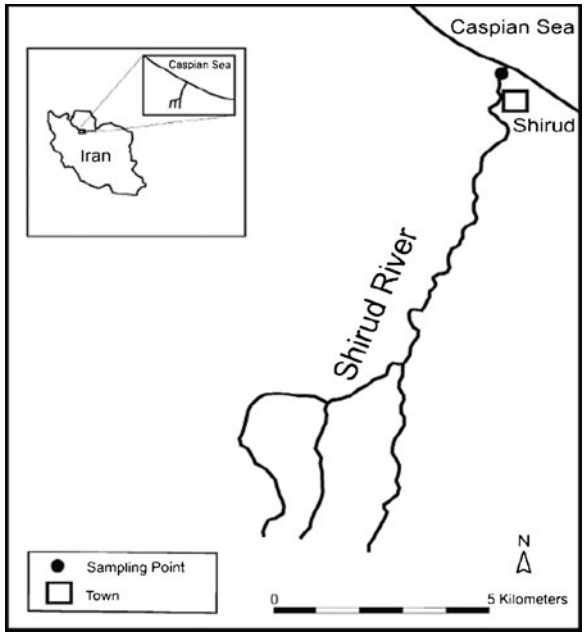

Fig. 1 Map of the study area in the Shirud River

the ELISA method using commercial kits (Diagnostics Biochem Canada Inc). Parts of gonads were removed and then were processed through routine paraffin embedding. After embedding in paraffin we carried out thin sectioning at 6 μm, and stained with Delafield's hematoxylin and Eosin (Luna 1968). The stages of gonadal development were described according to Mojazi Amiri et al. (1996) and Robillard et al. (2008).

The specimens (spring migrants n=89, 43 females 46 males and fall migrants n=15, 8 females 7 males) were preserved in 10% formalin after blood sampling. Total length (L±0.01 cm) and total mass (W±0.01 g) were measured. The length–weight relationships were determined according to the allometric equation (Bagenal 1978):

$$W = aL^b$$

After incision through the abdominal musculature and sex determination, the gonads and liver were removed from the body cavity, blotted dry, and a mass was determined to the nearest 0.01 g using an analytical balance. The gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated using the following equations (Bagenal 1978; Fukayama and Takahashi 1985):

$$\mathrm{GSI} = \left(\text{gonad weight} \times \text{body weight}^{-1}\right) \times 100$$
$$\mathrm{HSI} = \left(\text{liver weight} \times \text{body weight}^{-1}\right) \times 100$$

The individual absolute fecundity (F) was determined by the counting–weighing method, the number of eggs in 1 g (n) was counted. Then fecundity was determined according to the total weight of ovary (W) and weight of eggs in 1 g (w) by the following formula (Kucheryavyi et al. 2007):

$$F = nW\,w^{-1}$$

Total length was used as the measure of size in relation with fecundity (Docker and Beamish 1991).

The maximum (to the nearest 1 μm) egg diameter was determined using a binocular Nikon microscope at 4× magnification. We measured 40 eggs from each ovary.

Data analysis

SPSS version 9.0 (SPSS Inc., Chicago, IL, USA) and Excel 6.0 were used for statistical analyses. Significant differences in the sex ratio were estimated with the Chi-Square test. Significant differences in mean length and weight in both sexes were determined with the Student's t-test and analysis of Covariance, respectively. Mean data were compared using ANOVA test. T-test was used to compare serum steroids levels between sexes and seasons. Data are expressed as mean values ± SD. P-values <0.05 were considered significant.

Results

Hormonal data

Concentrations of serum E_2, T, and P in fall and spring migrants of Caspian lamprey are presented in Fig. 2.

Histological observation

The final maturation in fish is the resumption of meiosis and is reflected morphologically by the migration of the germinal vesicle (GV) and its breakdown in females, as well as generation of spermatozoa from spermatocytes in males (Yaron and Sivan 2005). We found that 25% of fall female migrants had central GV oocytes, 62.5% had migrating GV oocytes, and 12.5% had peripheral GV oocytes. We foundthat 14.3% of spring female migrants had central GV oocytes, 71.4% had migrating GV oocytes, and 14.3% had peripheral GV oocytes. Migrating GV oocytes and peripheral GV oocytes showed GV migration in both fall and spring migrants. All male migrants in the fall had testes full of spermatozoa, but in the spring 66.66% of male migrants had testes full of spermatocytes and 33.34% had testes full of spermatozoa. It is important to note that in the present study in both migrants GV break down, ovulation and sperm ejaculation were not observed.

GSI (Gonadosomatic index), HSI (Hepatosomatic index), total length, total weight, fecundity and egg diameter

The GSI of fall migrants were 8.56±4.54 (males) and 15.31±2.86 (females) and for spring migrants were 6.45±2.10 and 12.05±2.78 for males and females,

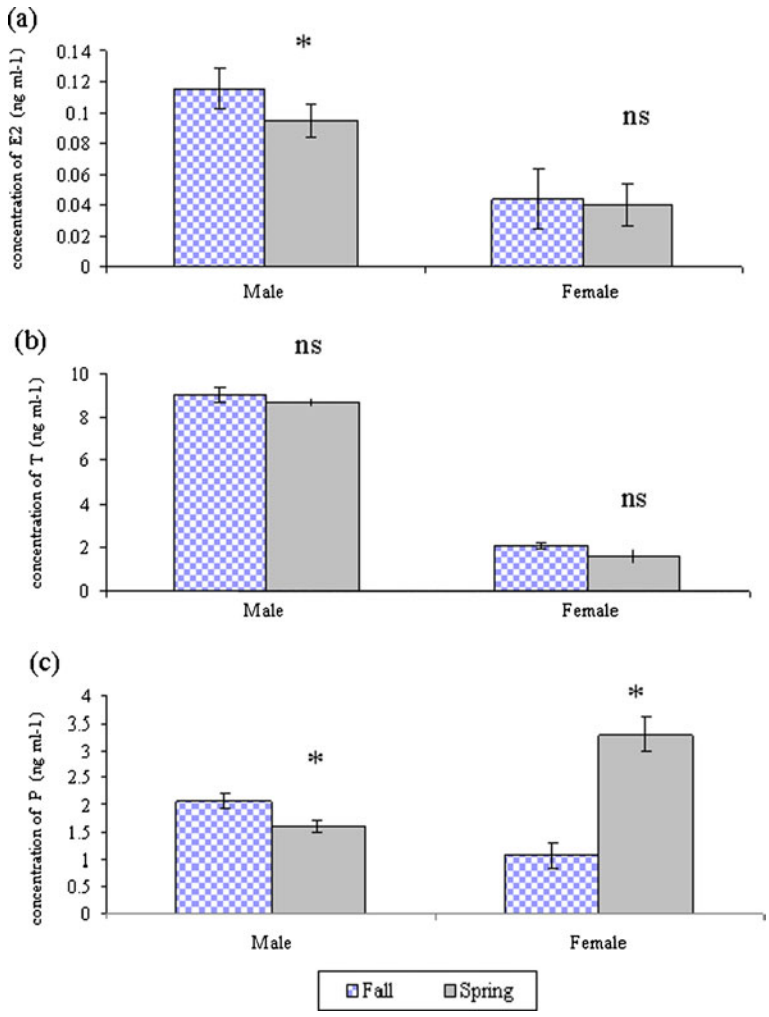

Fig. 2 Serum E_2 (**a**), T (**b**) and P (**c**) levels (mean ± SD) in fall and spring migrant of Caspian lamprey (ng ml^{-1}) (*=significant, *ns* not significant)

respectively. The HSI of fall migrants were 1.03±0.20 for males and 1.77±0.39 for females and for spring migrants were 0.98±0.23 and 1.21±0.30 for males and females, respectively. The GSI and HSI were significantly higher in the fall. Total lengths of Caspian lampreys were 33.63±1.05 for fall migrants (male=33.92±0.87 cm, female=33.40±1.94 cm) and 38.46±2.56 cm for spring migrants (male=38.49±2.29 cm, female=38.43±2.82 cm). Total weights of Caspian lampreys were 69.20±12.15 for fall migrants (male=68.38±13.04 g, female=69.9±12.58 g) and 96.79±15.73 g for spring migrants (male=96.20±15.33 g, female=97.40±16.29 g). There were no significant differences between body length and body weight of Caspian lamprey in the two seasons. Length (log)-Weight (log) relationship equations of fall and spring migrants of Caspian lamprey are presented in Eqs. 1 and 2, respectively.

$$\mathrm{Log(W)} = 2.2013(\mathrm{Log(L)}) - 3.5116 \quad (1)$$

$$\mathrm{Log(W)} = 1.8618(\mathrm{Log(L)}) - 2.2332 \quad (2)$$

Comparing the length-weight relationships of the fall and spring migrants using covariance analysis, no significant difference was found; but positive correlation was observed between total length and weight in both seasons (R^2=0.43 for fall migrants, R^2=0.60 for spring migrants).

The individual absolute fecundity of spring and fall migrants was 17,778±5,377 and 20,247±6,417, respectively. The egg diameter of fall migrants was

710±0.233 μm, and of spring migrants were 800± 0.709 μm. Fecundity (log)—Length (log) relationship equations of fall and spring migrants of Caspian lamprey are presented in Eqs. 3 and 4, respectively.

$$\mathrm{Log(F)} = 4.7704(\mathrm{Log(L)}) - 6.9823 \quad (3)$$

$$\mathrm{Log(F)} = 1.6942(\mathrm{Log(L)}) + 3.6715 \quad (4)$$

There was a significant positive correlation between absolute fecundity and total length (R^2= 0.88 for fall migrants, R^2=0.19 for spring migrants). Comparison of fecundity and egg diameter between fall and spring migrants showed no significant difference.

The overall sex ratio (males:females) in spring and fall was 0.94:1 and 1.14:1, respectively. Sex ratios were close to 1:1 in both seasons.

Discussion

Sex steroids play a key role in lamprey maturity (Sower 2003). In both the sea lamprey and Arctic lamprey (*Lampetra camtschatica*) E_2 concentrations increased during spermiation (Fukayama and Takahashi 1985; Sower et al. 1985; Fahien and Sower 1990) and decreased during ovulation (Sower et al. 1985; Linville et al. 1987; Bolduc and Sower 1992). Our results showed higher concentrations of E_2 in fall males than spring males. Based on histological results, GV migration and spermatozoa generation indicate that gonads in both fall and spring migrants were in an advanced stage of maturity. This may indicate a possible role of E_2 in final maturation of the Caspian lamprey.

T concentrations show a positive relationship with gonadal development stage in river lamprey (*Lampetra fluviatilis*) (Kime and Larsen 1987) and brook lamprey (*Lampetra planeri*) (Seiler et al. 1985). Arctic lamprey pre-spawning males have higher T level than pre-spawning females (Fukayama and Takahashi 1985). Sower et al. (1985) reported that there were no significant differences between serum T levels in female and male in sea lamprey. Likewise, T levels are similar in male and female of river lamprey (Kime and Larsen 1987). Based on our histological study, all migrants were in the advanced stage of maturity, and no difference in T concentrations was found between fall and spring males and between fall and spring females, so it seems that T is not involved in final maturation of Caspian lamprey.

Linville et al. (1987) reported that P levels: (1) did not change significantly at different stages of maturity in female sea lamprey; (2) were not correlated with various reproductive behavior in either sex; and (3) were, on average, higher in males during final reproductive development. In contrast, Bolduc and Sower (1992) reported elevated levels of P in female sea lampreys at the time of spawning. Our histological results indicated both fall and spring migrants were ready for spawning. Our histological study indicated the fall and spring migrants were in an advanced stage of maturity. Differences of P levels between fall and spring male migrants and fall and spring female migrants may indicate a specific role of P in final maturation of Caspian lamprey.

We compared the biological indices of Caspian lamprey migrants in fall and spring season that we investigated in this study to other reports. Total length of males and females reported from the Volga River were 36 and 36.9 cm, respectively (Dyuzhikov 1956), from the Kura River 42.6 to 43.2 cm for males, and 43.6 to 44 cm for females (Smirnov 1952), and from the Shirud River for males and females 38.34±3.06 and 38.65±4.49 cm, respectively (Nazari and Abdoli 2010). We observed similar total length ranges (fall migrants=33.63±1.05 cm, spring migrants=38.46±2.56 cm) of males and female lampreys in Shirud River as Nazari and Abdoli (2010) reported.

The GSI of female lampreys have been reported for pre-spawning stage from 2.67 to 11.7 and for spawning stage from 12.12 to 33.55 (Pravdin 1913a, b; Smirnov 1953; Holčík 1986; Noori 1990; Nazari and Abdoli 2010). In fully ripened females GSI value increase up to 28–29 (Holčík 1986). Fukayama and Takahashi (1985) observed that during the upstream migration period, mean GSI values in female Arctic lamprey increased gradually as vitellogenesis progressed, and reached 14.03± 1.49 in April when ovarian oocytes were close to the end of exogenous vitellogenesis. The GSI in the present study for fall and spring males were 8.56± 4.54, 6.45±2.10, and for fall and spring females were 15.31±2.86 12.05±2.78, respectively.

Caspian lamprey fecundity varies from 14,000 to 60,000 eggs (Pravdin 1913a, b; Smirnov 1953; Ginzburg 1969; Holčík 1986; Noori 1990). Also Nazari and Abdoli (2010) reported fecundity for these species as 31 758 to 51 198 eggs. However our results were in agreement with Abdoli and Naderi (2009) who reported that fecundity varied from 17,000 to 21,000. Our results showed significant correlation between the total length and the absolute fecundity of Caspian lamprey, However, Noori (1990) and Nazari and Abdoli (2010) reported no such significant correlation.

The sex ratio of Caspian lamprey has been reported to show a predominance of males. The sex ratio (male: female) of this species reported in the Volga River ranged from 1.13:1 to 1.94:1 (Pravdin1913a, b; Dyuzhikov 1956; Ginzburg 1969; Holčík 1986) and in the Babolrud, Talar and Shirud Rivers ranged from 2.33:1, 2.97:1 and 1:1, respectively (Noori 1990; Ghasempouri 1993; Nazari and Abdoli 2010). In this study, we found a sex ratio around 1:1. However Smirnov (1953) reported that the numbers of each sex are actually more or less equal as the males predominate at the beginning of the migration, and the females during the later part. Also the population density is not stable and fluctuates at 2 to 4 year intervals. This periodicity is apparently related to the short life cycle of this species (Holčík 1986).

Further studies are needed to clarify when spawning occurs in fall and spring migrants of the Caspian lamprey. Such basic information on physiology and reproductive biology increases our understanding of the life history of the Caspian lamprey. Investigation of the potential correlations between physiological status and the tendency to migration and spawning in these species is an obvious area of future research.

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