

Comparative study on the gastrointestinal tract of mature and maturing female and male of Caspian lamprey, *Caspiomyzon wagneri* (Kessler, 1870) in the southern Caspian Sea (Cephalaspidomorphi: Petromyzontidae)

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Abstract: Present study was conducted to elucidate the nutritional value and histological events of gastrointestinal tract (GT) between mature and maturing female and male Caspian lamprey (*Caspiomyzon wagneri*) during their spawning migration to the freshwater rivers in southern Caspian Sea (Shirud River, Mazandaran Province, Iran). From the upstream migrating Caspian lamprey population, 60 fish specimens were randomly collected. After checking the intestine content, samples from intestine and liver tissues were removed for histological studies for two groups of mature and maturing specimens either in female or male separated based on external and internal sexual characteristics. According to the histological observation, many folds, connective tissues, interstitial fluid, goblet-shaped cells, enterocytes and numbers of intestinal villi were observed in the intestine. Also, there was an intermediate structure named “typhlosole” in the entire intestine. Furthermore, height and folds of the typhlosole were smaller at the beginning and the end of it. Significant differences were observed in the numbers of fold of intestine in mature and maturing females ($P < 0.05$). Hepatocytes were consisted the majority of liver cells in mature and maturing females, whereas in the male group it was totally dwindled and gone.

Keywords: Shirud River, Mazandaran, Histology, Digestive system, Maturity, Feeding.

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Introduction

Lampreys (Petromyzontiformes) are an ancient vertebrate group comprising about 40 recognized species classified in 3 families and 10 genera which are distributed throughout the Northern and Southern hemispheres (Renaud 2011; Hume 2012). Despite a conserved morphology, lampreys show a diverse range of life history strategies (Hume 2012) that might be present at the histology level. Classification of lampreys based on nutritional habits has not been completely emphasized. Based on available

literature, 18 species are diagnosed as parasites when adult and others are considered as non-parasitic (Renaud 2011). Parasitic lampreys feed on blood and body fluids of the host fish and non-parasitic lampreys do not feed at all when mature (Jenkins & Burkhead 1993). These fish, depending on the species, will be external parasites, scavenger, carnivorous hunter or mix feeder (Hume 2012). The feeding of lampreys (either parasitic or non-parasitic) during the spawning migration depends entirely on gonadal development and body recourses. Thus, the

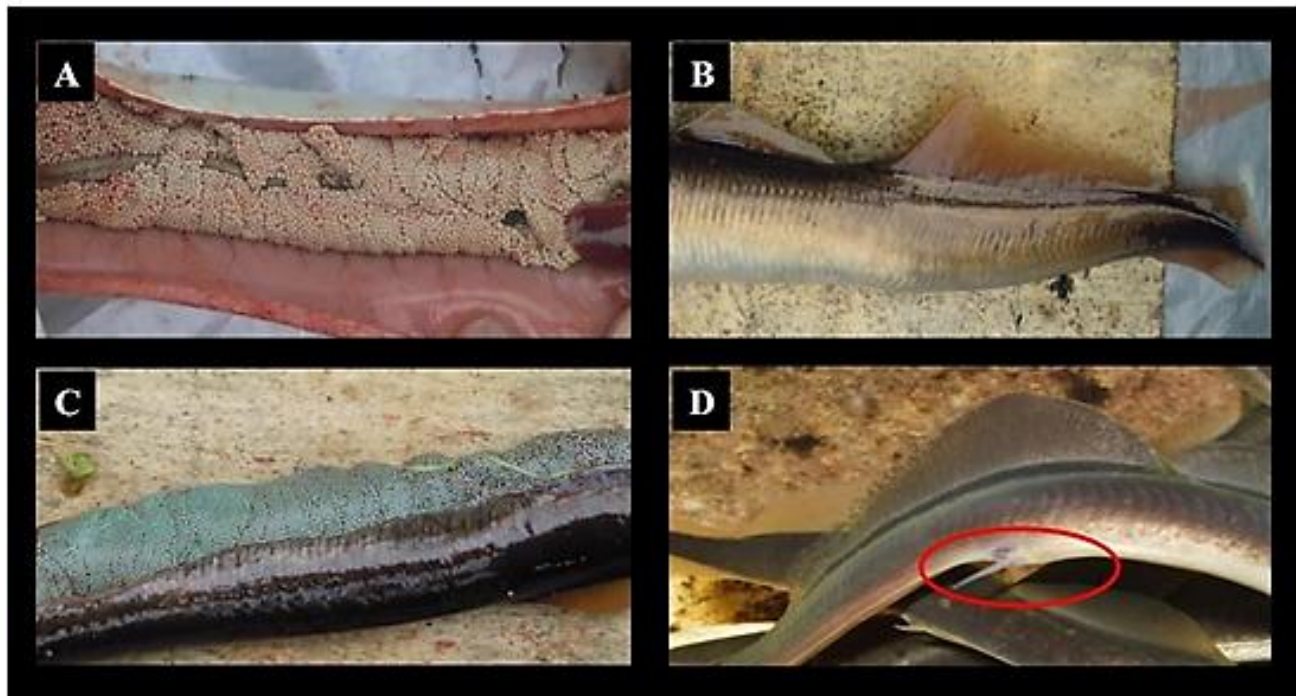


Fig.1. Caspian lampreys. (A): maturing female, (B): maturing male, (C): mature female, and (D): mature male (papillae).

migration of lampreys to the upstream parts is accompanied by physiological, morphological and behavioral changes (Imanpoor & Abdolahi 2011). Caspian lamprey, *Caspiomyzon wagneri*, is one of the anadromous species of the Petromyzontiformes. This non-parasitic species migrates for spawning two times a year i.e. in spring and autumn (Abdoli et al. 2009; Ahmadi et al. 2011), which migrates to some rivers in southern Caspian Sea e.g. Gorganrood, Tajan, Talar, Babolrood, Kheirood, Haraz, Sardabrood, Tonekabon, Shiroom, Nesarood, Chalkrood, Sefidrood, Aras rivers and Anzali lagoon (Nazari & Abdoli 2010; Ahmadi et al. 2011; Vatandoust et al. 2015; Jouladeh-Roudbar et al. 2015).

Caspian lampreys are usually divided into two maturing and mature cohorts during their spawning migration to the freshwater rivers of southern Caspian Sea. Mature fish show obvious secondary sexual characteristics and female and male can be easily recognized externally. Additionally, fish eggs and sperm are fluidic and release after a mild pressure on the abdominal area (Farrokhnejad et al. 2014), while maturing fish do not show secondary sexual

features and also not a long time has been passed since their start of migration from estuary to upstream. However, data regarding dietary and digestive system and also structural and histological characteristics of gastrointestinal tract (GI) of this species (mature/maturing male and female) is scarce. Since this species is listed as endangered species on red book of U.S.S.R (Coad 2005), and also reported “near threatened” according to the standards of the IUCN in the southern part of the Caspian Sea (Kiabi et al. 1999; Mostafavi 2007), cognition of different aspects of biology such as nutritional and histological changes in GI, especially during spawning period in different sexes is very important. Therefore, the present study was conducted to elucidate nutritional and histological statuses of GI in mature and maturing female/male Caspian lampreys.

Material and Methods

In the spring (March & April) 2013, a total of 60 specimens were caught 200m upstream from the river mouth, under the Shiroom river bridge (34°44'-34°51'N, 50°48'-50°49'E) by hand during their spawning migration. Genders of fishes were

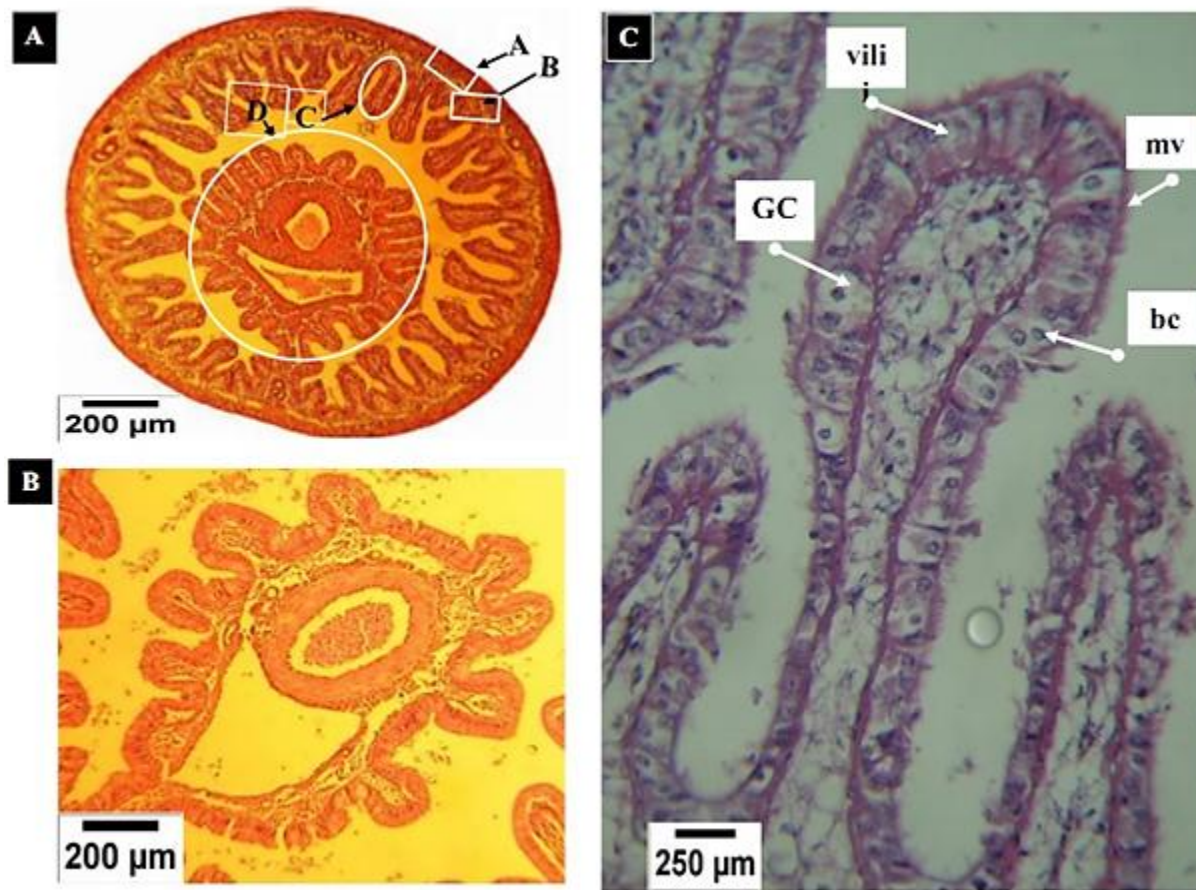


Fig.2. Optical microscope images of intestine sections of Caspian lampreys (thickness of 5 to 7 μm , H&E staining). (A) tissue sections of overall intestine structure; A: connective tissue, B: interstitial fluid, C: intestinal folds, D: typhlosole, (B): tissue sections of typhlosole overall structure and (C) tissue sections of intestinal folds, vili: villi, mv: micro villi, bc: basophiles, GC: mucous-like cells (Goblet-like cells).

determined based on secondary sexual characteristics and confirmed by autopsy and finding testis and ovary. Maturation status was recognized according to the ovary or testes external feature and also histological indices of gonads (Farrokhnejad et al. 2014).

With reference to fish with soft abdomen and filled with ovary in entire cavity, round and sticky oocytes and blue color, considered as mature and those with hard pressed abdomen and greenish- olive yellow ovary considered as maturing female. The fish with a well-developed sexual papillae and sperm ejaculation with a mild pressure on abdomen, was considered as mature male and those with firm abdomen and without secondary sexual characteristics deemed as maturing male (Fig. 1).

Fish anesthetized in 200ppm clove oil solution,

after biometry, parts of intestine and liver were removed and fixed in 10% formaldehyde for 48 hours and prepared for histological sections were prepared based on Mojazi Amiri et al. (1996) and Eagderi et al. (2013). Images of histological sections obtained from at least 3 samples of each fish after H&E staining. In order to verify digestive system contents, the colon completely rinsed with distilled water and its contents transferred to micro tubes. In order to prevent the degeneration, a few drops of pure formaldehyde added to each tube which then were used to calculate the contents dry weight after dried by Freeze-dryer machine and weighted to the nearest 0.001g.

For calculating numbers and the heights of intestinal folds respectively, 36 and 12 intestinal images of each group (mature and maturing males

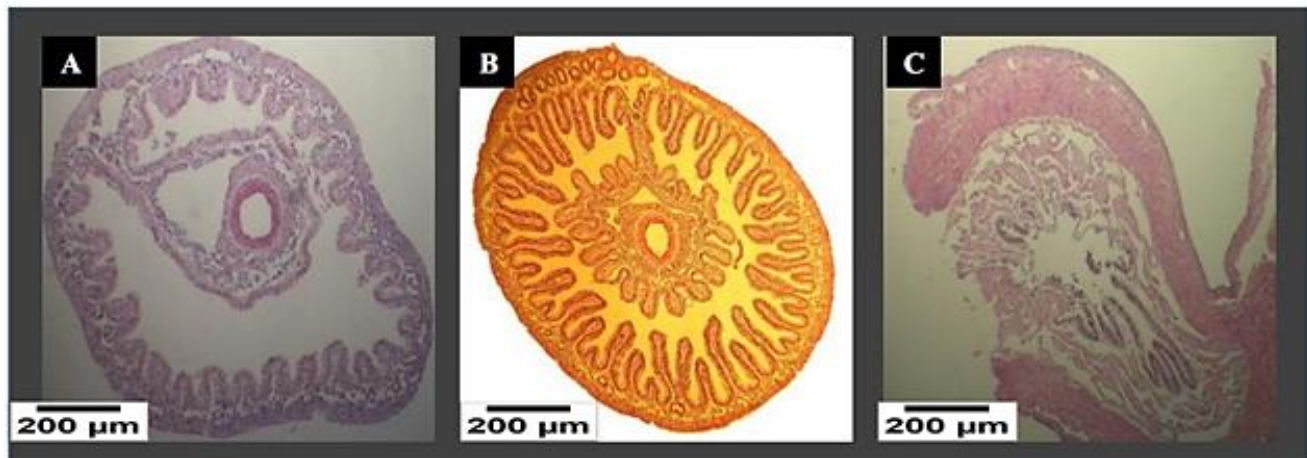


Fig.3. Optical microscope images of intestine section of Caspian lampreys (thickness of 5 to 7µm, H&E staining). (A): interior, (B): middle, and (C): posterior.

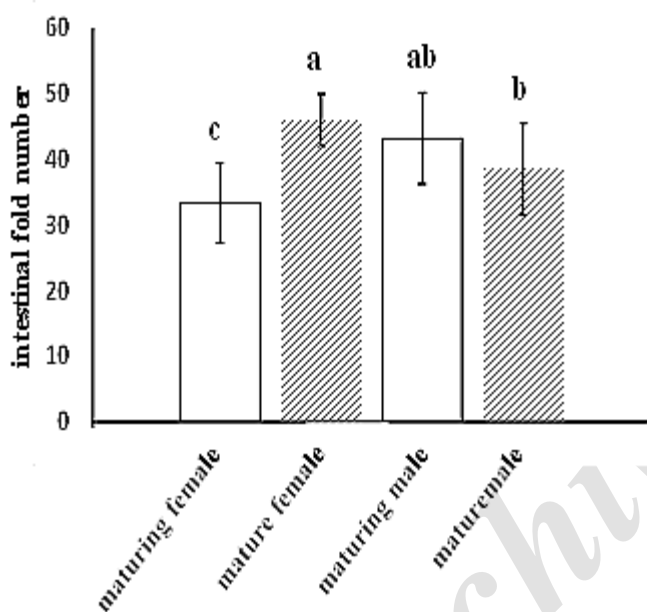


Fig.4. Intestinal fold number of Caspian lampreys in spring.

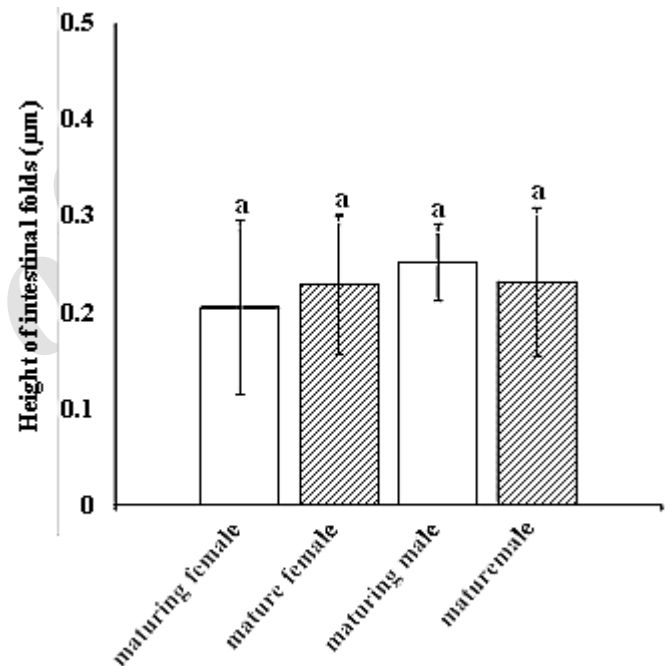


Fig.5. Height of intestinal folds of Caspian lampreys in spring (µm).

and females) randomly selected and the number of folds and intestinal protrusion height were measured using ImageJ software. For drawing charts, Excel 2010 was used and for statistical analysis t-student test was performed using SPSS version 19.

Results

Histological features of intestine: Histological observations on Caspian lampreys showed that there is no differences compared to teleost fishes in terms of the general stomach and structure of intestine. There was a middle structure namely typhlosole in

the intestine of the studied lampreys (Fig. 2). The intestine structure was consisted of connective tissue, interstitial fluid, mucous-like cells (goblet-like shape) and basophilic cells (enterocytes, Fig. 2). No significant differences were found between anterior, middle and posterior parts of intestine. Although, it was clear that the height of intestinal folds in interior and posterior parts was relatively lower than middle part and typhlosole progression (Fig. 3).

Digestive system content: The intestine of specimens, was mainly empty, and has no food, but

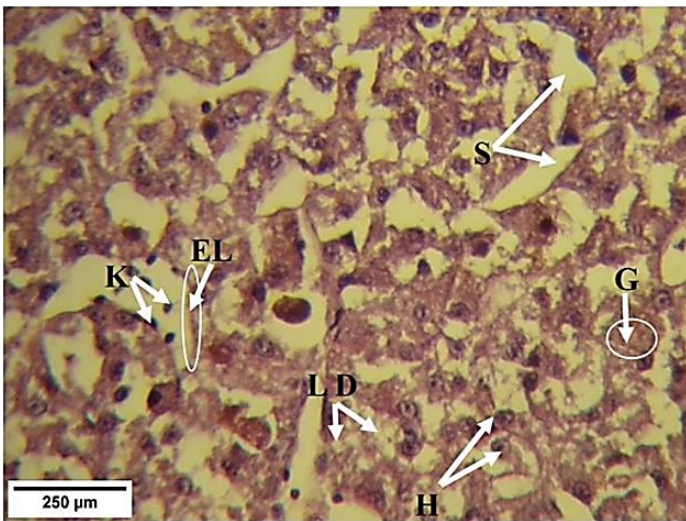


Fig.6. Microscope images of tissue sections of Caspian lampreys with a thickness of 5 to 7mm (H&E staining). S: sinusoids, H: hepatocytes, G: glycogen, EL: endothelial layer, LD: fat ditches, and K: macrophage cells (Kupffer cell).

five of them including 3 maturing and 2 mature fish, had a little food content that its average dry weight was 11.6 ± 0.72 and 9.8 ± 0.28 mg in maturing (male and female) and mature (male and female) fish, respectively.

Average numbers of intestinal folds in maturing females (33.33 ± 6.04) was significantly ($P < 0.05$) lower than those of mature females (46 ± 3.96). Calculated values of the intestinal fold number between maturing females and males, and also between mature females and males showed a significant differences ($P < 0.05$) (Fig. 4).

Average height of the intestinal folds in maturing and mature females was 0.205 ± 0.09 and 0.229 ± 0.072 μm and in maturing and mature males was 0.252 ± 0.04 and 0.232 ± 0.077 μm, respectively (Fig. 5).

Liver: Liver tissue is composed of the hepatic cells mainly with a clear nucleus and a glycogen accumulation. The endothelial layer was observed at the periphery of sinusoids (sinuses) in macrophage cells and lipid vacuoles were observed clearly as white globules between cells (Fig. 6).

In mature and maturing females, the liver has atrophy and was completely depleted so that none of the liver cells could not be identified; this is despite

the fact that this situation was not observed in males (Fig. 7).

Discussion

The results indicate the presence of two main parts in intestine of Caspian lampreys, including intestine wall-folds and typhlosole structure and also main gastrointestinal cells (mucous-like cells and basophiles). Existence of typhlosole in gastrointestinal tract of some other species of Petromyzontidae has been reported (Norman 1931; Lagler et al. 1977; Sterling et al. 2012). Typhlosole structure in Petromyzontidae is due to enhancing higher intestinal surface to volume ratio which has an important role in the absorption of nutrients in the intestine (Norman 1931; Lagler et al. 1977; Langille & Youson 1985) and its great development in intestinal ditch is the reason for reducing the entry of food content, especially during fish migration. In sea lamprey (*Petromyzon marinus* L.), reduction of intestine diameter, typhlosole extent and increase of the height of intestinal folds during metamorphosis is assumed associated with increasing in nutrition (Langille & Youson 1984). Existence of relatively high altitude marked folds in the colon is also another reason that indicates mentioned species in migration period doesn't have much nutrition activity (Sterling et al. 2012). Also observed large numbers of intestinal microvillus in the present study in mature male or female on the surface of intestinal folds indicated an increase in the absorption of nutrients in this organ in the fish (Pahlavan-Yali 2001).

According to the results, the intestine of studied specimens was empty. A few fishes showed some intestinal contents that were unidentifiable because of their complete decaying (an evidence for a long time fish hunger). These trace amounts of calculated dry weight, also showed no significant differences between the studied groups. In the light of foregoing, in line with the results of this study, this fish's lack of long-term feeding partly confirmed (Renaud 1982; Imanpoor & Abdolahi 2011)

The fact that no significant differences in the

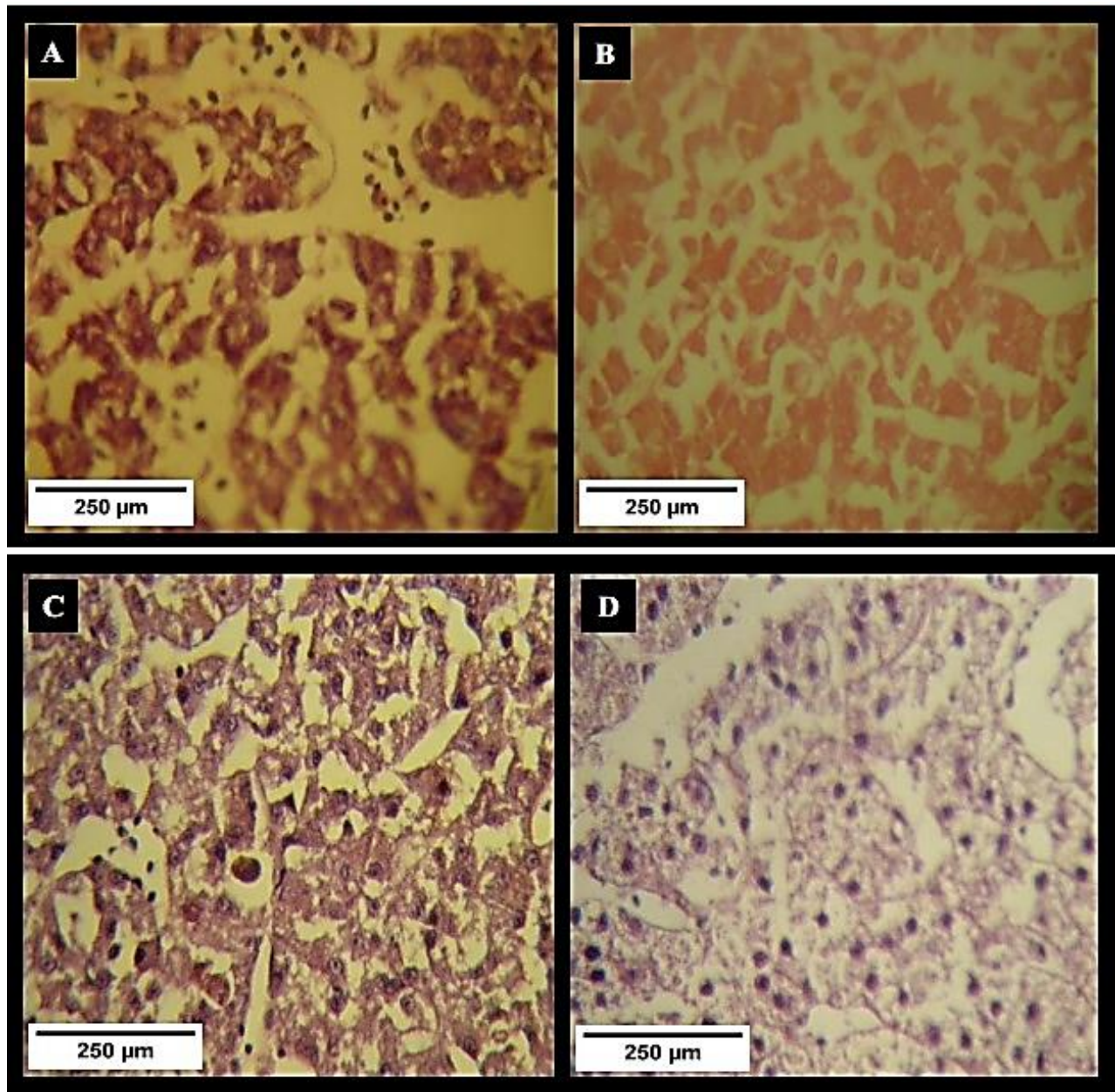


Fig.7. Histological sections images of liver tissue in Caspian lampreys (H&E staining). (A): maturing female, (B): mature female, (C): maturing male, and (D): mature male.

height of intestinal folds between mature and maturing fishes may associate with catching fish at the same time and same period of starvation during migration. The numbers of intestinal folds in mature fish (both males or females) may indicating higher energetic needs of mature females than maturing females (lower loss of energy reservoirs) and mature males (existence of yolk and ovulation in females and absence of these stages in males) during the migration period (Abramova & Balykin 1997;

Echina & Granado 2001; Komova 2001).

Hepatocytes, macrophages (Kupffer cells), sinusoids and fat storage cells in liver tissue of *C. wagneri* were diagnosed and also mass of stored glycogen in hepatocytes was clearly visible. Such structures in the liver of Caspian lamprey shows the similarity with most teleost fish despite the evolutionary differences (Chen et al. 2006; Treviño et al. 2011).

In the present study, the histological observation

of liver confirmed atrophy of liver cells in mature and maturing fishes in both groups may due to long period of starvation. During the migration digestive system and associated glands, such as liver and pancreas, are the organs that most affected by lack of food (Green & McCormick 1999; Gisbert & Doroshov 2003). In fact, primary sources of energy, in time of starvation and formation of yolk during migration in females are glycogen and stored lipids in liver cells (Gisbert & Doroshov 2003). In the present study, liver degeneration in females was stronger than males so that glycogen levels in hepatocytes were greatly reduced. Such conditions probably were related to higher energetic need of female fish than male fish to form of egg yolk. However, reduction of liver reserves probably occurred as a result of their use to provide the energy needed to maintain the basic level of metabolism during migration and lack of nutrition makes it harder (Green & McCormick 1999; Ostasewska et al. 2005).

As conclusion, this study indicated that alimentary canal in the Caspian lamprey has the histological similarity with other teleost fish except intermediate structure namely typhlosole that is species specific. Also migrating fish either in female or male, maturing or mature do starving when doing spawning migration.

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مطالعه تطبیقی دستگاه گوارش ماهیان مولد ماده و نر رسیده و در حال رسیدگی دهان گرد خزری (*Caspiomyzon wagneri*) (Kessler, 1870) مهاجر به حوزه جنوبی دریای خزر

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چکیده: پژوهش حاضر به منظور مطالعه تفاوت‌های تغذیه‌ای و بافت‌شناسی دستگاه گوارش ماهیان ماده و نر رسیده و در حال رسیدگی دهان گرد خزری (*Caspiomyzon wagneri*) در حین مهاجرت تخم‌ریزی در حوزه جنوبی دریای خزر (رودخانه شیروود، استان مازندران) طی دو فصل بهار و پاییز انجام پذیرفت. بدین منظور، از دهان‌گردان خزری مهاجر به سمت بالادست رودخانه، تعداد ۶۰ نمونه به صورت تصادفی صید شد. ضمن بررسی روده‌های این ماهی‌ها از لحاظ پر و یا خالی بودن، نمونه‌هایی از بافت‌های روده و کبد ماهیان مزبور جهت انجام مطالعات بافت‌شناسی استحصال شد. براساس شاخص‌های خارجی و داخلی رسیدگی جنسی، نمونه‌های صید شده برای هر جنس به دو دسته رسیده و در حال رسیدگی تفکیک شدند. بررسی و مشاهده روده در ماهیان صید شده مهاجر نشان داد که روده اکثر آنها خالی و فاقد مواد غذایی هستند. بر اساس مشاهدات بافت‌شناسی، روده دارای چین‌خوردگی‌های فراوان، بافت پیوندی، مایع میان بافتی، سلول‌های جامی شکل، انتروسیت‌ها و تعداد زیادی پرز روده‌ای بوده است. علاوه بر این یک ساختار میانی به نام تیفلوسول نیز در تمامی طول روده وجود داشت. ارتفاع چین‌خوردگی‌های روده و گستردگی تیفلوسول در ابتداء و انتهای روده کمتر از قسمت میانی آن بود. بین تعداد چین‌خوردگی‌های روده در ماهیان ماده رسیده و در حال رسیدگی تفاوت معنی‌داری مشاهده شد ($P < 0.05$). هیپاتوسیت‌ها بیشترین جمعیت سلول‌های کبدی را تشکیل داده و در ماهیان ماده رسیده و در حال رسیدگی نسبت به ماهیان نر دچار آتروفی شده و کاملاً تحلیل رفته بود.

کلمات کلیدی: رودخانه شیروود، مازندران، بافت‌شناسی، سیستم هضمی، بلوغ، تغذیه.