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Original Article

Morphometric analysis of olfactory organ and telencephalon in maturing and mature migrants of Caspian lamprey (*Caspiomyzon wagneri*, Kessler 1870)

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Abstract: This study was conducted to provide a detailed information about changes of the olfactory organ and telencephalon morphology in spring and fall spawning run maturing and mature Caspian lamprey, *Caspiomyzon wagneri*, in the Shirud River, Sothern Caspian Sea basin, Iran. A total of 71 maturing and mature fish were collected during their spawning migration. The results showed that the thickness of the olfactory epithelium and the density of ciliated olfactory receptor cells (ORC) were lower in mature migrants. In addition, the nasal cavity, relative weight of olfactory organ and relative telecephalon area in mature migrants were larger indicating its more sensitivity to external queues. Based on the results, the olfactory organ and telencephalon of maturing migrants of Caspian lamprey have not developed completely and needs a period of rest in the river to its full development for spawning.

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Introduction

The Caspian lamprey, *Caspiomyzon wagneri*, is an endemic species to the Caspian Sea basin and migrates into its northern, western and southern rivers (Coad, 2016). Its populations in the southern part of the Caspian Sea have dramatically decreased due to river pollution, damming, and loss of spawning grounds (Kiabi et al., 1999). Now with "near threatened" status (IUCN, 1996), there is need for special protection of this species.

Caspian lampreys inhabit freshwater rivers during larval development and migrate to the Caspian Sea for feeding after metamorphosis. When sexual maturation approaches, adults migrate to the rivers for reproduction (Holcik, 1986). Spawning migrations to the southern Caspian Sea rivers (e.g. Shirud River, Northern Iran) occur in the middle of March to late-April in spring, and mid-September to late October in fall (Ahmadi et al., 2011) and these migrants can be classified into two sexual types: mature and maturing.

The olfactory organ of lampreys is located in a shallow nasal cavity on the dorsal surface of the head and its olfactory receptors are long having thin dendrites with rounded apical nodes (Thornhill, 1967). During sexual maturation and particular season, the olfactory sensitivity increases (Moore and Scott, 1992), e.g. Sea lampreys (*Petromyzon marinus*) use a specific chemical or pheromone to migrate back to the river, also males secrete pheromones to attract females to spawning grounds (Teeter, 1980; Bjerselius et al., 2000). Pheromones stimulate the olfactory receptors to send the appropriate message to the brain (Weiming et al., 2007).

Lamprey has a small brain comprising the telencephalon, diencephalon, mesencephalon and metencephalon. Their telencephalon, in particular, is very small. The shape of the brain does not change much through the adult period, but increase in size (Scott, 1887). There is significant coupling between the olfactory system and brain controlling areas. The

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Table 1. Body weight (g) and total length (mm) of maturing and mature males or females of the Caspian lamprey in spring and fall. Each value represents the mean ±SE.

season	sex		total length (mm)	weight (g)
Spring	Male	mature	3788±362	79±9
		maturing	3917±110	93±8
	Female	mature	3876±40	102±5
		maturing	4274±390	127±8
Fall	Male	mature	3675±234	83±6
		maturing	3745±388	90±7
	Female	mature	3723±168	93±6
		maturing	3827±277	104±9

telencephalon is the olfactory center and mediates a variety of behaviors such as classical conditioning (Overmier and Hollis, 1983), processing of sensory information (Davis and Kassel, 1983; Hofmann, 2001), mating and reproduction (Demski and Beaver, 2001), social behavior (Huber et al., 1997; Kotrschal et al., 1998; Hofmann, 2001; Pollen et al., 2007), aggression (Davis and Kassel, 1983), schooling behavior (Davis and Kassel, 1983; Shinozuka and Watanabe, 2004), and avoidance learning (Portavella et al., 2003, 2004; Vargas et al., 2009). In addition, the size of the telencephalon is associated with residence in structurally complex habitats (Bauchot et al., 1977; Huber et al., 1997), superior spatial learning (Vargas et al., 2000, 2009), greater parental care (Gonzalez-Voyer et al., 2009), monogamy (Pollen et al., 2007), and sociality (Pollen et al., 2007). Sexual maturation can trigger changes in brain morphology (Jacobs et al., 1990; Jacobs and Spencer, 1994; Clayton et al., 1997; Sherry, 1998).

Little studies are available about the reproductive biology and physiology of the Caspian lamprey (Kiabi et al., 1999; Nazari and Abdoli, 2010; Ahmadi et al., 2011). To the best of our knowledge, no information is available about the olfactory organ and its target regions in the brain i.e. telencephalon of the Caspian lamprey. Hence, this study aimed to analysis morphometric features of the olfactory organ and telencephalon in maturing and mature migrants of Caspian lamprey. The findings of the present study can increases our understanding about the changes of the olfactory organ and telencephalon during reproductive spawning migration in mature and maturing Caspian lamprey and provide valuable information on the spawning migration physiology in this endangered species.

Materials and Methods

Study site: Specimens were collected by hand at night in spring (middle of April) and fall (middle of October) 2013. A total of 71 maturing and mature fish were collected under the Shirud river bridge (200 m upstream from the river mouth; 36°51′N, 50°47′E). The mean water temperature was 14±3°C and 11±2°C during sampling period in spring and fall, respectively.

Sampling and biometry: Fish were anaesthetized using a solution of clove powder (250 ppm). A total of 40 specimens, including mature and maturing males and females were selected from the collected specimens in each sampling season (5 per each group). Mature males were opted from maturing males by applying moderate pressure to their abdomen to stimulate sperm ejaculation. Ejaculation was observed in mature males but not in maturing males. Mature and maturing females were separated according to their ovary color i.e. mature female had ovaries in bluish-green color, whereas those of maturing were yellowish. In addition, mature migrants had empty intestines unlike those of maturing migrants which had green and full intestines (Ahmadi et al., 2011). The body weight (g) and total length (mm) of fish were measured using a digital balance and measuring board, respectively (Table 1). The nasal cavity opening was measure from 2D pictures using ImageJ Software. Then, the olfactory organ (without inlet tube and surrounding

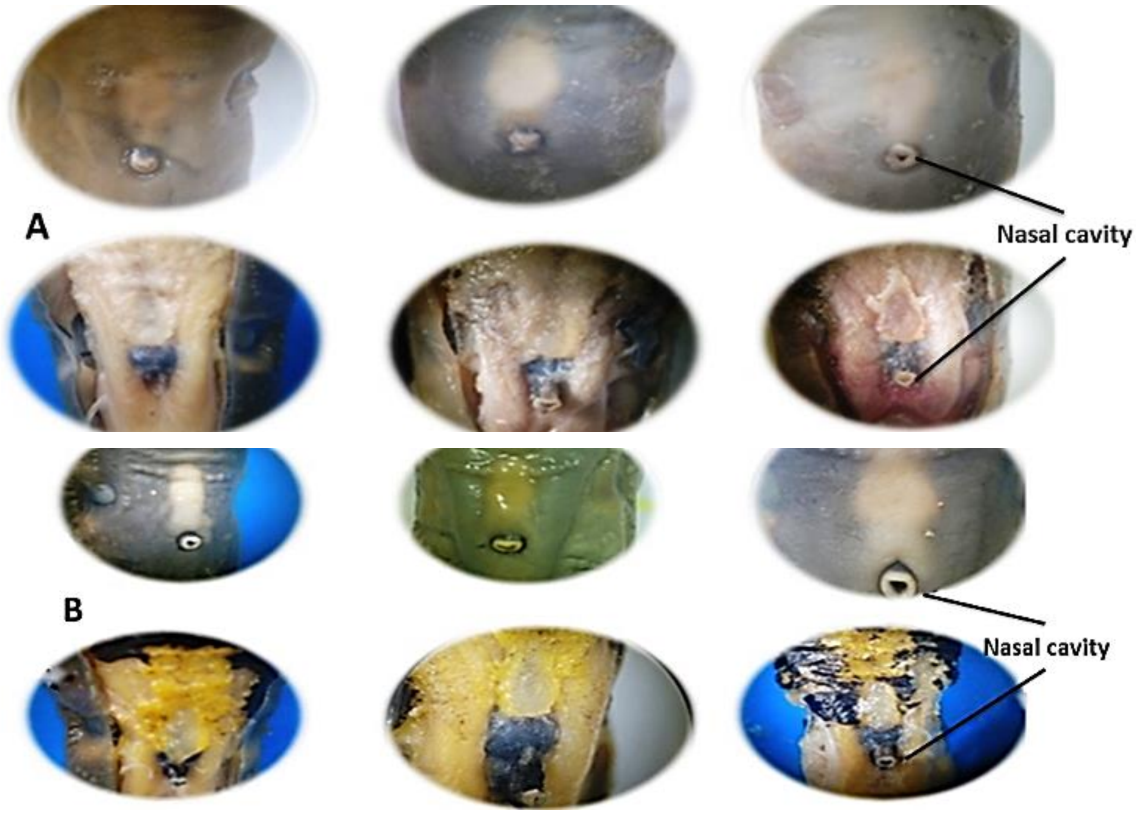

Figure 1. Nasal cavity (external feature, up and internal feature, down) in the Caspian lamprey (A: maturing and B: mature migrants).

cartilages) and brain of fish were removed (Figs. 1, 2) and their weight were measured using a digital balance to the nearest 0. 01 g. The relative weight of olfactory organ was calculated using the following equation: nose weight/ body weight × 100. Finally, the nasal and brain were fixed in Bouin's fixative.

Histological study: The olfactory organ of specimens was embedded in paraffin and histological sections were prepared at 4-6 µm thickness and stained with Delafield's hematoxylin and eosin based on Eagderi et al. (2013). Numbers of olfactory receptor cells (ORC) per 100 µm of the olfactory epithelium, and olfactory epithelium width were measured using Olympus BH-2 optical microscope equipped to a Dino-Capture camera and ImageJ Software. ORCs were distinguished by their ciliated olfactory knobs with basal bodies at the outer margins and analyzed according to van Denbossche et al. (1995). The area of brain and telencephalon region (as µm^2 in dorsal view) were examined under stereomicroscopy equipped to a Dino-Capture camera and ImageJ Software. The relative size of telencephalon was calculated using the equation: telencephalon area/ brain area × 100.

Statistical Analysis: Differences between sex ratios were determined using Student's t-test. Mean data were compared using analysis of variance (ANOVA). T-test was used to compare data between maturing and mature fish. Data are expressed as mean ±SD. P-value<0.05 was considered significant.

Results

Nasal cavity opening in mature Caspian lampreys was larger than that of maturing fish in both spring and fall migrants (Fig. 1). The relative weight of the olfactory organ was significantly greater in mature fish than those of maturing migrants (0.3223±0.002 vs. 0.2847±0.0011 and 0.3347±0.0023 vs. 0.3013± 0.0022 in spring and fall migrant males, respectively; 0.3067± 0.0019 vs. 0.2793±0.0017 and 0.324± 0.0023 vs. 0.295±0.0015 in spring and fall migrant females, respectively) (P<0.05) (Fig. 3).

The thickness of olfactory epithelium (µm) in mature fish were significantly lower than that of maturing migrants (393±1.2 vs. 249.67±3.65 and

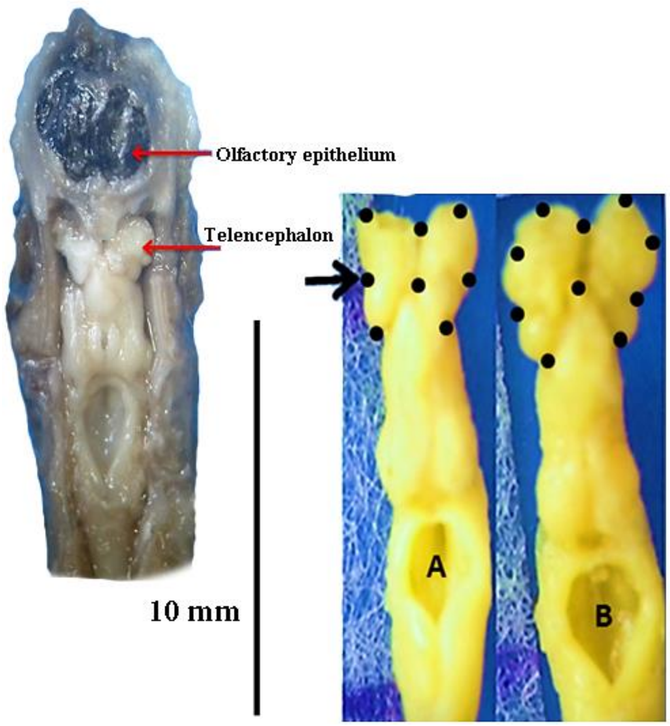

Figure 2. Nasal cavity (external feature, up and internal feature, down) in the Caspian lamprey (A: maturing migrants B: mature migrants).

389±1 vs. 256.6±2.2 in spring and fall migrant males, respectively; 354.9±1.1 vs. 276.63±3.31 and 364.2±1.6 vs. 279.3±3.5 in spring and fall migrant females, respectively) (P<0.05) (Fig. 4). The numbers of ORC/100 µm of olfactory epithelium in maturing migrants were closely spaced and greater, compared to those of matures (8.67±3.65 vs. 3.33±1.2 and 8.67±2.2 vs. 3.66±1 in spring and fall migrant males, respectively; 6.67±3.31 vs. 4.67±1.1 and 7.33±3.5 vs. 4.67±1.6 in spring and fall migrant females, respectively) (P<0.05) (Fig. 4).

Telencephalon area/ brain area (μm^2) (relative telecephalon area) was significantly greater in mature migrants than that of maturing fish (37.3±0.2 vs. 29.4±0.23 and 33.4.33±0.17 vs. 28.1±0.19 in spring and fall migrant males, respectively; 35.8 ±0.15 vs. 29.1±0.23 and 30.5±0.12 vs. 26.9±0.28 in spring and fall migrant females, respectively) (P<0.05). There was a high positive correlation (R^2=0.98) between the weight of olfactory organ and telecephalon weight in maturing and mature migrants during spring or fall.

Discussion

This study provided a detailed information about change of the olfactory organ and telencephalon region of maturing and mature migrants of *C. wagneri* during spring and fall migrations. The results showed that the nasal cavity and relative weight of the olfactory organ in mature migrants were larger. In sea lamprey, progressive enlargement of the nasal sac during metamorphosis and maturation has been reported (Lowe et al., 1973; van Denbossche et al., 1997).

The thickness of olfactory epithelium and ORC

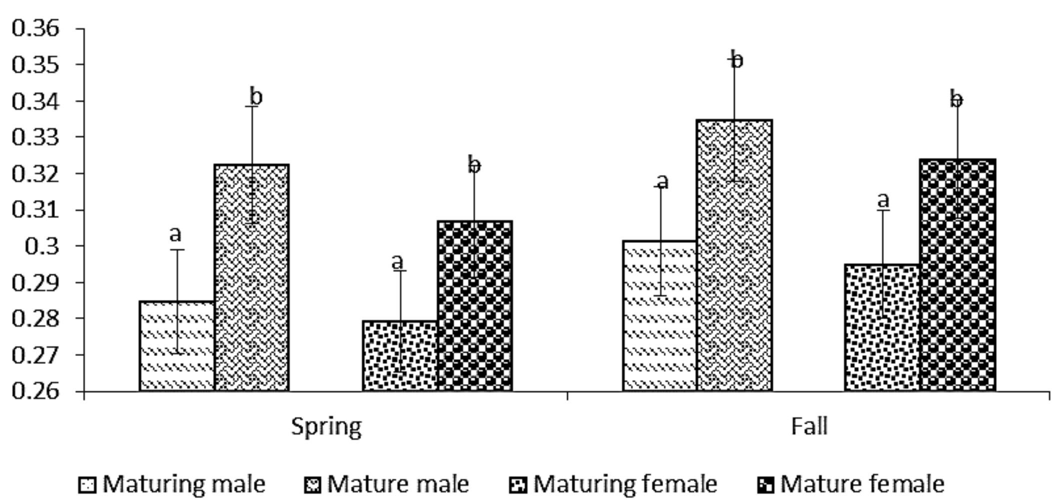

Figure 3. The relative weight of the olfactory organ in maturing and mature male and female migrants in spring or fall (a, b=significant, a, a= not significant, P<0.05).

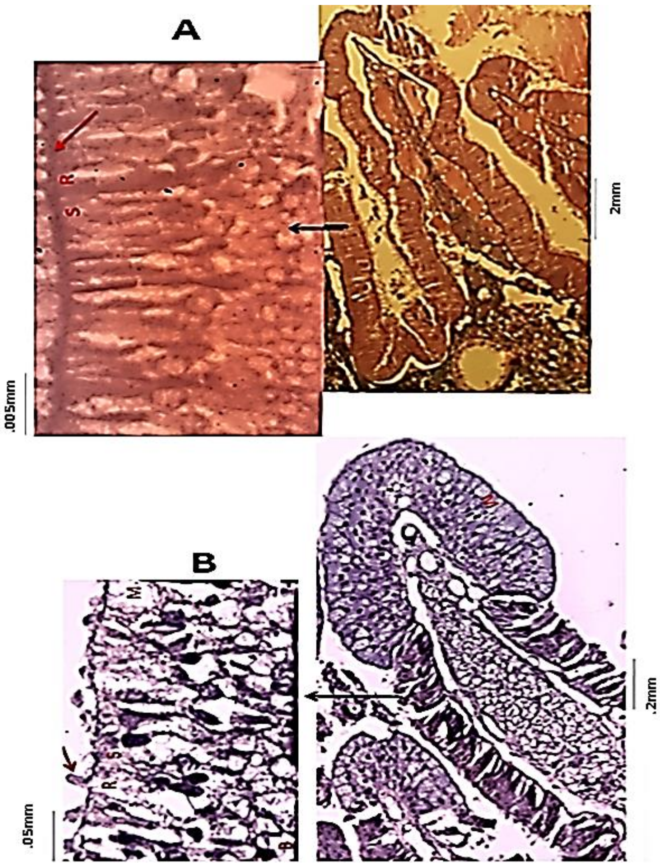

Figure 4. The olfactory lamella and the arrangement of sensory cells (A: maturing migrant, B: mature migrant, R: ORC (Arrowheads), B: basal cell, S: supporting cell, and M: mucous cells).

density were greater in non-mature specimens than in mature fish. Thomhill (1967) reported that the olfactory epithelium in adult *Lampretra fluviatilis* had ciliated ORC and our results also extend this observation to the maturing and mature stages. The range of ORC numbers in the olfactory epithelium of upstream migrant *P. marinus* was 2-12 (Zielinski et al., 1994). The enlarged olfactory organ may enable fish to receive large quantities of water and becomes more efficient at chemoreception as more water enters in the cavity and more odors can be detected (Kleerekoper, 1972; VanDenbossche et al., 1995). Maturing migrant lamprey must find food with a smaller nasal cavity, which may lead to an increase in ORC. In contrast, mature migrants not eat and build the reproductive products, resulting in a lower body weight and larger nasal cavity, which may explain the deformed ORC in current study.

Telencephalon area/ brain area was significantly greater in mature fish compared to maturing migrants. During the larval period, the telencephalon grows slowly, then doubles in size during metamorphosis, and increases linearly until adulthood (Hardisty and Potter, 1971). Furthermore, olfactory sensitivity increases and changes are occurred in olfactory receptors during sexual maturation due to presence of pheromones in the water (Moore and Scott, 1992) maybe causing enlargement of the telencephalon region as observed in this study. Nanomolar concentrations of a sexual pheromone in males can stimulate the olfactory epithelium of adult females (Li et al., 2002). Behavioral responses in vertebrates are closely related to the olfactory system and olfactory-brain regions (Healey, 1972). Olfactory input is relayed on the medial part of the olfactory bulb, the posterior tuberculum, the mesencephalic locomotor region, and finally reach reticulospinal cells in the hindbrain. Activation of this olfactory-motor pathway generated rhythmic ventral root discharges and swimming movements (Derjean et al., 2010).

Based on the findings of the present study, mature fish (males or females) show larger relative nasal sac and telencephalon in both migration seasons indicating more sensitivity to external queues compare to maturing fish. Also, it can be concluded that the olfactory organ and telencephalon of maturing migrants of Caspian lamprey have not developed completely and needs a period of rest in the river to its full development for spawning.

Acknowledgments

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چکیده فارسی

تحلیل ریخت‌سنجی اندام بویایی و تلن‌سفالون مغز در مارماهی دهان‌گرد خزری بالغ و در حال بلوغ (*Caspiomyzon wagneri*, **Kessler 1870**)

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چکیده:
این مطالعه با هدف فراهم نمودن اطلاعات در مورد تغییرات ریختی اندام بویایی و تلن‌سفالون مغز مارماهی دهان‌گرد خزری بالغ و در حال بلوغ در زمان مهاجرت‌های تولیدمثلی بهاره و پاییزه به رودخانه شیرود، حوضه آبریز جنوب دریای خزر، ایران به اجرا درآمد. برای این منظور تعداد ۷۱ ماهی بالغ و در حال بلوغ در زمان مهاجرت تولیدمثلی جمع‌آوری شدند. نتایج نشان داد که ضخامت اپیتلیوم بویایی و تراکم سلول‌های گیرنده مژک‌دار بویایی در ماهیان بالغ کمتر بودند. به‌علاوه حفره بویایی، وزن نسبی اندام بویایی و مساحت نسبی تلن‌سفالون در ماهیان مهاجر بالغ بیشتر بودند که بیانگر حساسیت بیشتر آن به محرک‌های خارجی می‌باشد. براساس نتایج اندام بویایی و تلن‌سفالون مغز مارماهی دهان‌گرد مهاجر در حال بلوغ هنوز به‌طور کامل توسعه نیافته و نیازمند یک دوره استراحت در رودخانه برای تکمیل توسه به‌منظور تخمریزی است.

کلمات کلیدی: مارماهی دهان‌گرد خزری، بالغ، در حال بلوغ، ریخت‌سنجی، اندام بویایی.