Histological development of eye in Caspian roach, *Rutilus lacustris* (Pallas, 1814*)* (Teleostei: Cyprinidae) during early ontogeny

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Abstract

Fish larvae are equipped with several sensory systems that are functional at or soon after hatching and their function are modified further throughout the larval and juvenile periods. The development of the functional eye generally is correlated with the onset of feeding. This study aimed to determine the development of the eye structure in Caspian roach, *Rutilus lacustris*, during its early ontogeny. For this purpose, the histological sections of *R. lacustris* eye from hatching up to 90 dph were prepare, examined and photographed. According to the results, the retina of the newly hatched larvae was almost completely differentiated. The most differentiations of the eye structures had been occurred until 6 dph concomitant with initiation of exogenous feeding. This fact illustrates the importance of visual sense as an eye-dependent species during its larval period.

Keywords: Eye, Retina, Ontogeny, Development, Caspian roach

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Introduction

Eyes are major sensory organs in fishes to detect photic stimuli and form images of the environment (Chai *et al*., 2006; Lim *et al*., 2014). Fish visual capability is highly related to the eye structure; therefore, study of its histological aspects can provide insight on the visual capability of the fish (Lim *et al*., 2014). In addition, development of the functional eye is generally correlated with the onset of feeding, i.e. distinct changes in the retinal morphology is occurred concomitant with a shift from pelagic (planktonic) to a benthic habitat (Hall and Wake, 1999).

The Caspian roach, *Rutilus lacustris,* is a commercially important cyprinid fish and native to the Caspian Sea (Hasanpour *et al*., 2015, 2016a, b). Due to over fishing and destruction of its spawning grounds, this species has experienced a remarkable decline in its fishing yields. Therefore, its artificial propagation in hatcheries to recruit its natural stocks has fulfilled during last decade (Ghelichpour and Eagderi, 2012; Hasanpour *et al*., 2015). Since in restocking programs providing basic biological information is crucial for breeding and rearing of larvae, therefore, this study was conducted to study the development of the eye in the Caspian roach using histological analysis from hatching up to 90 days post hatching (dph) with emphasis on retinal morphology.

Material and methods

The adult Caspian roach were caught in the estuary of the Gorgan River by gill nets during their spawning migration during April and May 2017. The Caspian roach larvae were obtained from the semi-artificial breeding of 20 brood stocks in an earthen pond in the Sijval Restocking Center (Bandar-e-Turkmen, north of Iran) with an ambient temperature. The pond was filled three weeks prior to the introduction of the fish and fertilized to sustain primary production and benthos as natural food by adding cattle manure and urea fertilizer. During rearing period, the larvae were fed by fertilizing ponds and a diet based on Fontagne and Silva (2009). The water temperature, dissolved oxygen and pH were 21.4-24.4 °C, 6.5-8 ppm and 7.6-8.4, respectively, during rearing period. Fish were reared under the natural photoperiod. In addition, the semi extensive condition was applied to provide a natural habitat and to produce high quality larvae with low anomalies (Sfakianakis *et al*., 2004, 2005; Lewis and Lall, 2006; Hasanpour *et al*., 2015, 2016a). A total of 270 larvae and fry were randomly sampled from hatching up to 90 dph, prior to feeding, in the morning. Sampling was done every day till 20 dph and then every 5 days up to 90 dph (n=10). The samples were sacrificed by overdosing with MS222 (Sigma-Aldrich). Then, the specimens were preserved in 5% buffered formaldehyde and transferred to 72% alcohol after 48 hours

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Five fixed specimens per sampling day were randomly selected and subsequently dehydrated in a graded series of ethanol (70-100%) and cleared with Xylene and finally embedded into paraffin. The histological sections were prepared with 6 µm thickness, mounted on the glass slides and stained with hematoxylin and eosin (Eagderi *et al*., 2013). The sections were examined under a light microscope and photographed by a Nikon camera (13 MP resolutions).

Results

On 1 dph, the retina was relatively thick, and by growing eye, the vitreal gap developed and the retina, as the inner sensory (photosensitive) tissue, was completely differentiated. Pigmented epithelium of the retina as non-nervous region was thin, and its inner nervous region was composed of seven layers, including (according to Atta, 2013) (1) the photoreceptor cell layer (Ph), (2) outer or external limiting membrane (OM), (3) outer nuclear layer (ON) representing the nuclei of the photoreceptor cells, (4) outer plexiform layer (OP), position of the synaptic relationship between photoreceptor, bipolar, amacrine and horizontal cells as well as mullers cells, (5) inner nuclear layer (IN) containing the nuclei of several types of the neurons mainly bipolar, amacrine and horizontal cells as well as mullers cells, (6) inner plexiform layer (IP) i.e. location of the synaptic connection of the bipolar and ganglionic cells, and (7) ganglionic layer (G) composed of a narrow chain of the granular and spherical cells surrounded by a fine connective tissue network (Fig. 1).

At the hatching, the inner nuclear layer was significantly thicker than the ganglionic layer. The median uveal layer was not completely developed. The lens, as an avascular spherical ball, was made up of 4 layers, including an extra cellular matrix (capsule), a monolayer of the nucleated flattened or cuboidal cells capable division and secretion, a hyaline layer and last layer consisting of parallel rows of long, slender, transparent and non-nucleated fibers.

At 2 dph, the nerve fiber layer i.e. axons of the ganglionic layer was observed. The blood vessels of the rete choroid were strongly developed to support the retina. There was no significant change during 3-5 dph. By formation of the inner limiting membrane at 6 dph (beginning time of the exogenous feeding), all layers of the retina were present with an order similar to other teleost i.e. development of the retina and choriocapillar layer in *R. lacustris* are completed in accordance to exogenous feeding. After this time, the only noticeable changes in the retina’s layers were either increase or relative decrease in diameter and density of the rod, cone and ganglion cells. In addition, the pigment epithelium was gradually thickened in 25 dph, and thickness of the nerve fiber layer was increased (Fig. 2).

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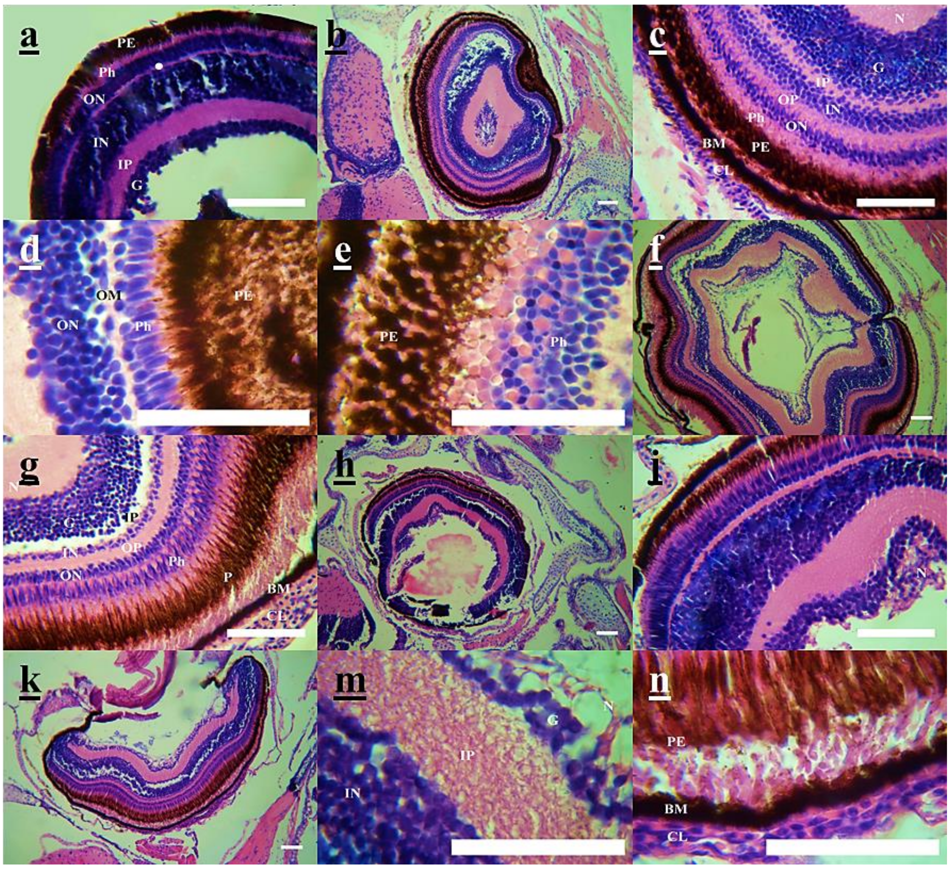

Figure 1: Eye development of the *Rutilus lacustris*. a: 1-dph, b-e: 2-dph, f-g: 3-dph, h-j: 4-dph, k-n: 6-dph, f: 80-dph. N: nerve fiber layer, G: ganglionic layer, IP: inner plexiform layer, IN: inner nucleus layer, OP: outer plexiform layer, ON: outer nucleus layer, OM: outer membrane, Ph: photoreceptor cell layer, PE: pigment epithelium, LC: lens capsule, LF: lens fiber, HL: hyaline layer, BM: brush membrane, CL: choriocapillar layer (Scale bar = 100 µm).

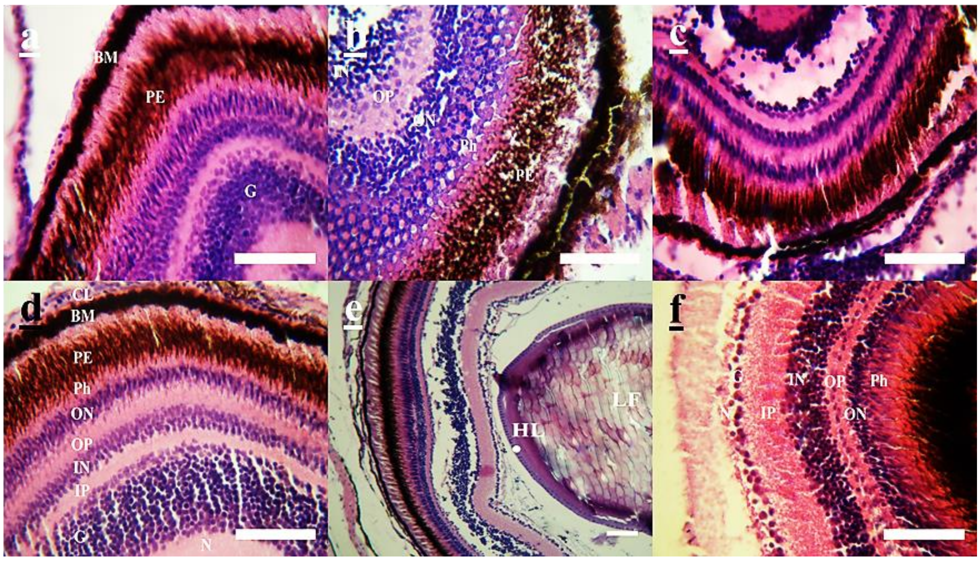

Figure 2: Eye development of the *Rutilus lacustris*. a: 7-dph, b: 14-dph, c: 18-dph, d:20-dph, e: 30-dph, f: 80-dph. N: nerve fiber layer, G: ganglionic layer, IP: inner plexiform layer, IN: inner nucleus layer, OP: outer plexiform layer, ON: outer nucleus layer, OM: outer membrane, Ph: photoreceptor cell layer, PE: pigment epithelium, LC: lens capsule, LF: lens fiber, HL: hyaline layer, BM: brush membrane, CL: choriocapillar layer (Scale bar = 100 µm).

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Discussion

Major events in the functional ontogeny of the visual system are closely correlated with life history event where the fish experiences changes in the photic environment due to a change in vertical or horizontal position or changes in behavioral repertoire (Blaxter and stains, 1970; Hall and Wake, 1999). The photoreceptor cell layer is usually composed of rods and cons (Atta, 2013). The rod cells, which are long and slender in shape, have low threshold to light stimulation and are effective in dim light. However, the cone cells have high threshold to light stimulation (Atta, 2013). Several studies found that many fishes possess only cones at the onset of exogenous feeding, as the larvae live near the surface of the water where sun light penetrates (Blaxter and Stains, 1970; Hall and Wake, 1999; Lenkowski and Raymond, 2014). While the appearance of the rod photoreceptors in the retina delay until the larvae move to deeper waters (Hall and Wake, 1999; Chai *et al*., 2006; Ebbessen *et al*., 2007; Lenkowski and Raymond, 2014), similar to *R. lacustris* as a bottom feeder.

At hatching, the retina of *R. lacustris* possesses well-differentiated photoreceptors in contrast to *Acipenser sinensis* (Chai *et al*., 2006) *A. stelatus, A. rutenus* and *Huso huso* (Detlaff *et al*., 1993) illustrating that *R. lacustris* larvae are highly dependent on the visual capability. In addition, *R. lacustris* starts its exogenous feeding on 6 dph (Hasanpour *et al*., 2015, 2016a, b), when the layers of retina have been completely formed and this shows the importance of visual sense during early developmental stage. In addition, complete development of the choriocapillar layer until 6 dph is in accordance to the high oxygen demand of the eye.

Rod cells become more important to visual function when *R. lacustris* switches its habitat preference to deep waters similar to other bottom feeders (Whal *et al*., 1993). The pigment epithelium of the retina was very thick, similar to the most of the bottom feeders (Atta, 2013). As the greater eye size commonly accommodates the larger eye lens, more light can be gathered and a higher resolution image can be generated in the brain (Lim *et al*., 2014). As conclusion, Caspian roach is an eye-dependent species during its larval period and its eyes structure particularly the retina is completed before the onset mixed feeding.

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