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Original Article

Histological and allometric growth analysis of eye in Caspian kutum, *Rutilus kutum* Kamensky, 1901 (Teleostei: Cyprinidae) during early developmental stages

Shaghayegh Hasanpour[1], Soheil Eagderi*[1], Seyed Valli Hosseini[1], Mohamad Hasan Jafari Sayadi[2]

[1]Department of Fisheries, Faculty of Natural Resources, University of Tehran, P.O. Box: 4111, Karaj, Iran.
[2]Department of Agriculture and Natural Resources, University of Payam Noor, Karaj, Iran.

Abstract: Fish larvae have several sensory systems that are functional at or soon after hatching and then are developed further during larval and juvenile stages. This study was conducted to investigate development of the eye in *Rutilus kutum*, based on histological and allometric growth analysis during early developmental stages up to 35 day post hatching with emphasis on retinal morphology. For this purpose, the histological sections were prepared and allometric growth pattern of the eye was calculated. The results showed that the most eye's structures along with the retina of the newly hatched larvae, as the inner sensory (photosensitive) tissue were completely differentiated. Allometric growth pattern of the eye diameter up to the inflexion point (7 dph) was somewhat positive and then it became negative. The results revealed that the Caspian kutum is dependence on visual capability as visual feeder during their larval period which itself explains completion of eye structures and the high growth rate of eye before 3 dph i.e. beginning of mixed feeding.

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Introduction

Fish larvae have several sensory systems that are functional at or soon after hatching and then are developed further during larval and juvenile stages (Wahl et al., 1993; Hall and Wake, 1999; Loosey et al., 2000). The feeding habits of fishes are reflected on the structure and size of the sense organs particularly the eyes (Atta, 2013). Eyes are among the major sensory organs in fishes for detecting photic stimuli and forming images of the environment (Chai et al., 2006). Fish visual capability is highly related to the eye structure, therefore study of the eye structure and its retinal morphology can provide insight to the visual capability of fish (Lim et al., 2014). In addition, the development of the functional eye is correlated with the feeding habits of fishes for instance distinct changes in the retinal morphology occur concomitant with a shift from pelagic to a benthic habitat (Hall and Wake, 1999).

Different growth rate of various parts of the body or allometric growth is a common phenomenon during early development of fishes (Osse and van den Boogart, 1995), which it is responsible for a progressive transformation of recently hatched specimen from a larval body shape to juvenile or adult form in a relatively short time (Khemis et al., 2013). Hence, understanding normal growth pattern and morphological changes are crucial to reduction of the hatchery losses (Khmis et al., 2013; Pena and Dumas, 2009).

Caspian kutum, *Rutilus kutum* is an important commercial and edible fish in the southern Caspian Sea distributed from the southwest (the Atrak River) to northward (the Volga River) (Abdolhay et al., 2011). Due to over fishing and deterioration of its spawning grounds and natural habitats, this species has experienced a dramatic reduction in its fishing yields. Therefore, its artificial propagation in hatcheries was established to recruit its natural stocks by releasing its fingerlings into rivers that drain to the Caspian Sea basin (Jafari et al., 2010).

* Corresponding author: Soheil Eagderi
E-mail address: soheil.eagderi@ut.ac.ir

Since in restocking programs, providing basic biological information is crucial for breeding and rearing of larvae; therefore, this study was conducted to investigate the development of the eye in the Caspian kutum based on histological and allometric growth analysis during early developmental stages from hatching up to 35 day post hatching (dph) with emphasis on retinal morphology. Its ontogeny will provide insight on the visual capability of this species and can help to optimize its larval culture conditions.

Materials and Methods

Specimens rearing and sampling: Larval specimens were obtained from artificial propagation of 15 female and 30 male broodstocks in April-May 2012, in Dr. Yousef-pour Fish Hatchery Center (Siahkal, Guilan, Iran). The eggs were incubated in 10 L vase incubators with flow-through system at 22°C. After six days of incubation, eggs were hatched and transferred to a large larval collector tanks (200 L). After 3 days, at the beginning of exogenous feeding, 30,000 larvae were transferred to an earthen pond (0.1 ha) with a flow-through system with a mean temperature, pH and dissolved oxygen of 25±2.2°C, 8.1±0.5 and 7.4±1.1 mg L^{-1}, respectively. The natural water flow provided some natural prey but additional artificial feed was supplied from 7 dph. The feed was a specialized feed for Caspian kutum larvae and juveniles based on a mix of protein and cereal meals. Larval specimens were randomly sampled from 1-20 dph every days and then every 5 days up to 35 dph (n=30) from the same larval batch prior to feeding, in the morning.

Allometric analysis: The samples were sacrificed by overdose of MS222 (Sigma-Aldrich), weighed to the nearest 0.0001 mg and fixed in 5% phosphate buffered formaldehyde solution. The left side of the fresh larvae, aged 1-12 dph, were photographed using a dissecting microscope equipped with a Cannon camera (5MP resolution) and the older specimens were photographed using a Copy-stand equipped to the camera. Total length (TL: from tip of the snout to the end of the caudal fin) and eye diameter were measured from the digital images to the nearest 0.01 mm using the ImageJ software (version 1.240). TL was measured as the reference point in the description of the ontogeny because it is a proper measure of ontogenetic state than age (Hasanpour et al., 2015, 2016; Saka et al., 2008; Sfakianakis et al., 2004, 2005).

Allometric growth pattern was calculated as a power function of TL using non-transformed data: $Y=\alpha X^{b}$. Where Y is the dependent variable, X: the independent variable (TL), α the intercept and b the growth coefficient. Allometric growth pattern is considered as positive, when b is larger than the isometric value (b=1) and as negative when b>1 (Gisbert, 1999). The inflexion point of growth curve was determined according to van Sink et al (1997). Both preparation of the plots and data analysis were performed using Manitab (version 16), PAST and Microsoft Excel (version 2013) softwares.

Histological analysis: Six 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 (Hewitson and Darby, 2010; Eagderi et al., 2013). The sections were examined under a light microscope and photographed by a Nicon camera (13MP resolution).

Results

At 1 dph, the eye was developed and had almost similar structures as adults showing importance of this organ during eleuthero-embryonic stage. In addition, the retina was completely differentiated as inner sensory (photosensitive) tissue of eye at this stage. The pigment epithelium layer of the retina as a non-nervous area, was thin. The inner nervous area of the retina is composed of 9 layers, including (definitions are according to Atta, 2013): (1) Photoreceptor cell layer (Ph), (2) Outer or the external limiting membrane (OM), (3) Outer nuclear layer (ON) representing the nuclei of the

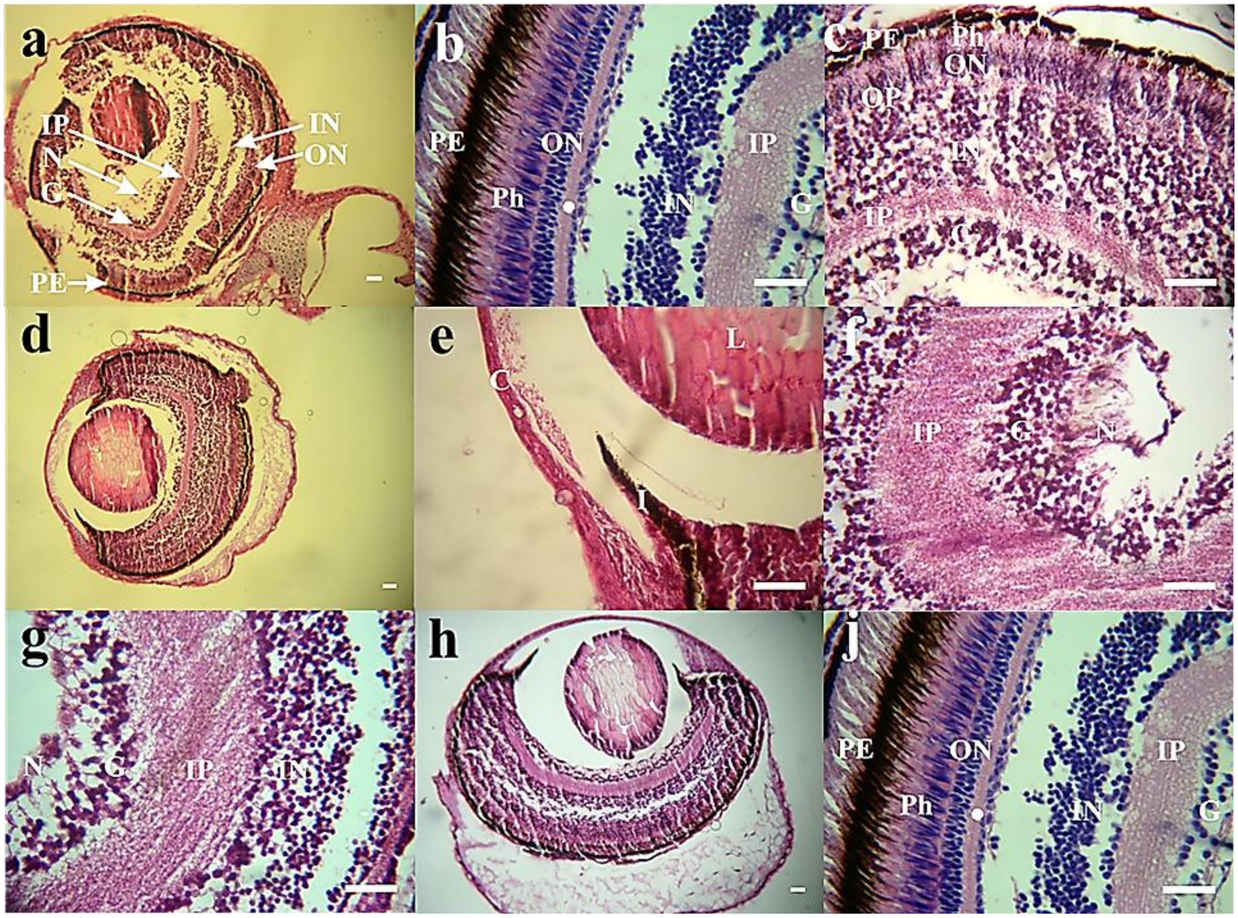

Figure 1. Eye development of *Rutilus kutum*. a-b: 1-dph, c: 2-dph, d-e: 3-dph, f: 4dph, g-h: 5-dph, j: 35-dph. N: nerve fiber layer, G: ganglionic layer, IP: inner plexiform layer, IN: inner nucleus layer, OP: outer plexiform layer, ON: outer nucleus layer, OM: outer membrane, Ph: photoreceptor cell layer, PE: pigment epithelium, BM: brush membrane, CL: choriocapillar layer, L: lens, I: iris, and C: cornea (scale bar = 100 µm).

photoreceptor cells, (4) Outer plexiform layer (OP) i.e. the location of synaptic relationship between photoreceptor, bipolar, amacrine and horizontal cells as well as mullers cells, (5) Inner nuclear layer (IN) contains the nuclei of several types of neurons mainly of bipolar, amacrine and horizontal cells as well as mullers cells, (6) Inner plexiform layer (IP), the location of synaptic relationship between the bipolar and ganglionic cells, (7) Ganglionic layer (G) that is composed of a narrow chain of granular and spherical cells surrounded by a fine connective tissue network, (8) Nerve fiber layer (N) represents axons of the ganglionic layer, and (9) Inner limiting membrane (IM) (Fig. 1). In addition, the diameter of the inner nuclear layer was significant compared to the ganglionic layer.

The median uveal layer had not been completely developed at 1 dph. The lens as an avascular spherical ball was made up of 4 tissue layers, including an extra cellular matrix (capsule), a monolayer of nucleated flattened or cuboidal cells capable of division and secretion, hyaline layer, and fourth layer consisting long, slender, transparent, non-nucleated fibrous cells that are arranged as parallel rows (Fig. 1).

At 2 dph, the blood vessels of the rete choroid was strongly developed to support the retina. There was no significant structural changes in the eye expect their size from 3 dph onward that is coincided with starting mixed feeding, i.e. development of the retina and choriocapillary layer is completed before exogenous feeding. After 3 dph, the only noticeable changes in the retina were increasing some layers' diameter and the density of the rod, cone and

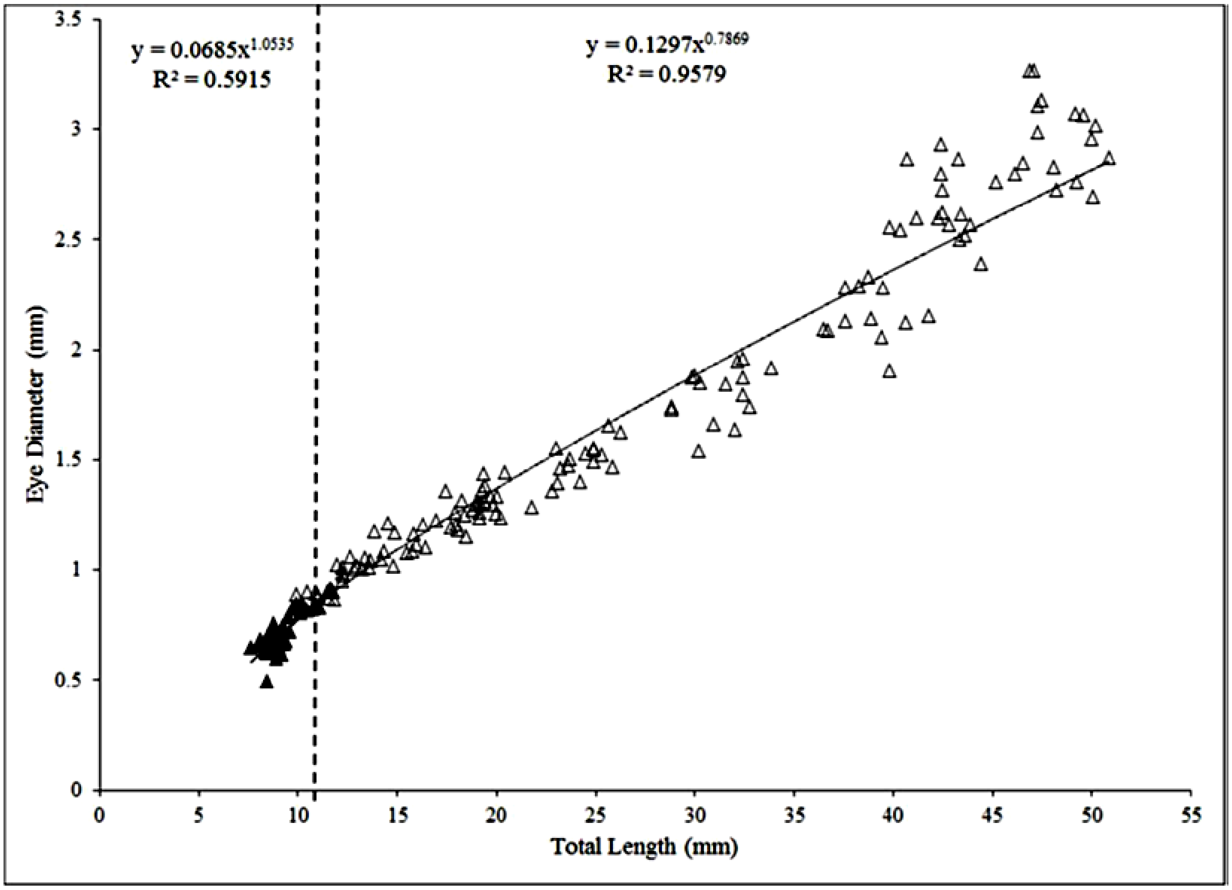

Figure 2. The allometric growth pattern of the eye of *Rutilus kutum* (The dashed line represents the inflexion points of growth).

ganglion cells. During this period, the pigment epithelium was gradually thickened.

The eye was externally unpigmented at hatching and pigmented at the beginning of the mixed feeding stage (3 dph). Allometric growth pattern of the eye diameter up to the inflexion point (7 dph, TL=12.48±0.67) was somewhat positive (b = 1.05, r^2=0.59), and it became negative after this point (b=0.79, r^2=0.95) (Fig. 2).

Discussion

The eye determines the ability to feed, search, distinguish objects and orient in a three dimensional light environment (Chai et al., 2006). Therefore, major events in the functional ontogeny of the visual system are closely correlated with life history events where 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).

Several studies found that many fishes possess only cone photoreceptor cells 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). The appearance of rod photoreceptor cells in the retina delay until the larvae move to deeper water (Chai et al., 2006; Ebbessen et al., 2007; Hall and Wake, 1999; Lenkowski and Raymond, 2014). At hatching, the retina of the Caspian roach larvae had composed of well-differentiated photoreceptor cells in contrast to many fish species (Chai et al., 2006), showing their dependence on visual capability as visual feeder during early development. Prior to initiation of exogenous feeding at 3-5 dph, whole layers of the retina were present in the Caspian roach larvae, illustrating the importance of visual sense for its exogenous feeding. In addition, the complete development of the choriocapillar layer, which supply the high oxygen demand of the eye, up to 3 dph was in accordance with formation of other structures of the eye in this species. Increasing of the rod photoreceptor cells of in older Caspian roach specimens i.e. about 35 dph shows switching its habitat preference to deep water like many bottom feeders e.g. yellow perch (*Perca flavescens*) (Whal et al., 1993).

Allometric growth pattern of the eye diameter up to the inflexion point was somewhat positive, and it became negative after this point i.e. the most of the ontogenesis and differentiation of the eye structure had been completed before 3 dph. Larger eye diameter commonly accommodates the larger eye lens. Therefore, more light can be gathered and a higher resolution image can be generated in the brain. The greater eye size also provides the better visual sensitivity to the fish especially under dim light conditions with its better light gather feature (Lim et al., 2014). In addition, changes in allometry of morphometric characters are hypothesized to be related to many functions such as predator avoidance and feeding (Yúfera and Darias, 2007). After exhaustion of the yolk-sac, larvae need to shift from endogenous to exogenous feeding, as a result, the positive allometry of those structures involved in exogenous feeding i.e. eye is predicted. Positive allometry of eye diameter in early larvae (0-7 dph) confirmed this hypothesis and its critical role in feeding, prey detection, schooling behavior and predator avoidance (Rodríguez and Gisbert, 2001; 2002). Our results shows that the Caspian kutum is mainly eye-dependent during their larval period which itself explains completion of eye structure particularly the retina and the high growth rate of eye before starting mixed feeding.

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