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Eye Structure and Chemical Details of the Retinal Layer of Juvenile Queen Danio *Devario regina* (Fowler, 1934)

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ABSTRACT

The eye structures and chemical details of the retinal layer in juvenile Queen Danio—*Devario regina*, an ornamental fish—were histologically investigated under a light microscope. Sample fish were collected from the Tapee River, Nakhon Si Thammarat province, Thailand and their heads were exclusively prepared using a standard histological technique. The results revealed that the eye of *D. regina* was composed of three layers—inner, middle and external—based on histological organization and cell types. The inner layer was composed of 10 layers; 1) pigment epithelium, 2) photoreceptor layer, 3) outer liming membrane, 4) outer nuclear layer, 5) outer plexiform layer, 6) inner nuclear layer, 7) inner plexiform layer, 8) ganglion cell layer, 9) optic nerve layer and 10) inner limiting membrane, respectively. The localization and chemical details showed that a periodic acid-Schiff reaction for the detection of glycoprotein was intensive in the pigment epithelial layer whereas the inner plexifrom layer had only a slight reaction. Reaction of aniline blue was employed for the detection of mucopolysaccharide which was slightly positive for three layers—the outer limiting membrane, outer plexiform and inner plexiform.

Keywords: eye, histology, *Devario regina*, histochemistry

INTRODUCTION

The eye is a specialized organ for the detection and analysis of light. The eye of a fish is broadly similar to other vertebrates, like amphibians, reptiles, birds and mammals, but it is generally denser and more spherical in structure (Genten *et al*., 2008). In an aquatic environment, there is no difference in the refractive index of the cornea and the surrounding water and therefore, the lens has to do the majority of the refraction (Land and Nilsson, 2012). "Due to a refractive index gradient within the lens exactly as one would expect from optical theory", the spherical lenses of fish are able to form sharp images free from spherical aberration (Wehner, 2005). Unlike humans, fish normally adjust focus by moving the lens closer to or further from the retina (Campbell

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and Reece, 2005; Trevor, *et al.,* 2007). Light passes through the lens and is transmitted through a transparent liquid medium until it reaches the retina. The eyes are an important organ for teleosts but the histological information is not known. Therefore, the eye has been investigated for both histological structures and functions including in *Bovichtus diacanthus, Cottoperca gobio* (Eastman and Lannoo, 2007), *Astronotus ocellatus*, *Barbus caudovitatus* (Genten *et al*., 2008), *Danio rerio* (Haug *et al*., 2010), *Siganu javus* (Mansoori *et al.,* 2012; Sattari *et al*., 2012) and *Puntius stoliczkanus* (Senarat *et al.,* 2013) from which the following information was sourced (Genten *et al*., 2008; Senarat *et al.,* 2013). All examinations revealed that the eye is a fluid chamber enclosed by three layers of tissue. The outer layer of the eye consists of the cornea and sclera. Both these tissues consist mainly of collagen fibers. The cornea is the principal refractive element of the eye whereas the sclera is rigid and resistant to penetration and thus is able to protect the more delicate inner layer. The middle layer includes the iris, ciliary body and choroid. The iris is a layer disposed in the anterior pole of the eye that limits the quantity of light passing into the eye through the pupil. The ciliary body is adjacent to the iris and it consists of a ring of muscle cells (Germain *et al*., 2010). The ciliary body muscle is part of the system for altering the refractive power of the lens. The choroid is composed of blood vessels and pigmented epithelium. These epithelia are composed of dense melanin pigment to absorb and eliminate scattered light that might otherwise degrade the image. The inner layer is the retina that receives inputs from photodetectors. "The retina works by detecting light in the retinal image and sending it to the brain" (Germain *et al*., 2010).

Devario regina (Fowler, 1934) is important as an ornamental fish but information on its eye histology is not known. Therefore, this study began to clarify the detail of the structural organization and chemical details of the eye of juvenile *D. regina*. This information offers to increase not only the histological knowledge of the juvenile stage of this fish but also within the Cyprinids group.

MATERIALS AND METHODS

Juvenile *Devario regina* (sample number = 30; total length 2–3 cm) were collected during the fishing season (January to April 2012) from the Tapee River, Chawang district, Nakhon Si Thammarat province, Thailand (8°28'10" N, 99°29'45" E). All fish were euthanized using a rapid cooling technique (Wilson *et al.,* 2009). The specimens were kept and rapidly fixed in Davidson's fixative (24–36 hr). They were processed using standard histological and histochemical techniques (Bancroft and Gamble, 2002). The sections were cut at 5–6 μm thickness and stained with Harris's haematoxylin and eosin (H&E) for histological study. Other sections were stained with periodic acid-Schiff (PAS) and aniline blue (AB) for histochemical study. The structure and details of the eye of these fish were investigated under a light microscope.

RESULTS AND DISCUSSION

Histological structure of the eye

Based on H&E staining, the histological micrograph of the *D. regina* juvenile eye was composed of three layers comprising an inner, middle and external layer (Figures 1a–1f). The inner layer of the eyes was the retina that was composed of ten layers: 1) the pigment epithelium was a black or brown layer that contained melanin pigment. This layer was close to the choroid layer (Figure 1c); 2) the photoreceptor layer was associated with rod and cone cells. The rod cell was long and ellipsoid in shape while the cone cell was conical and a bulbous ellipsoid (Figure 1d)**.** The function of the rod cell was to sense light and dark conditions while the function of the cone cell was to be sensitive to the color spectrum. However, in deep-sea fish only rod cells are found

in this layer because they are adapted for low light conditions. The rod cells are very sensitive to low light signals. (Genten *et al*., 2008); 3) the outer liming membrane was not clearly visible; 4) the outer nuclear layer contained the nuclei of the photoreceptor cells; 5) the next layer was the outer plexiform layer; 6) the inner nuclear layer consisted of bipolar cells that were connected to photoreceptors (Figure 1d); 7) the next layer was the inner plexiform layer; 8) the ganglion cell layer was composed of a narrow chain of granular and spherical cells that were surrounded by a fine connective tissue network (Figure 1d). In this layer, the nucleus receives stimuli from the bipolar elements and sends them to the fibers (Germain *et al.,* 2010); 9) the optic nerve layer of ganglion cell axons forms the optic nerve and nerve fiber along the optic nerve to the brain; and 10) the last layer was the inner limiting membrane. In addition, this layer also contained preretinal vessels (Figure 1c). Generally, the retina of vertebrates is formed by different cells and contains major cell types; for example the photoreceptors (rods and cones), bipolar and ganglion cells (Méndez-Vilas and Díaz, 2010). Some fish species such as the cave fish can be distinguished by showing a slightly lesser degree of reduction in eye size and pigmentation (Wilkens and Strecher, 2003).

The middle uveal layer of *D. regina* contained the choroid, lens and iris (Figure 1b–1f). Based on the current study, the choroid in this stage was rarely seen. However, its functions in teleosts is to transfer the nutrients and regulate the temperature. Moreover, it is associated with a network of capillaries which are active in oxygen secretion and is considered to be related to ensuring a high level of oxygen for the retina (Germain *et al.,* 2010; Sattari *et al*., 2012). The lens of juveniles in this fish species is prominent and appeared as a transparent, spherical ball that is agloboidal in shape (Figure 1b). The inner eye muscles can change the globoid shape of the lens to focus the image. The lens also consisted of three parts (Figure 1e). First, were the encapsulated sheaths of non-cellular transparent material, which was secreted as protein by the cell. An underlying layer of encapsulated sheaths consisted of a monolayer of epithelial cells which nucleated and were capable of division and secretion. The third tissue consisted of the lens fibers, which constituted most of the lens volume. These fibers were long, slender, transparent, non-nucleated cells lying in layers of long, parallel rows (Mumford *et al.,* 2007). Each layer of the fish lens was loosely made up of cells whereas the mammalian lens is elastic and can be modified in shape by contraction of the eye muscles (Mumford *et al.,* 2007). The iris was covered by loose, connective tissue of iridial stroma, blood vessels and the layer of pigment cells (Figure 1f). The external layer of the eyes, sclera and cornea (the sclera-corneal layer) exhibited poor development.

Histochemistry of retinal layer

Detection and localization of mucopolysaccharide

AB staining was employed for the detection of mucopolysaccharide as a bluish color which was apparent as slight positive staining in the three layers of the outer limiting membrane, the outer plexiform and the inner plexiform. Therefore, this confirmed that these layers can produce mucopolysaccharide (Figures 2a and 2b).

Detection and localization of glycoprotein

In the organization and cell layers, the PAS reaction showed a purple color which was intensively present in the pigment epithelial layer whereas the inner plexiform layer showed a slight reaction. Thus, these layers indicated the production and secretion of glycoprotein (Figures 2c and 2d).

CONCLUSION

This study first reported on the organization of the eye in the juvenile fish especially chemical information. This research indicated that some layers of the retina contained

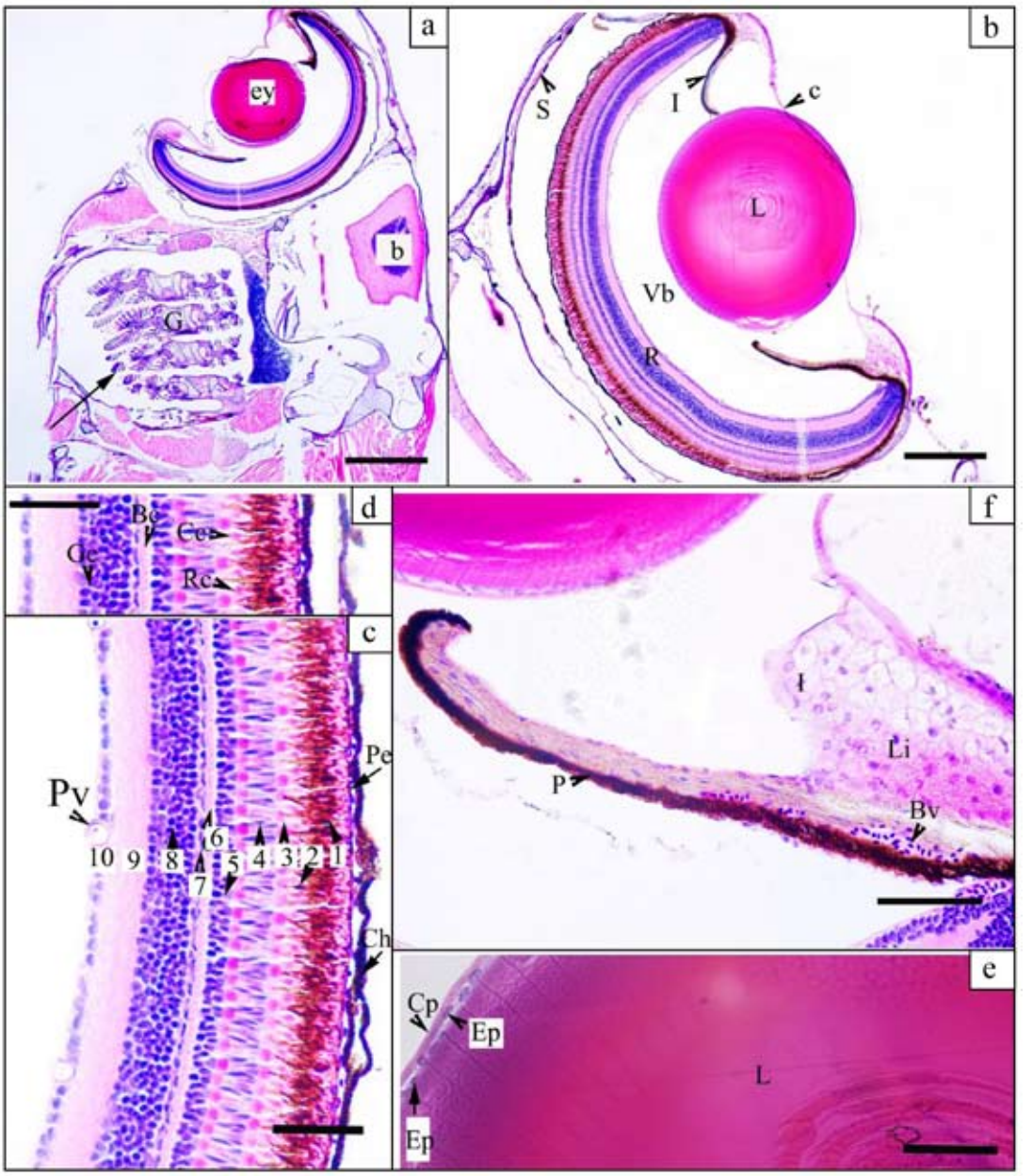

Figure 1 Micrograph showing eye structure and retinal layer: 1) Pigment epithelium (Pe) mostly contains melanin; 2) photoreceptor layer (rod and cone processes); 3) outer liming membrane; 4) outer nuclear layer consisting of the nuclei of the photoreceptors; 5) outer plexiform layer; 6) inner nuclear layer; 7) inner plexiform layer; 8) ganglion cell layer; 9) nerve fiber layer; 10) internal liming membrane. (b = brain; Bc = bipolar cell; Bv = blood vessel; c = cornea; Cc = cone cell; Ch = choroid; Cp = encapsule; ; ey = eye; G = gill; Ep = monolayer of epithelial cells; Gc = ganglion cell; I = iris; L = lens; Li = loose connective of iridial stroma; P = pigment layer; Pv = preretinal vessel; R = retina; Rc = rod cell; S = sclera; Vb = vitreous body; Bar scales = 150 µm (a); 100 µm (b); 20 µm (c–f); staining with Harris's haematoxylin and eosin).

both glycoprotein and mucopolysaccharide. This finding provides basic information for histopathology and fisheries science. Further research should involve comparative study with the adult fish eye based on structural, chemical and ultrastructural analysis.

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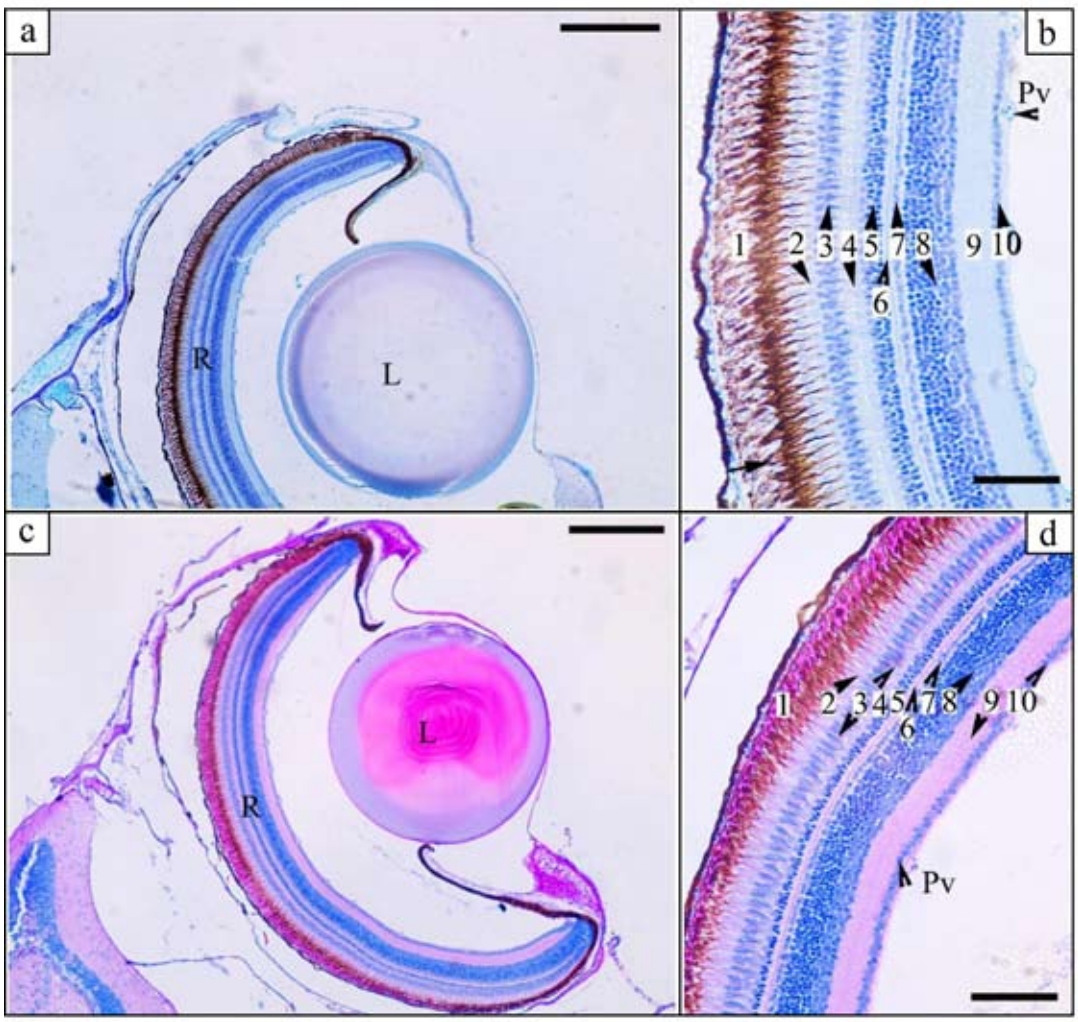

Figure 2 Micrograph showing chemical detail of renal layers: 1) pigment epithelium mostly contains melanin; 2) photoreceptor layer (rod and cone processes); 3) outer liming membrane; 4) outer nuclear layer consisting of the nuclei of the photoreceptors; 5) outer plexiform layer; 6) inner nuclear layer; 7) inner plexiform layer; 8) ganglion cell layer; 9) nerve fiber layer; 10) internal liming membrane. (L = lens; Pv = preretinal vessel; R = retina; bar scales = 100 μm (a; c); 20 μm (b;d); Staining = aniline blue for (a and b); and periodic acid-Schiff (c and)).

LITERATURE CITED

Bancroft, J.D. and M. Gamble. 2002. **Theory and Practice of Histological Techniques.** Churchill Livingstone. London, UK.

Campbell, N.A. and J.B. Reece. 2005. **Biology,** 9th ed. Benjamin Cummings. San Francisco, CA, USA. 1472 pp.

Eastman, J.T. and M.J. Lannoo. 2007. Brain and sense organ anatomy and histology of two species of phyletically basal non-Antarctic thorn fishes of the *Antarctic suborder* Notothenioidei (Perciformes: Bovichtidae). **J. Morphol.** 268: 485–503.

Genten, F., E. Terwinghe and A. Danguy. 2008. **Atlas of Fish Histology.** Science Publishers. Enfield, NH, USA, 223 pp.

Germain, F., C. Pérez-Rico., J. Vicente and P. de la Villa. 2010. Functional Histology of the Retina, pp. 914–925 *In* A. Méndez-Vilas and J. Díaz (eds.). **Microscopy: Science, Technology, Applications and Education.** Formatex Research Center. Badajoz, Spain.

Haug, H.F., O. Biehlmaier, K.P. Mueller., C.F. Stephan and S.C. Neuhauss. 2010. Visual acuity in larval zebrafish: behavior and histology. **Front Zool.** 7: 1–8.

Land, M.F. and D. Nilsson. 2012. **Animal Eyes.** Oxford University Press. Oxford, UK. 221 pp.

Mansoori, F.S., A. Sattari, R. Kheirandish and M. Asli. 2012. A histological study of the outer layer of rabbit fish (*Siganus javus*) eye. **Comp. Clin. Pathol.** DOI 10.1007/s00580-012-1582-7.

Mumford, S., J. Heidel., C. Smith., J. Morrison., B. MacConnell and V. Blazer. 2007. **Fish Histology and Histopathology.** U.S. Fish and Wildlife Service's National Conservation Training Center. Shepherdstown, WV, USA

Sattari, A., M. Asli., F.A. Mansoori., R. Kheirandish and H. Yavari. 2012. Histological study of middle layer of rabbit fish eye (*Siganus javus*). **Asian Pac. J. Trop. Biomed.** 52: 1086–1089.

Senarat, S., J. Kettretad., W. Yenchum., P. Poolprasert and N. Kangwanrangsan. 2013. Microscopic organization of the eye of Stoliczkae's barb *Puntius stoliczkanus* (Day, 1869). **Kasetsart J. (Nat. Sci.).** 47: 733–738.

Trevor, D.L., S.P. Collin and E.N. Pugh. 2007. Evolution of the vertebrate eye: Opsins, photoreceptors, retina and eye cup. **Nat. Rev. Neurosci.** 8: 960–976.

Wehner, R. 2005. Sensory physiology: brainless eyes. **Nature** 435: 157–159.

Wilkens, H. and U. Strecher. 2003. Convergent evolution of the cave fish *Astyanax* (Characidae, Teleostei): Genetic evidence from reduced eye-size and pigmentation. **Biol. J. Lin. Soc.** 80: 545–554.

Wilson, J.M., R.M. Bunte and A.J. Carty. 2009. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). **J. Am. Assoc. Lab. Anim. Sci.** 48: 785–789.