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Research Article

A comparison of the retinal structure in the zebra-snout seahorse (*Hippocampus barbouri* Jordan & Richardson, 1908) between juveniles and adults in captivity

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Abstract
The activity of the sensory organ in the eye structure of the teleost fish is essential, as it plays an important role in regulating fish-feeding behaviors. Unfortunately, the above information in reference to the zebra-snout seahorse *Hippocampus barbouri*, an aquaculture species in Thailand, has not been described. In this study, the eye structure, together with the retinal structure, of juvenile [5th and 20th day after birth (DAB)] and adult (35th DAB), *H. barbouri* reared in captivity was investigated. All DABs were carried out and histologically observed. Light microscopic level explored the external-lateral surface of the eye structure of *H. barbouri*, which consisted of the external, middle, and inner layers, as similarly reported in other teleost species. Well-differentiated retinal and photoreceptor cell layers were observed at 35th DAB compared to that of other DABs. This finding supports feeding strategies in captivity, helping the Thailand "circular aquaculture" of this fish.

1. Introduction

The eye of fish is considered to be the major visual system and serves as an important sensory organ to support mating, feeding, and predator avoidance (Genten et al., 2008; Yokoyama & Yokoyama, 1996; Bowmaker, 1991). The anatomy and histological structure of the eye is an important topic in several fishes (Perez et al., 2017; Boonyoung et al., 2015; Senarat et al., 2013; Genten et al., 2008). The remarkable retinal layer of most fish includes the neuron and neuronal

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fiber, especially cone and rod cells (Genten et al., 2008). The retinal layer is thought to be mediated by the absorption of photons under the process of visual transduction. Single and double rods accompany the cones with a regular arrangement, which allows for possibility of color and visual acuity (Genten et al., 2008). In contrast, the structure of rods is hardly identifiable or able to function under low light conditions (Flamarique & Harosi, 2000). Based on the different photic habitat conditions and ecological life, the variation of these cells in terms of their density and patterns might differ among fishes (Thomas & Craig, 2010; Kim et al., 2014) and life cycle stages (Pavón-Muñoz et al., 2016).

During the early larval stage, the eye ontogeny of teleost fish, i.e., *Paralichthys olivaceus* (Kawamura & Ishida, 1985), *Tilapia nilotica* (Kawanura & Washiyama, 1989), and *Oxyeleotris marmoratus* (Senoo, Kaneko, Cheah, & Ang, 1994), show only pure-cone cells. Only rod cells were exclusively detected in *Anguilla anguilla* and *Macruridae* larvae (Blaxter & Staines, 1970). Two important fish species, namely *Tinca tinca* (Bejarano-Escobar et al., 2009) and *Solea senegalensis* (Bejarano-Escobar et al., 2010), exhibited an undifferentiated retina. Further, their retinal development is not completed until the late larval stage. Based on the fundamental importance of the eye development mentioned above, the possibility exists that rod-cone interactions play a vital role for the feed consumption and behavioral activation of fish.

The zebra-snout seahorse *Hippocampus barbouri* (Jordan & Richardson, 1908) is an economically valuable species belonging to the family Syngnathidae. The circular aquaculture systems of this seahorse have been principally conducted by the Phuket Marine Biological Center (PMBC), Thailand. However, the fundamental question concerning the eye, with an emphasis on the retinal layer, of *H. barbouri* has never been addressed. The eye structure, with the initial evidence of the eye and retinal structure between juveniles ([5th and 20th day after birth (DAB)] and adults (35th DAB), was investigated concerning predictive behavioral activity.

2. Material and methods

2.1 Seahorse sampling and hatchery area

Dead specimens of zebra-snout seahorses (*H. barbouri*), were gifted as voucher specimens from Kamnurdnin (2017). Briefly, they were reared in a recirculating aquaculture system. They were kept in cement concrete tanks filled with seawater (temperature ~26 - 28 °C, salinity ~31 - 33 ppt) and fed with wild krill twice per day. All animals were maintained under a 12/12 h light/dark cycle. Every three samples of seahorses were randomly chosen at 5th, 20th, and 35th days after birth (DAB). A preliminary investigation warranted that the adult stage of *H. barbouri* in captivity was proposed as individuals over 30-days of age, from which the appearance of secondary growth developed (Kamnurdnin, 2017). The experimental protocol was approved by the Animal Care and Use Committee of the Faculty of Science following the guidelines for the care and use of laboratory animals prepared by Chulalongkorn University (Protocol Review No. 1623004).

2.2 Anatomy and histological analysis of eyes

All dissected eyes from different sampled times were processed using a standard histological technique (Presnell & Schreibman, 2013; Suvarna et al., 2013). Paraffin blocks were sectioned at 4 µm thickness and then stained with Harris's hematoxylin and eosin (H&E), Periodic acid-Schiff (PAS), and Masson's Trichrome (MT). The localization and histology of the eye were observed and photographed by a TE750-Ua camera (Leica, Heidelberg, Germany).

2.3 Morphometric analysis of retinal and photoreceptor cell layers

All histological slides of the eye were collected. The parameters of the retina and photoreceptor cell layers were measured from three located areas of the retina using Image J software version 4.9. The results were presented as mean ± S.D. Statistical comparisons of the data were performed using the ANOVA test, followed by Tukey's HSD Post Hoc analysis with SPSS (version 25.0) software package for Windows (IBM Corp, 2017).

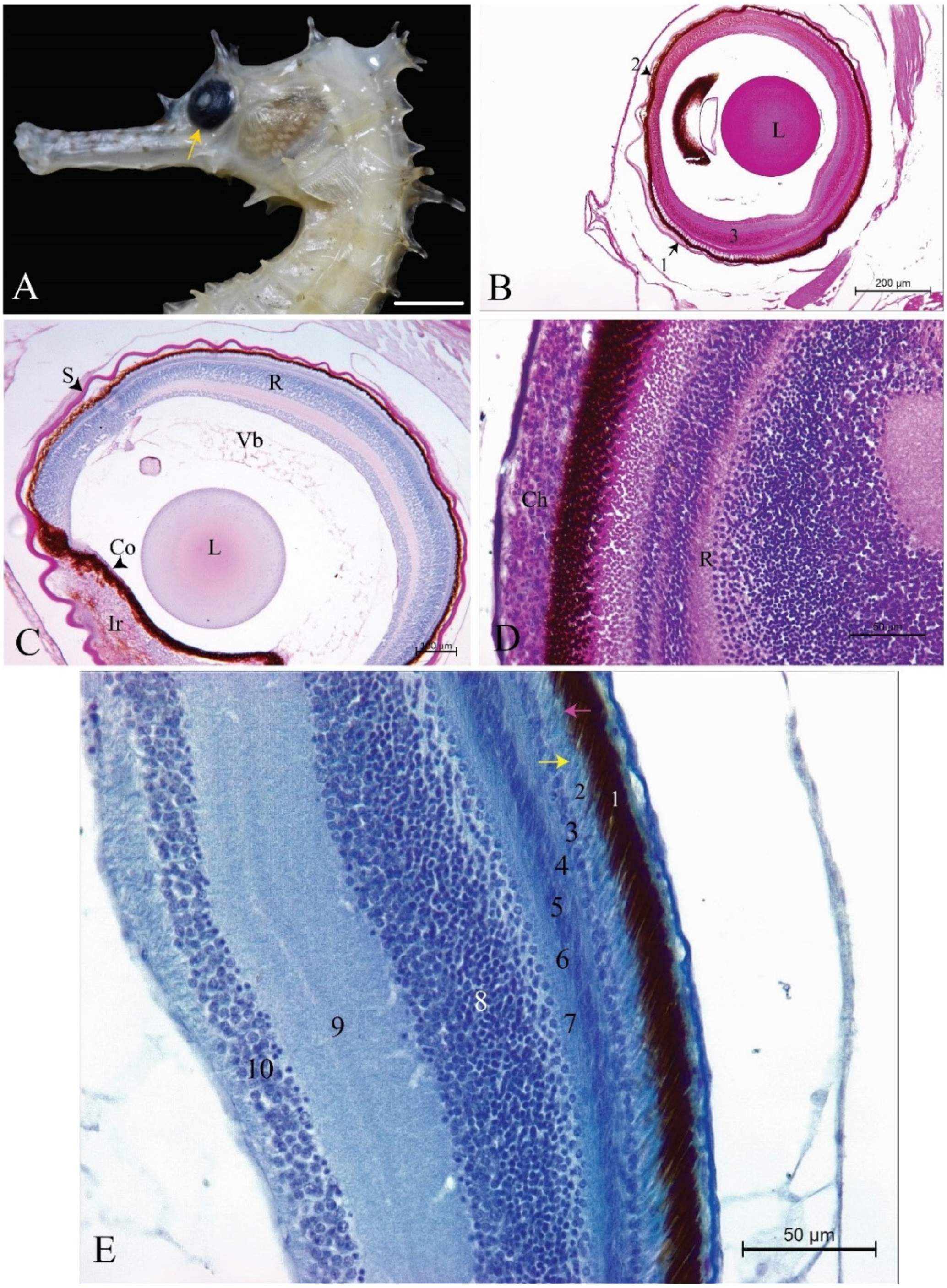


Figure 1 Morphology (A) and light microscopic level (B-E) of *H. barbouri* eye. A: eye morphology (yellow arrow). B: Overall structure of the eye, composed of external (1), middle (2), and inner (3) layers. C: Histology of the eyeball showed several compositions including the lens (L), iris (Ir), cornea (Co), sclera (S), vitreous bod (Vb), and retina (R). D: A network of capillaries showing in the choroid (Ch) was identified. E: The retinal histology comprised: 1) pigment epithelium; 2) photoreceptor layer (rod and cone processes); 3) outer limiting membrane; 4) outer nuclear layer; 5) outer plexiform layer; 6) inner nuclear layer; 7) inner plexiform layer; 8) ganglion cell layer; 9) nerve fiber layer; 10) internal liming membrane.
Scale bra A = 0.3 mm

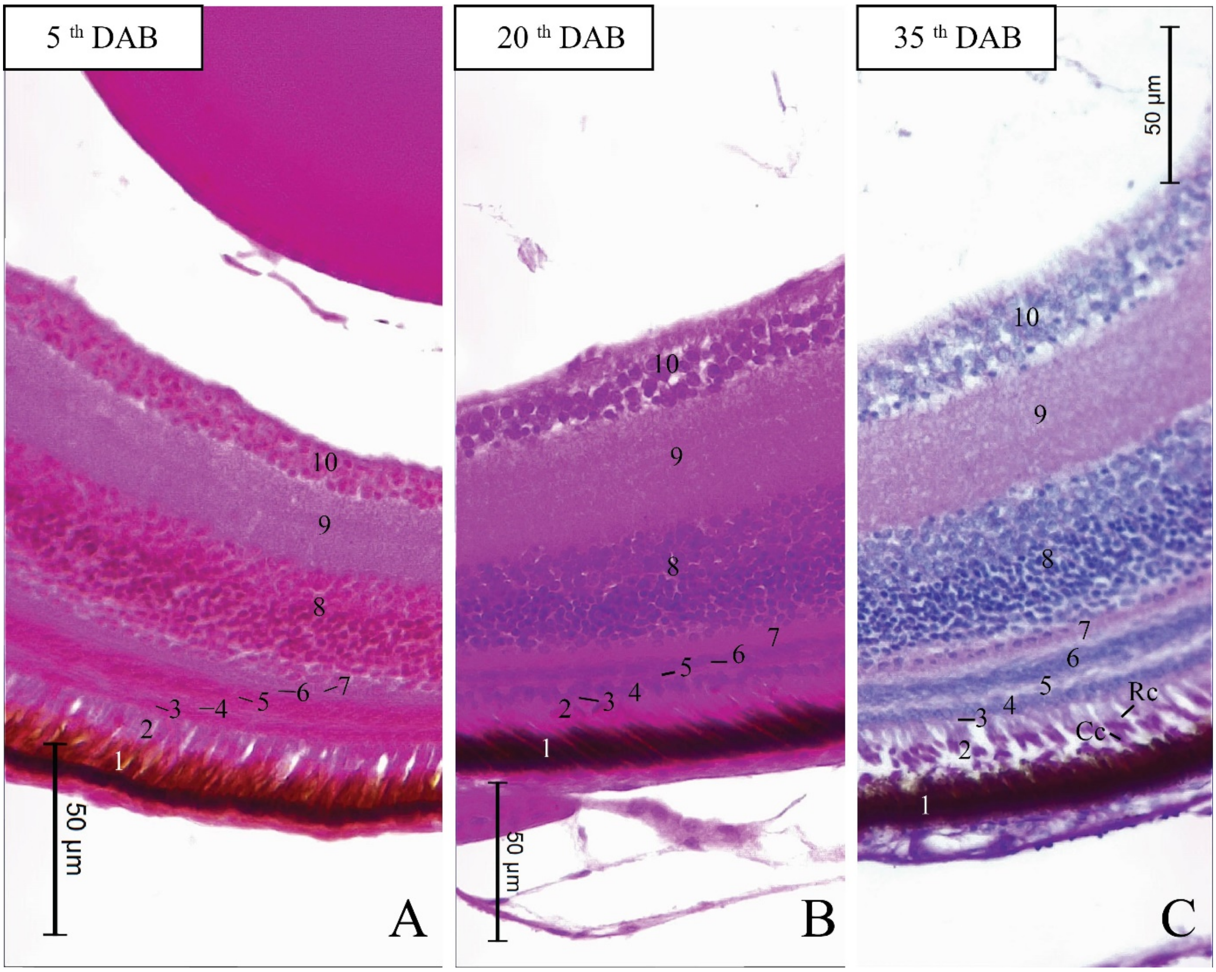


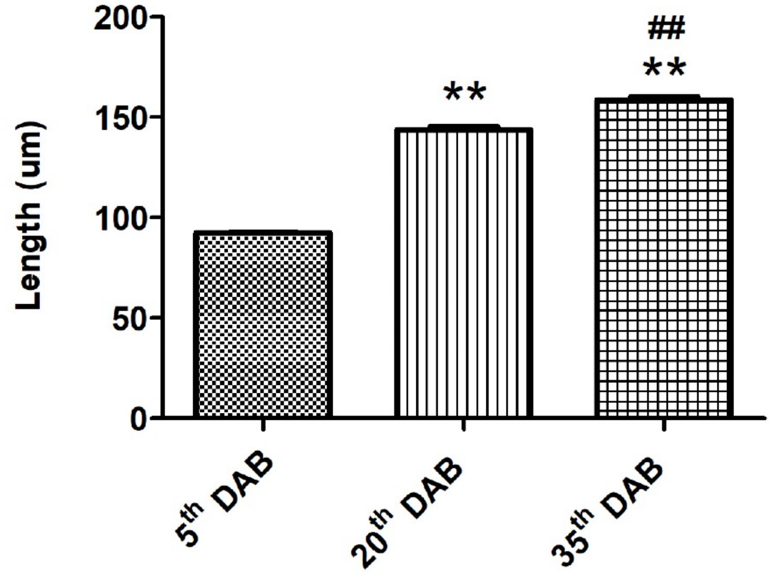
Figure 2 Light microscopic level of the retinal development of *H. barbouri* during the 5th DAB (A), 20th DAB (B), and 35th DAB (C). They were identified and composed of ten layers, including 1) pigment epithelium; 2) photoreceptor layer (rod and cone processes); 3) outer limiting membrane; 4) outer nuclear layer; 5) outer plexiform layer; 6) inner nuclear layer; 7) inner plexiform layer; 8) ganglion cell layer; 9) nerve fiber layer; 10) internal liming membrane.

3. Results and discussion

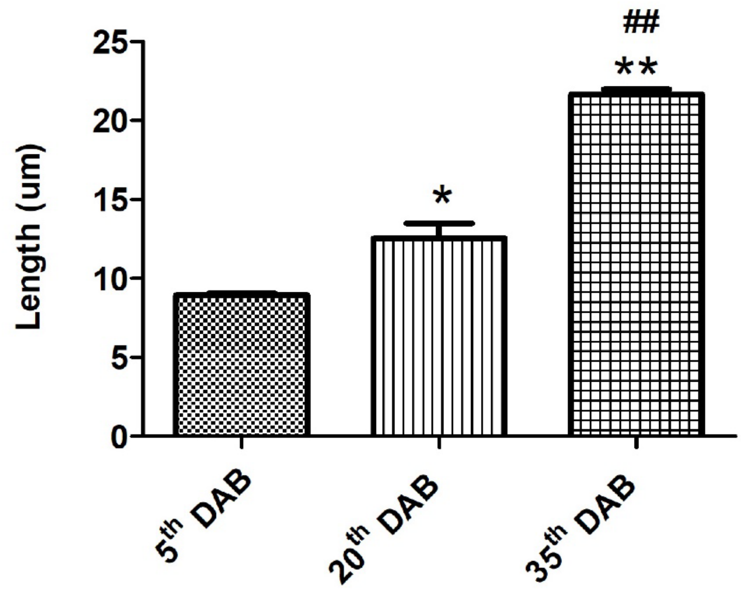
Macroscopically, a lateral-eyed structure of *H. barbouri* was observed from the distinctive conical snout (**Figure 1A**). A spherical lens eye was observed (**Figure 1B**), which reflected the loss of the refractive power of the cornea when the seahorse lives in a shallow-water environment (Collin, 1997). Most adult fish have well-developed eyes, which are likely to be effective for prey location as well as predator detection (Hawryshyn 1991; Browman et al., 1990).

The external-lateral surface of the *H. barbouri* eye was histologically divided into the external, middle, and inner layers (**Figure 1B**), similar to the other investigations (Boonyoung et al., 2015; Genten et al., 2008). The external layer (the sclera-corneal layer) contained the cornea and sclera (**Figure 1C**). The middle uveal layer of the eye was identified by the choroid and iris (**Figure 1C**). A choriocapillaris with a network of capillaries of the choroid was identified (**Figure 1D**). The likely role of this layer is to supply oxygen to the retina and an inner pigmented cell layer (Zyznar & Ali, 1975).

Inside the retina of the *H. barbouri*, an inner layer was found. This structure could be further divided into ten specific layers, including 1) pigmented epithelium, mainly containing melanin; 2) photoreceptor layer (rod and cone processes); 3) outer lining 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 that synapses with the optic nerve; and 10) internal lining membrane (**Figure 1E**). A photoreceptor vision-cell layer of *H. barbouri* displayed a long rod and a single small cone, as is consistent with a previous report of syngnathid fish (Collin & Collin, 1999). This was probably related to an increasing sensitivity of fish under low light conditions (Warrant, 2004; Wagner, 1990).



A



B

Figure 3 The retinal (A) and photoreceptor cell (B) layer thickness of *H. barbouri* eye. Values represent means ± SE; symbols (* and #) indicate significant differences at $p < 0.05$.

Light microscopic level and morphometric analysis of the retinal development of the *H. barbouri* (**Figures 2** and **3**) showed that the *H. barbouri* at 5^{th} DAB with clear classification of the ten layers was found (**Figure 2A**). Both retinal thickness and the photoreceptor cell layer from the 5^{th} to 35^{th} DAB were increased dramatically, which were of a statistically significant difference ($p < 0.05$) (**Figures 2A, 2B, 3A, 3B**), indicating that this is concerned with visibility, capturing prey, and feeding. Similar reports concerning seahorses showed that this fish group is a visual feeder and can capture prey like small shrimp and plankton (Lee, 2013). Similar to a previous report, seahorses are predators that use high resolution acuity for prey detection (Lee & Bumsted O'Brien, 2011). A previous study by Mass (2007) focused on photoreceptor density, including the retinal layer thickness of *H. abdominalis*. It was found that this change was related to environmental light and background color. In *H. abdominalis*, there was an adaptation, as seen by the varied layer thicknesses of the retina to different colors during a span of 56 days. The results of this study were also similar to that of Ofelio and colleagues (2018). They found that the thickness of photoreceptors and retinal layers in *H. guttulatus* increased significantly at 118 days of age.

4. Conclusions

The eye structure of seahorse *Hippocampus barbouri*, as the photoreceptor cell layer, developed from the 5^{th} to 35^{th} DABs. This finding supports feeding strategies in captivity, helping the Thailand "circular aquaculture" of this fish.

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