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Original Article

The relative eye size, visual cells, cone mosaic and retinal tapetum in the spotted barb *Puntius binotatus* (Valenciennes, 1842)

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Abstract: The relative eye size, types of visual cell and mosaic, and the existence of retinal tapetum in the eyes of the spotted barb *Puntius binotatus* were determined to gain baseline information on its visual capability. The *P. binotatus* acquired relatively larger eye size than the other similar sized freshwater fish species with its retina being contained both cone and rod visual cells (cone ellipsoid to outer nuclei ratio = 1: 5.7). Three types of cone were identified (double-, central single- and corner single- cones), arranging in the square mosaic, and the retinal tapetum was determined to be existed. These results evidenced that the *P. binotatus* has good visual capability as it possessed both the photopic and scotopic visions. Vision can be the primary sense for the *P. binotatus*. Further study is needed to gain more information on the vision of this species.

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Introduction

Vision is generally known as the primary sense for most fish species, as they rely on it to detect preys and predators for survival. Fish visual capability is highly related to the eye structures. Therefore, studying on the eye and its retinal morphology can provide insight to the visual capability of the fish (e.g. Nag and Bhattacharjee, 2002).

The spotted barb *Puntius binotatus* is a cyprinid (Roberts, 1989; Robins et al., 1991), found in the mountainous streams, rivers and lakes of the Southeast Asian countries, including Cambodia, Indonesia, Laos, Malaysia, Philippines, Thailand and Vietnam and introduced species to Singapore (Talwar and Jhingran, 1991; Rainboth, 1996; Jenkins et al., 2009). Due to its common availability, *P. binotatus* has been recognized as an important bio-indicator to the environmental bio-degradation status of these freshwater resources (Baumgartner, 2005; Zakeyudin et al., 2012). In addition, it is an economically important species in Mindanao of Philippines (Dorado et al., 2012). Despite of its common availability, little information is available about its biology.

In Sabah, *P. binotatus* is locally known as "Turongou" and popular to the native people as a deep-fried delicacy. With aim to promote the delicacy at commercial level, the first hatchery and pond culture has been setup by a local company, Innovasi Sedia Private Limited, for the production of this fish (Lim et al., 2013). Such operation has provided a great opportunity for studying its biology, which is useful for understanding the ecology and to improve the culture techniques of this species. In the pond culture, the *P. binotatus* has been observed to swim to water surface and snap on the given floating commercial pelleted feed accurately. Such feeding behaviour is similar to those observed in the visual feeder species, such as the trout and grouper (Ahlbert, 1976; Tan et al., 2013). Therefore, the

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Table 1. Calculation of the relative eye size for *P. binotatus* (n = 33).

	Minimum	Maximum	Mean	Standard deviation
Standard length, SL (cm)	3.60	4.90	4.22	0.320
Eye diameter, ED (cm)	0.25	0.40	0.31	0.035
Relative eye size, ED /SL	0.06	0.09	0.07	0.006

Table 2. Calculation of the cone ellipsoid-outer nuclei ratio within 100 μm for *P. binotatus* (n = 5).

Cone ellipsoids (CE)				Outer nuclei (ON)				CE: ON
Min	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**(from means)**
22	33	25.6	4.3	133	158	145.2	11.3	1: 5.7

P. binotatus may rely much on its vision to detect objects. Nonetheless, there has been no information on the vision of this species. For this reason, the present study aimed to examine the relative eye size and its retinal morphology to find baseline information on the visual capability of the cultured *P. binotatus*.

Materials and methods

Sampling and measurements: A total of 33 cultured *P. binotatus* were sampled from several ponds, managed by the local company Innovasi Sedia Private Limited. The specimens were then transferred to the Wet Laboratory in Borneo Marine Research Institute, Universiti Malaysia Sabah. Subsequently, each fish were measured for their standard length (SL, in mm), and eye diameter (ED, in mm). Prior to measurement, the specimens were anesthetized with 200 ppm Tricaine methanesulfonate (MS222) and measurements were carried out with a ruler. The relative eye size (RES) of *P. binotatus* was calculated through dividing ED by SL.

Histological procedures: After the measurements, the specimens were decapitated, and the head were fixed in Bouin's solution for 24 hours. The samples were subsequently transferred into a series of ethanol with ascended concentrations (70 % to 100 %), cleared with xylene, and finally embedded into paraffin. The samples were sectioned at 6 μm and stained with haematoxylin and eosin. The sections were examined under a light microscope (Eclipse 80i, Nikon, Japan) attached to a computer, and photos were taken using the imaging software NIS-Elements F 3.0 (Nikon, Japan).

Retinal Observation: From the histological sections, the types of visual cell and cone mosaic were identified, and the existence of retinal tapetum was determined. For identifying the types of retinal cone and its mosaic, tangential sections of the retina were observed. Retinal rods were difficult to resolve by light microscope hence its existence was determined using the calculation on the ratio of the cone ellipsoids (CE) number to the outer nuclei (ON) number within 100 μm length of retina. The retinal rods were assumed existed if the CE to ON ratio was more than 1: 1 (Blaxter and Staines, 1970). The presence of retinal tapetum was ascertained using a microscope with a guide light.

Results

Relative eye size: Table 1 demonstrates the measurements on the standard length, eye diameter and RES of the cultured *P. binotatus*. The size of fish examined in the present study was in the range of TL from 3.60 cm to 4.90 cm. The RES value was 0.07 ± 0.006 (mean ± S.D.).

Cone and mosaic types: Figure 1a shows the tangential section on the retinal temporal region of the *P. binotatus*. Three types of cone: the double cone, the central- and the corner-single cones were identified, arranging in a square mosaic (Fig. 1b).

Presence of retinal rods: Table 2 shows the calculation on the number of cone ellipsoids (CE), outer nuclei (ON) and the ratio of CE to ON. The CE: ON was determined to be 1: 5.7 from the mean

Table 3. Reviewed information on the relative eye sizes of several cyprinids from Nag and Bhattacharjee (2002).

Cyprinids	SL (cm)	ED (cm)	ED /SL
Garra gotyla gotyla	12.0	0.52	0.04
Garra lamta	9.0	0.38	0.04
Barilius bendelisis bendelisis	8.0	0.43	0.05
Barilius vagra vagra	8.0	0.45	0.06
Schizothorax richardsonii	24.0	1.12	0.05
Acrossocheilus hexagonolepis	7.0	0.42	0.06
Danio aequipinnatus	7.0	0.41	0.06

numbers of the CE and ON, proving the presence of the retinal rods.

Existence of retina tapetum: The retina of *P. binotatus* was shining when it was torch-lighted under the microscope without a light source (Figs. 2a-b). Therefore, retinal tapetum existed in the eye of the *P. binotatus*.

Discussion

This is the first report on the vision of the *P. binotatus*. In the present study, the RES mean value was 0.07, indicating that the *P. binotatus* acquired quite large size of eyes, in comparison with some other similar or larger sizes of cyprinids, which their RES ranged generally from 0.04 – 0.06 (Table 3). The greater eye size commonly accommodates the larger eye lens than the smaller one. 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 under dim light conditions with its better light-gather feature. The relative large eye size in *P. binotatus* therefore, suggested that this species may have good vision under bright condition, and also able to see in dim light. Indeed, the presence of retinal rods and tapetum further supports the idea that the *P. binotatus* may possess scotopic vision. Retinal rods is the visual cells, which is responsible for scotopic vision due to its wider area of outer segment for light absorption (Evans, 2004) while the retinal tapetum is the light-reflecting system which reflects light back into the retinal visual cells layer hence more light can be absorbed by the retinal rods (Bone et al., 2004), increasing the fish visual sensitivity under the dim light (Somiya, 1980).

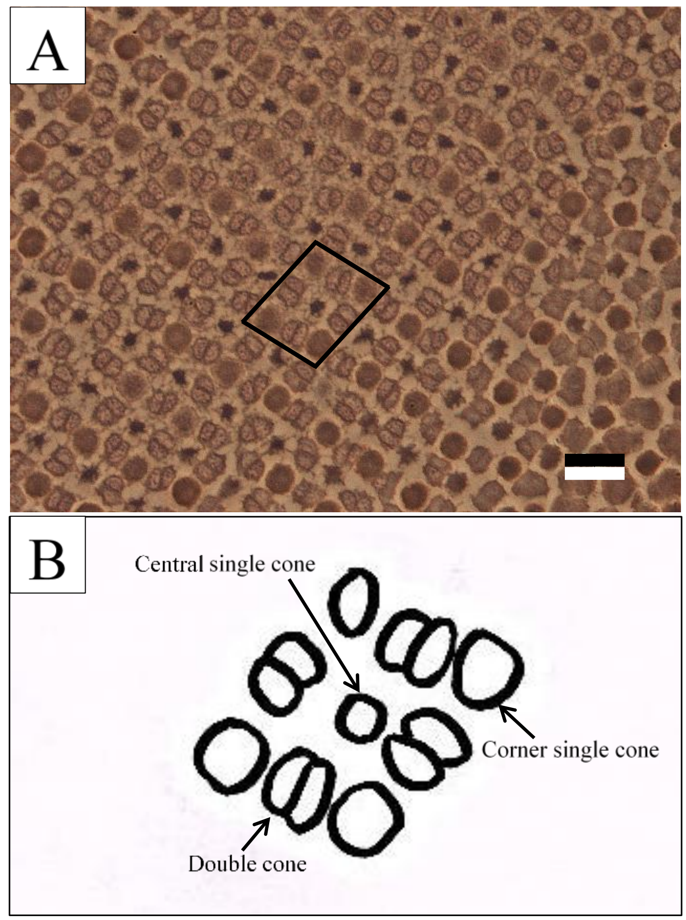

Figure 1. Square mosaic in the retina (a) the square box with black line shows a basic square mosaic, scale 10 µm; (b) illustration on the basic square mosaic with labels on the types of cone visual cell.

The possession of several types of retinal cone suggested that the *P. binotatus* may have both the color and ultraviolet (UV) visions because each type of cone is sensitive to different light spectrum (Evans, 2004), as demonstrated by Jordan et al. (2006) on 15 species of the Lake Malawi cichlids through microspectrophotometry. The capability of UV vision in *P. binotatus* can be further assured as it possesses the corner cone, which was reported to be

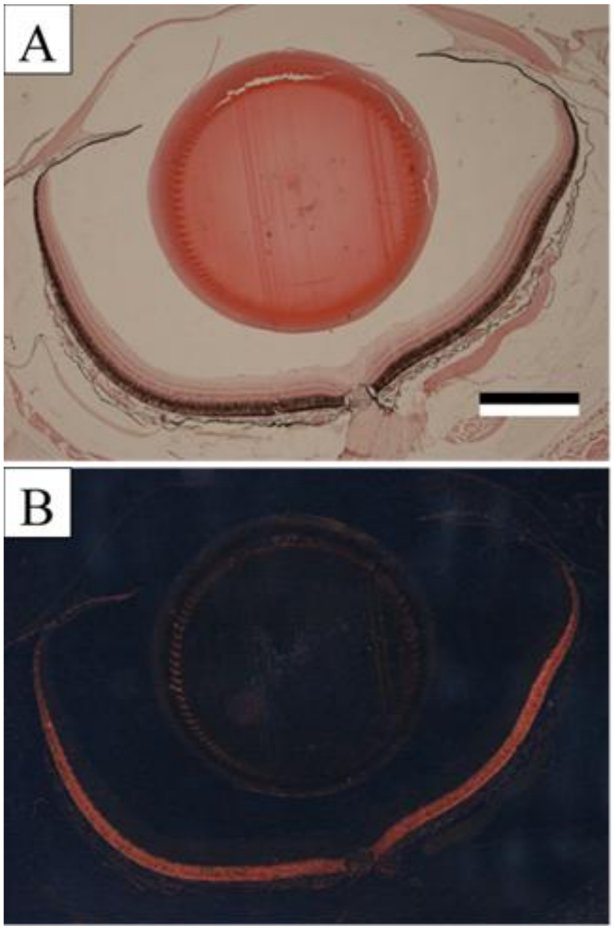

Figure 2. Retinal tapetum in the eye of *P. binotatus* (a) section under light microscope; (b) section under guided light. Shining at the retina region evidenced the existence of the tapetum. Scale 500 µm.

UV sensitive (Bowmaker and Kunz, 1987; Beaudet et al., 1997).

In the present study, well-arranged square cone mosaic with additional corner cones was identified in the retina of the *P. binotatus*. According to the reviews by Evans and Browman (2004), well-arranged square cone mosaics were found only in fish species rely typically on photopic vision. Those fish species which live in habitats with reduced ambient light intensity and typically nocturnal possess usually less-arranged mosaics or absence of mosaic. With the possession of several types of retinal cone and the square cone mosaic, therefore, *P. binotatus* can be considered as a diurnal species.

In fact, how the cone mosaic contributes to the fish visual capabilities is still not fully known, despite so many studies have been conducted to find out the answer (Cook and Chalupa, 2000). Nevertheless, the square cone mosaic is believed to enhance many visual characteristics of the fish. Several studies suggested that the square cone mosaic is predominant in acute vision (Engström, 1963; Kawamura and Tamura, 1973; Kawamura et al., 1981), although there are also studies suggested that it is important for the fish to detect motion (Lyall, 1957; Ahlbert, 1973). In wild, *P. binotatus* mainly feed on zooplanktons and insect larvae (Rainboth, 1996) which are very tiny in size. Therefore, good visual acuity appeared to be more essential than motion detection for the *P. binotatus* to detect its prey. The function of square cone mosaic in the *P. binotatus* should be mainly for acute vision.

In conclusion, *P. binot*atus has good visual capability. They have big eyes, acquiring good visual acuity and both the color and UV visions under the photopic environments, allowing them to track on the tiny zooplanktons and insect larvae. In addition, they possess scotopic vision. It is confirmed that vision is an important sense to the *P. binotatus*. Therefore, further studies should be carried out to gain more information on the visual capability of this species.

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