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MORPHOLOGICAL TYPES AND ARRANGEMENT OF CONE CELLS, AND THE VISUAL ACUITY OF SUTCHI CATFISH *PANGASIANODON HYPOPHTHALMUS*

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Abstract

The density and spatial arrangement of photoreceptor cells in the retina reflect the visual environment of a fish. The density of photoreceptor cells also determines the visual acuity. In this study, the morphological types and arrangement of cone cells, and the visual acuity of sutchi catfish *Pangasianodon hypophthalmus* were determined to obtain fundamental understanding of its vision. The left eyes of the adult sutchi catfish were enucleated, fixed in Bouin's solution for 24 hours and then preserved in 70% ethanol. The fixed retinae were cut into 17 regions. The nine major regions were the dorso-nasal (DN), dorsal (D), dorso-temporal (DT), nasal (N), bottom (B), temporal (T), ventro-nasal (VN), ventral (V), and ventro-temporal (VT). The 17 regions were then immersed separately in a series of ethanol (from 70% to 100%), cleared with histolene, embedded in paraffin, cut into 6 μm thick tangential sections, and stained with haematoxylin-eosin. The density of cone cells per 0.01 mm^2 in each region was counted from the stained sections. Visual acuity was then calculated using cone cell densities and lens radii. Only one type of cone cells, which is the single cone cell, was identified and these single cone cells were closely spaced. The area around the bottom region showed tendency of higher density of single cone cells. These findings provide the fundamental understanding on the adaptation of retinal structure of sutchi catfish to its feeding behaviour.

Keywords: Sutchi catfish, *Pangasianodon hypophthalmus*, cone cells, retina, visual acuity

1.0 INTRODUCTION

The main function of fish eyes is to detect light from the surroundings [1]. In response to the variation in the environmental light which occur in their natural habitats, fish eyes have evolved and shown morphological and functional adaptations to the different photic conditions [1, 2, 3 and 4]. The cells in retina that respond to light are rod and cone photoreceptor cells. In general, the rod photoreceptor cells (rods) respond to dim light whereas the cone photoreceptor cells (cones) confer high acuity and are used for vision in daytime [5]. For cones, they can be divided into single, double, triple or quadruple based on their anatomy and physiology [6]. The density of cones in the retina determines the visual acuity of fish [1]. To increase visual acuity, the cones are usually closely spaced and high densities of cones are observed in the retinal regions that are correlated to the feeding behaviour and natural habitat of fish [1, 7 and 8]. Thus, the morphological types of cones present and their arrangement throughout the retina are determined by the specific light environment of the fish [1].

Sutchi catfish *Pangasianodon hypophthalmus*, also known as Asian catfish and striped catfish, is an important freshwater fish species in the aquaculture industry of Southeast Asian countries including Malaysia, Indonesia, Lao People's Democratic Republic, and Cambodia [9, 10, 11 and 12]. In nature, sutchi catfish can be found in main channels and floodplains of large rivers such as Tonle Sap and Mekong River and seasonally moves up to floodplains and marshland for feeding and nursing [13]. It is an omnivore, feeding primarily on plants, zooplankton, insects, fruits, crustaceans, and fish [14]. Role of vision in catfish has received little attention in behavioural studies because catfish relies on other nonvisual senses, especially taste buds found on their entire body, for catching their prey. However, previous studies show that the larvae and juveniles of catfish are sensitive to light environment which means that catfish prefer dim light environment [15, 16, 17, 18, 19, 20 and 21]. Therefore, through this study, fundamental biological data were obtained in understanding the adaptation of retinal structure/visual system of sutchi catfish to its feeding habit/behaviour and the light conditions in its respective habitat. Besides that, these results will be useful to conserve the natural population of sutchi catfish which is listed as endangered species in the International Union of Conservation of Natural Resources (IUCN) red list of threatened species [22].

2.0 EXPERIMENTAL

For histological analysis, the left eyes of three adult sutchi catfish (TLs 38.0, 39.0 and 46.8 cm respectively), were enucleated, fixed in Bouin's solution for 24 hours and then preserved in 70% ethanol. The fixed retinae were cut into 17 regions (Figure 1) [23]. The nine major regions of the 17 regions were the dorso-nasal (DN), dorsal (D), dorso-temporal (DT), nasal (N), bottom (B), temporal (T), ventro-nasal (VN), ventral (V), and ventro-temporal (VT). The 17 regions were subsequently immersed separately in a series of ethanol (starting from 70% to 100% ethanol) and cleared with histolene. After paraffin embedding, the regions were cut by using a microtome into 6 μm thick tangential sections. The sections were then stained with haematoxylin-eosin and were examined under a light microscope connected to a camera.

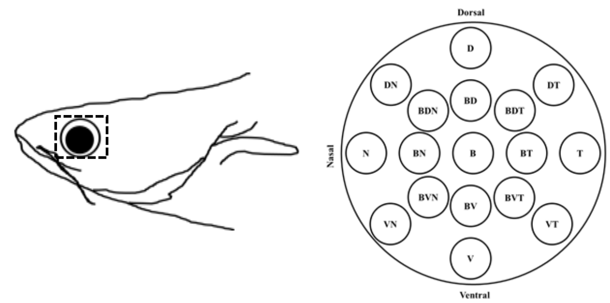

Figure 1 A schematic diagram of the regions of the left retina used for histological analysis

In order to calculate the visual acuity (VA), the density of cone cells per 0.01 mm^2 in each region was counted from the stained sections. Visual acuity was calculated using cone cell densities and lens radii based on the following two formulae [23, 24, 25 and 26]:

$$\sin\alpha \approx \alpha = \frac{1}{f}\left[\frac{0.1 \times (1 + 0.25) \times 2}{\sqrt{n}}\right]$$

$$VA = \frac{1}{\alpha} \times \frac{2\pi}{360} \times \frac{1}{60}$$

where, α = minimum separable angle, f = focal length of the lens (mm), 0.25 = ratio of shrinkage caused by fixation with Bouin's solution, and n = number of cones per 0.01 mm^2 in the retinal region. The focal length of the lens was estimated by multiplying the lens radius by 2.55 which is the Matthiessen's ratio [27].

3.0 RESULTS AND DISCUSSION

The morphological types and arrangement of cone cells in the retina of sutchi catfish were identified through the observation of the tangential sections of the left retina. There is only one type of cone cells, which is the single cone cells, being identified throughout the retina (Figure 2). All the single cone cells were closely spaced. Neither square- nor row-type mosaic, which was the repeating pattern of the arrangement of different types of cone cells, was observed from the tangential sections in each region of the retina.

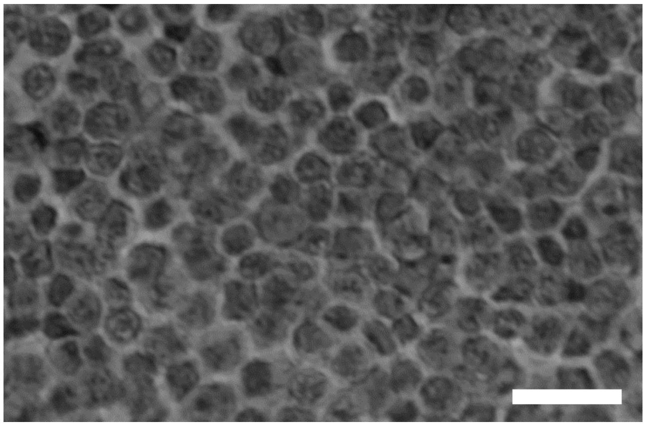

Figure 2 A tangential section in the bottom ventral (BV) retinal region of the adult sutchi catfish. Scale bar: 10 μm

In order to estimate the density of cone cells in each region of the retina, the number of cone cells per 0.01 mm^2 was counted from the stained sections. The cone cell densities varied among the regions of the retina. The retinal regions with the maximum number of cone cells were observed in the bottom ventral (BV; Patin P4 L) and temporal (T; Patin P5 L and Patin P6 L) regions (Figures 3a, b and c). The maximum number of cone cells in those regions were 595, 508 and 460 cone cells per 0.01 mm^2 respectively. In addition, the numbers of cone cells were relatively high in the regions around the bottom (B) region of the retina. The lowest number of cone cells were observed in ventro-nasal (VN), ventral (V) and ventro-temporal (VT) regions. The visual acuity, calculated from the cone cell densities and lens radii (Table 1), were 0.2245, 0.2034 and 0.2062 in those regions with highest density of cone cells (Figures 3d, e and f).

Table 1 Summary of the morphological characteristics and the minimum separable angles (in min of arc) in the retinal regions of bottom ventral (BV) and temporal (T) respectively, of three adult sutchi catfish

Fish (Code)	Eye	Total length (cm)	Lens radius (mm)	Focal length (mm)	Minimum separable angle (min of arc)
Patin P4 L	Left	46.8	3.10	7.91	4.46
Patin P5 L	Left	39.0	3.04	7.75	4.92
Patin P6 L	Left	38.0	3.24	8.26	4.85

In this present study, all the 17 regions of the retina were comprised entirely of single cone cells. This result is supported with previous studies on other catfish species such as glass catfish *Kryptopterus bicirrhis*, walking catfish *Clarias batrachus*, channel catfish *Ictalurus punctatus* and suckermouth armoured catfish *Liposarcus pardalis* [28, 29, 30 and 31].

In the fish retina, cone cells are typically present in either square- or row-type mosaic [1]. However, no cone mosaic was observed in sutchi catfish. This result indicates that sutchi catfish is a nocturnal fish because previous study has suggested that fish active at night do not have a cone mosaic [32]. In addition to the absence of cone mosaic, double cone cells were absent in the retina. Despite these, the single cone cells in the retina of sutchi catfish were closely spaced without any organisation and pattern. The closely spaced arrangement of the single cone cells may imply the increment of sutchi catfish visual acuity and the important roles of vision in sutchi catfish. Previous study showed that vision is relatively important to river catfish *Pangasius pangasius* (of Asian catfish family Pangasiidae) through the quantitative comparison of the catfishes' brain pattern [33]. The absence of double cone cells and the cone mosaic suggests that sutchi catfish does not prey on fast moving preys. This view is supported by the previous findings which reported that sutchi catfish and other pangasiid catfishes feed mainly on fish, aquatic insects, water plants, mollusks and crustaceans [34, 35 and 36].

The high cone cell densities in bottom ventral and temporal regions suggest that the visual axis of sutchi catfish is forward and upward. Previous study reported that the visual axis of each fish species is typically related to fish feeding behaviour and swimming layers in the respective natural habitat [37]. The visual axis of pelagic species is forward to upper direction for targeting preys near the surface [38]. In the wild, sutchi catfish is a migratory and benthopelagic species [13, 14]. The finding on sutchi catfish's visual axis and visual acuity in this study coincided with its feeding habits and natural habitat.

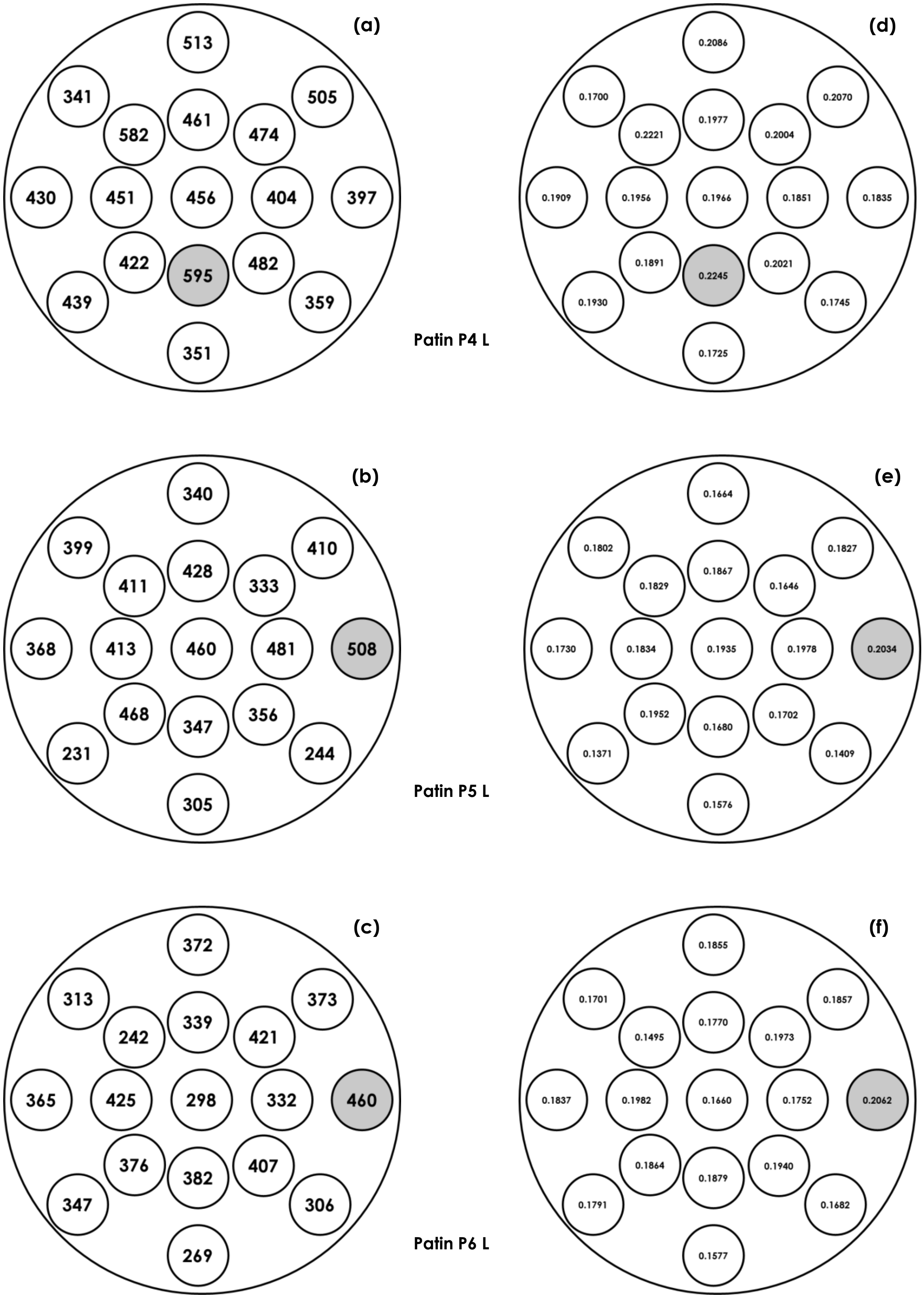

Figure 3 The cone cells densities (a, b and c) and the visual acuities (d, e and f) of three adult sutchi catfish. Grey filled circles indicate the regions of the left retinae with maximum number of cone cells (a, b and c) and the highest visual acuity (d, e and f)

It was observed that, in the aquarium, sutchi catfish showed simple foraging behaviour near the surface as well as the bottom with its head segment directed about 45 degrees upward and downward. This foraging behaviour imply that sutchi catfish feeds on fish and aquatic insects available in front of it in midwaters and near the surface while on mollusks, crustaceans and aquatic plants near the bottom. Visual acuity of sutchi catfish obtained from this study was in the range of 0.2034—0.2245. The relatively high visual acuity and the migratory behaviour of sutchi catfish support the view of previous study which suggested that higher visual acuity may be a requirement for pelagic migrating species [38]. Besides this, high visual acuity may be important as the turbidity in the waters in its natural habitat ranged from 10 to 40 cm.

4.0 CONCLUSION

The results of this study provide the fundamental understanding on the adaptation of retinal structure of sutchi catfish to its feeding habit/behaviour. Based on the histological results obtained, closely spaced single cone cells were identified throughout the retina of sutchi catfish. The highest cone cell densities were found in the bottom ventral and temporal regions of the retina, which ranged from 460 to 595 cone cells per 0.01 mm^2. These high cone cell densities regions indicate the visual axis of sutchi catfish to be forward and upward. The visual acuity of sutchi catfish, obtained through histological method, was in the range of 0.2034—0.2245. These results showed that sutchi catfish is nocturnal fish which exhibit a tendency of taking food available in front of it and vision is relatively important for targeting preys in generally turbid water.

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