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SHORT REPORT

Early appearance of the retinal tapetum, cones, and rods in the larvae of the African catfish *Clarias gariepinus*

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Abstract In the retina of the African catfish *Clarias gariepinus,* the pigment epithelium and the tapetum were formed in newly hatched larvae, the cones developed within 2 days, and the rods within 3 days after hatching. The retinal tapetum shone under surface light under a light microscope; the shine was located in the apical projections of the pigment epithelial cells. Early appearance of the retinal elements enables African catfish larvae to see and feed well even in dim light.

Keywords *Clarias gariepinus* · Visual elements · Dim light vision · Pigment epithelium · Ontogeny

Introduction

Freshwater catfishes populate the bottom of ponds, lakes, and the stagnant parts of rivers. The habitats are often sand and mud covered with vegetation, in which the catfishes dig small burrows or use natural shelters under logs and rocks. The visibility is usually low and vision is often of little use. Catfishes are generally considered to be nocturnal scavengers (Pohlmann et al. 2001) and photophobic (Parzegall and Trajano 2010). The *Clarias* species feed mostly at night on active benthic organisms (Bruton 1979a, b; Hocutt 1989). In laboratory experiments, larval African catfish *Clarias gariepinus* showed better feed ingestion and growth in the dark than in the light (Mukai et al. 2013). All this information suggests limited importance of vision to catfishes. However, in *C. gariepinus* and the river catfish *Pangasius pangasius*, the optic tectum, the second largest part of the brain, is well developed and indicates the importance of vision in these catfishes (Ching et al. 2015). Indeed, colour vision has been determined behaviourally in *C. gariepinus* juveniles (Lee et al. 2014). The activity of *C. gariepinus* is not restricted to nighttime in the natural habitat (Bruton 1979b; Hocutt 1989).

The eyes of freshwater catfishes (adults) are characterized by conspicuous eyeshine (Arnot et al. 1974; Kawamura et al. 2015), a common feature among nocturnal animals and those inhabiting dimly lit habitats (Nicol 1981). Eyeshine is due to the retinal tapetum, a specialized reflecting surface that increases the number of times that low-intensity light can pass through the rod cells and thus confers greater scotopic sensitivity to the eye (Nicol 1981). Whereas the development of the fish retina has been reported for the catfishes *C. gariepinus, Pangasianodon hypophthalmus,* and *Mystus nemurus* (Mukai et al. 2008, 2010; Osman et al. 2008; Rahmah et al. 2013), information on the retinal tapetum has not been included. Here, we present evidence of the appearance of the retinal tapetum in very early larvae of *C. gariepinus*, new information that improves understanding of the visual life of this catfish.

Materials and methods

We examined the retina and retinal tapetum of *Clarias gariepinus* reared at the Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS). Eggs were incubated at 28 °C in a plastic rearing tank and larvae were reared in the same tank at 26–30 °C. The larvae were fed a UMS-

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formulated diet starting 2 days after hatching. Animal care and handling was in accordance with the guidelines for the care and use of laboratory animals set by the World Health Organization in Geneva, Switzerland; the Malaysia Animal Handling Code of Conduct; and the National Research Council (1996). Catfish retinae under light and dark adaptation were obtained for histological observation. Since rod cells can be identified more easily in light-adapted retina, larvae under sunlight (i.e., light adapted) were sampled every day from hatching (0 day) to 4 days. In addition, 16-day-old larvae and an adult specimen were dark adapted to observe the retina in dark adaptation. During sampling, the larvae were anesthetized in 200 ppm MS-222 and preserved in Bouin's solution. The eyes of the

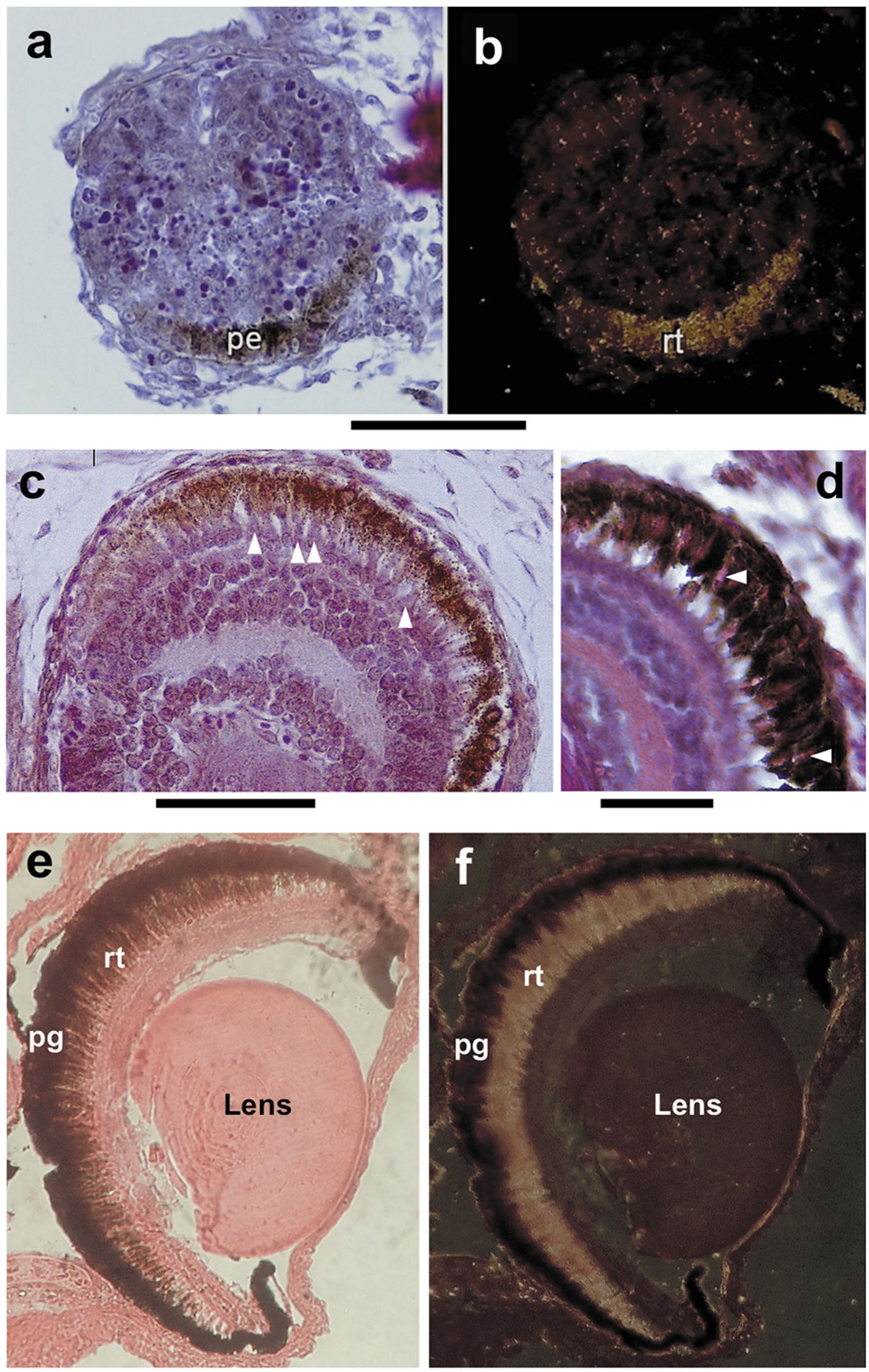

Fig. 1 Photomicrographs showing retinal development of larval *Clarias gariepinus* (**a**, **c**, **d**, **e**, under conventional lighting; **b**, **f**, under surface lighting). **a**, **b** On the day of hatching; **c** 1-day-old, cones are indicated by arrowheads; **d** 2 days old, rods are indicated by arrowheads; **e**, **f** 16 days old. *pg* Pigment granules concentrated in pigment epithelium, *rt* retinal tapetum. *Scale bar* 50 µm

dark-adapted older larvae and adult were removed from anesthetized specimens and preserved in Bouin's solution. The samples were dehydrated in an ethanol series, embedded in paraffin, cut into 6-µm-thick sections, and stained with hematoxylin–eosin for examination of the retina under a light microscope (ECLIPSE 80i, Nikon). The presence of the retinal tapetum was ascertained by illuminating histological preparations with oblique surface light from a light guide (LS-JHA, Sumita Optical Glass, Inc., Saitama, Japan) under a light microscope. The retinal tapetum shone under surface lighting due to birefringence.

Results

Newly hatched larvae had large yolk sacs and transparent eyes. Within a day from hatching, the cells of the pigment epithelium were formed in the outer layer of the retina (Fig. 1a). The pigment epithelium shone under surface lighting, indicating the reflective retinal tapetum in eyeshine (Fig. 1b). The retinal tapetum was retained in both Bouin's solution and lipid solvents (ethanol and xylene). The eyeshine became brighter (Figs. 1b, f, 2b) as the retinal tapetum became denser or thicker with growth of the fish. The retinal elements, i.e., ganglion cells, inner nuclear cells, inner plexiform layer, and outer nuclei cells were layered. The retina was innervated with the brain, and the cone ellipsoids were first recognizable in 1-day-old larvae (Fig. 1c) and rods in 2-day-old larvae (Fig. 1d). The cones were exclusively single cones and no cone mosaic pattern was seen in tangential sections of the adult retina. In the dark-adapted retina of a 16 days old larva, pigment granules were aggregated at the base of the epithelium and the long apical projections of the epithelial cells contained the retinal tapetum (Fig. 1e, f).

In the dark-adapted retina of an adult, the cones moved outward and rods inward (Fig. 2a), and both were spatially separated from, and not covered by, the pigment granules (Fig. 2b). Thus, both cones and rods could receive the light reflected back from the retinal tapetum. The retinal tapetum could increase the sensitivity of cones in scotopic vision, lower the threshold of colour vision, and enable fish to forage in dim light. The eyeshine of light-adapted juvenile *Clarias gariepinus* can be observed immediately after placing it in the dark, evidently without time lapse for retinomotor response.

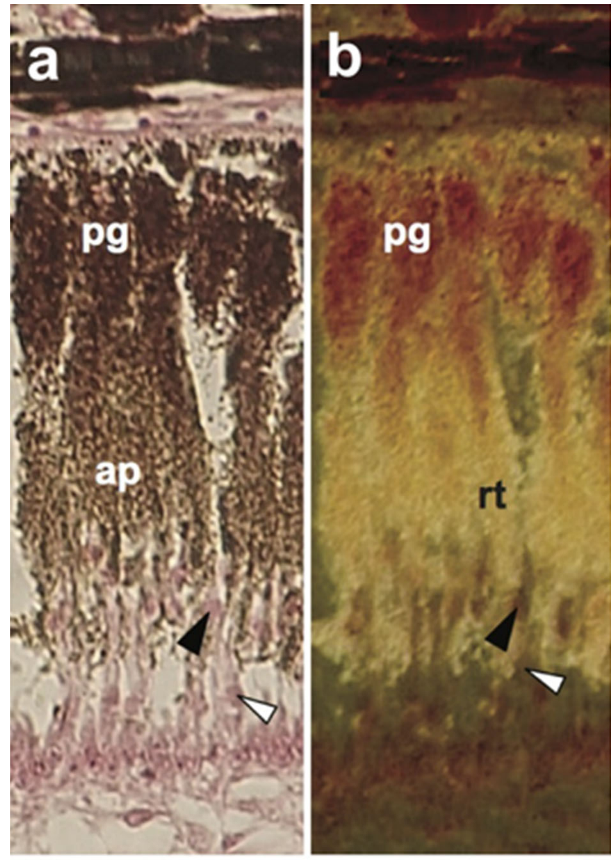

Fig. 2 Photomicrographs of cross section of dark-adapted retina of adult *Clarias gariepinus* (bottom of photographs shows retinal layers facing the lens). **a** Under conventional lighting, cone and rod ellipsoids are indicated by black and white arrowheads, respectively; **b** under surface lighting. *pg* Pigment granules concentrated at the base of the pigment epithelium, *ap* long apical projections of the epithelial cells, *rt* retinal tapetum made up of the apical projections containing reflective material responsible for eyeshine. *Scale bar* 50 µm

Discussion

The retina of *Clarias gariepinus* became functional as a photoreceptor within 2 days after hatching, given the early innervation, pigmentation, presence of the retinal tapetum, and the formation of cones and rods. All retinal elements were formed within 3 days after hatching. These findings were consistent with earlier work on African catfish (Mukai et al. 2008), except that they reckoned the timing of rod formation at 15 days after hatching, based on the formation of the double-layered outer nuclear cells. The cone formation in 1-day-old and rod formation in 2-day-old larvae were earlier at 28 °C in this study than the cone formation in 5 days at 24 °C (Osman et al. 2008). The African catfish is like *Clarias batrachus* in having only single cones and no cone mosaics (Nag and Sur 1992). Shortly after hatching and before the cones are formed, African catfish larvae detect and escape from light, a response mediated by the pineal organ which is functional before the eyes (Appelbaum and Kamler 2000; Rahmah et al. 2013).

The development rate and acquisition of retinal structures vary among fish species. The larvae of many species of teleosts at first have a pure-cone retina and the rods appear several days after hatching and often as late as metamorphosis (Evans and Fernald 1990). The halfbeak *Hemiramphus sajori* hatches with a duplex retina (Kawamura et al. 1990), but the Japanese flounder *Paralichthys olivaceus* and Atlantic halibut *Hippoglossus hippoglossus* develop rods only at the end of metamorphosis (Kawamura and Ishida 1985; Helvik et al. 2001).

The presence of the retinal tapetum soon after hatching enables *C. gariepinus* larvae to start feeding even in dim light. A retinal tapetum also appears early in 6-hour-old bagrid catfish *Mystus nemurus* (Rahmah et al. 2013). In contrast, the retinal tapetum formed much later in the walleye *Stizostedion vitreum vitreum*, at about 30 mm in total length (TL), and the rods and cones even later at 60–70 mm TL (Braekevelt et al. 1989). The presence of retinal tapetum in larval fish eyes was reported in wild 18–20-day-old larvae of milkfish *Chanos chanos* (Kawamura and Hara 1980) and in 29-day-old grenadier anchovy *Coilia nasus* (Haacke et al. 2001). Fishes that have a retinal tapetum have higher visual sensitivity and can feed for longer periods.

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