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Retinomotor Response in Larvae of Brown-marbled Grouper, *Epinephelus fuscoguttatus*

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ABSTRACT

The brown-marbled grouper *Epinephelus fuscoguttatus* is an important species for aquaculture, however, there is no data about visual threshold in order to estimate the optimum light intensity for larval rearing. This study examined the retinomotor responses of 14, 28 and 42 days old larvae of *E. fuscoguttatus* under seven orders of magnitude of light intensities from 0 1x to 100 1x to determine the visual thresholds. The retinae of 14 days old larvae had a single layer of outer nuclei, of the same number as the cone cells, indicating absence of rod cells. The 28 days old larvae had more nuclei in the outer nuclei layer than cone cells, indicating the appearance of rod cells. The retinomotor response was quantified as an increase in the expansion of the pigment epithelium and a decrease in the contraction of the cone myoids. The retinomotor response was absent in 14 days old larvae and weakly evident in 28 days old larvae. The retinal pigment layer was thinner at light intensity <1 1x than at 10-100 1x. The retinae of 42 days old juveniles showed a clear retinomotor response during 0.1-10 1x and 10-100 1x is necessary for cone vision of *E. fuscoguttatus*. Therefore, the larvae should be exposed to the light intensity >10 1x in the larval rearing tanks. Full retinal function at age 42 days would be prepared for their benthic life style in next stage of 45-50 days juveniles.

Key words: Brown-marbled grouper, retinomotor response, *Epinephelus fuscoguttatus,* light intensity, visual threshold

INTRODUCTION

Groupers belong to the family Serranidae that are highly valued marine finfish for food in Southeast Asia, more than 20 species have been raised commercially (Mohammadi *et al.*, 2007; Sarjito *et al.*, 2009; Gonzaga *et al.*, 2010; Shapawi *et al.*, 2011). One of family Serranidae species, the brown-marbled grouper *Epinephelus fuscoguttatus* (Forsskal) is also important species for aquaculture and widely distributed in the Indo-Pacific region and the Red Sea (Heemstra and Randall, 1993) and is considered a threatened species that is heavily fished for the live fish trade (this fish has been listed as the nearly threatened opecies by the IUCN Red lists). Work on aquaculture of *E. fuscoguttatus* started about 20 years ago in Southeast Asia (Lim *et al.*, 1990), but there is only limited information about the early life history (Kohno *et al.*, 1993).

There are so many studies about larval feeding behavior (Blaxter, 1986; Arimoro, 2007; Ara *et al.*, 2009; Gholami, 2010; Mukai, 2011b; Mukai and Lim, 2011). The vision is the major sense used by marine fish larvae to detect zooplankton regarding feeding behavior (Blaxter, 1968a, b; 1986; Evans and Browman, 2004) but *E. fuscoguttatus* has not yet been studied. The right illumination for larval rearing tanks depends on the visual sensitivity of the reared species. Many studies have been done on retinomotor responses and visual sensitivity of fish larvae (Blaxter and Jones, 1967; Blaxter and Staines, 1970; Kawamura, 1979; Neave, 1984; Masuma *et al.*, 2001) and on feeding under different light intensities (Blaxter, 1968a, b; Mukai *et al.*, 2010; Mukai, 2011a,b; Mukai and Lim, 2011) but none yet on *E. fuscoguttatus*.

Boeuf and Bail (1999) described the effect of light intensity on growth of many fish species: larvae require more light than older stages for feeding and predator avoidance, but too intense light is stressful and even lethal. In order to provide appropriate illumination for holding facilities of aquaculture species, there must be information on the light intensity required by a particular species at different stages of the life cycle. Thus, this study has been conducted on *E. fuscoguttatus* larvae to determine the light intensity that elicits the retinomotor response (physiological response of the eyes) and that enables optimum feeding on the brine shrimp *Artemia* in hatchery tanks (behavioural response). The larvae of *E. fuscoguttatus* have a long pelagic stage for approximately 7 weeks, so in this study, we examined the retinomotor response at 2, 4 and 6 weeks old.

MATERIALS AND METHODS

***E. fuscoguttatus* larvae:** *Epinephelus fuscoguttatus* broodstock spawned spontaneously in tanks at Tanjung Badak Marine Fish Centre in Sabah State, Malaysia. Fertilized eggs and larvae were brought to the laboratory of Borneo Marine Research Institute in Universiti Malaysia Sabah. The eggs were incubated in a 1 m^3 tank and hatched within 24 h at 29-31 ppt salinity and heater-controlled water temperature of 27-29°C. The larvae were reared in the same tank, in which the microalga *Nannochloropsis* sp. was added and maintained at a density of 2×10^6 cells m L^{-1}. Larvae were fed first with rotifers (*Brachionus* sp.), then *Artemia* nauplii, then omega-3 emulsion and powdered formulated feed according to their growth. In this study, larval age (in days) was reckoned from the time of hatching.

Retinomotor response experiment: The retinomotor response of *E. fuscoguttatus* larvae was measured at ages 14, 28 and 42 days. Fluorescent lamps (Power-Glo, 20 w Hagen Inc. Canada) and neutral density filters (HOYA, ND×8) provided seven orders of magnitude of light intensity; 0, 0.001, 0.01, 0.1, 1, 10 and 100 1x in the dark room. Under each light intensity three larvae were placed in 2 L glass bowls, exposed for 90 min, then anaesthetized with 200 ppm MS-222 (ethyl 3-aminobenzoate, methanesulfonic acid salt) and fixed in Bouin's solution. The fixed larvae were embedded in paraffin and the eyes were cut into 6 μm thick sections and stained with haematoxylin-eosin for histological examination of the retina. The sectioned retinae were examined under a light microscope (Eclipse 80i, Nikon Co.) and measured for the distance from the outer edge of the pigment epithelium to the external limiting membrane (v), the width of the pigment epithelium (p) and the length of the cone myoid (m). Then the retinal indices were computed: p/v to indicate the expansion of the pigment epithelium and m/v for the contraction of the cone myoids.

The retinomotor response was thus quantified as an increase in p/v and a decrease in m/v. This study was conducted from January, 2008 to June, 2009.

RESULTS

Retinal development: Figure 1 shows development of the outer nuclei layer (visual cell nuclei) in the retina of *E. fuscoguttatus*. The retina of 14 days larvae had a single layer of nuclei and the number of nuclei was the same as the number of cone cells (Fig. 1a). But the retinae of 28 and 42 days larvae had two or three layers of nuclei and many more nuclei than the number of cone cells (Fig. 1b, c). The extra nuclei belonged to rod cells that were otherwise too small to be seen. Thus, rod cells appeared in the grouper retinae by 28 days.

Retinomotor responses: The retinal indices p/v and m/v of *E. fuscoguttatus* at different light intensities are shown in Fig. 2a-c. The retinomotor response was absent in 14 days old larvae, indicating the absence of the rod visual cells and a reliance on pure-cone retina for vision. The 28 days old larvae showed weak retinomotor response; the retinal pigment layer was slightly thicker at 10-100 1x than at 1 1x light intensity. The retinae of 42 days old grouper showed a clear retinomotor response at 0.1-10 1x (Fig. 2) and were completely light-adapted at 10-100 1x Fig. 3a-e.

DISCUSSION

Retinomotor responses in fishes commence when rod cells develop in the larval retina (Blaxter and Staines, 1970). In larval grouper *E. fuscoguttatus*, rod cells were detected at 28 days and the retinomotor response was then observed.

The threshold light intensity for the retinomotor response (0.1-10 1x) of *E. fuscoguttatus* was in the middle of the range reported for many species of fishes. According to Ali (1959), the retinomotor response occurs at 0.1-10 1x in juvenile pink salmon *Oncorhynchus gorbuscha,* at 1-10 1x in chum salmon *O. keta,* at 0.1-10 1x in sockeye salmon *O. nerka* and at 0.01-1 1x in coho

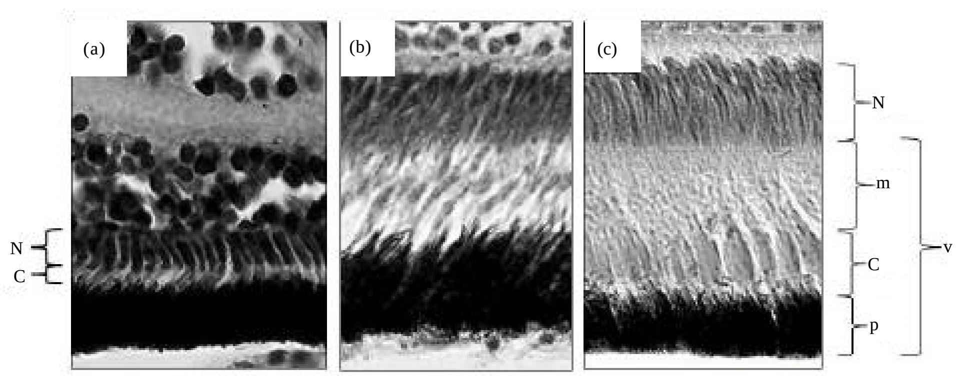

Fig. 1(a-c): Photomicrographs of the retina of *Epinephelus fuscoguttatus* larvae under dark conditions (0 1x). a, 14 d larvae; b, 28 d; c, 42 d. N, nuclei of visual cells; C: cone cells; v: distance from the outer edge of the pigment epithelium to the external limiting membrane; p, width of the pigment epithelium; m, length of the cone myoids. Scale bars, 10 μm

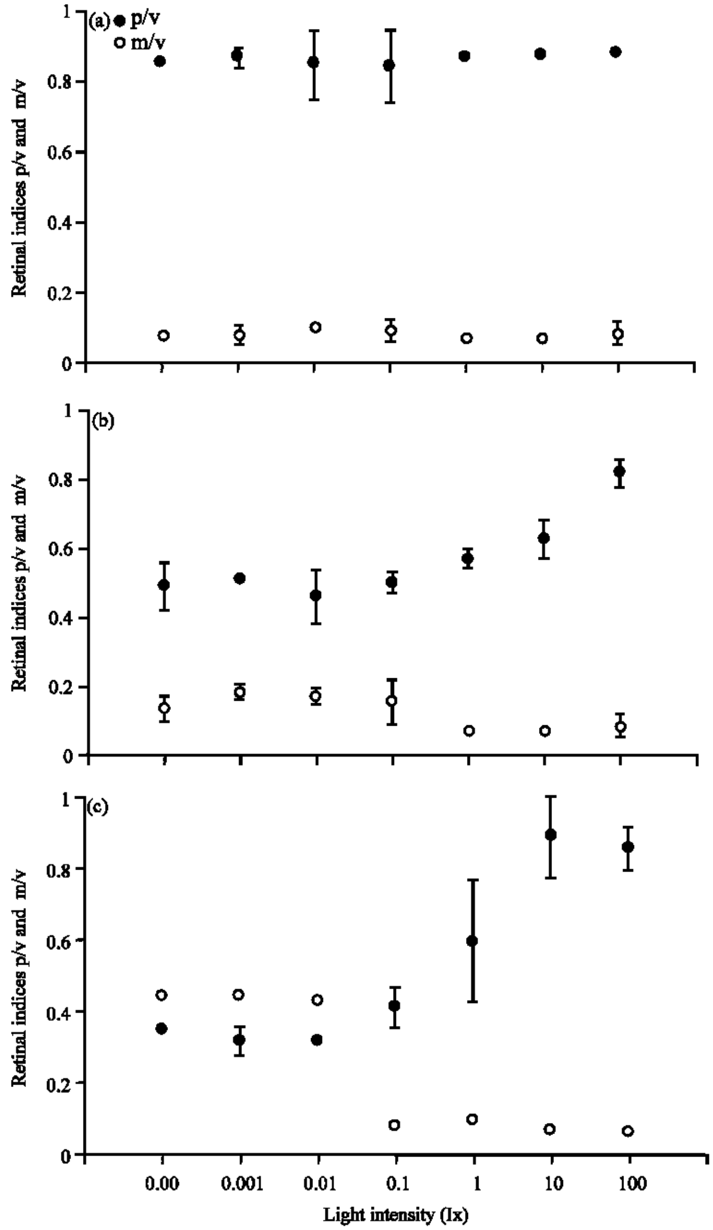

Fig. 2(a-c): The retinal indices p/v (•) and m/v(◦) of *Epinephelus fuscoguttatus* exposed to various light intensities ages (a) 14, (b) 28 and (c) 42 days. The index p/v indicates expansion of the pigment epithelium; m/v the contraction of cone myoids

salmon *O. kisutch*. The larvae of plaice *Pleuronectes platessa* L. show the retinomor response at 0.01-1 1x (Blaxter, 1968b). Plaice larvae show the retinomotor response at 1-10 1x and turbot larvae at 0.01-0.1 1x (Neave, 1984). In larvae of Asian seabass *Lates calcarifer,* the retinomotor response occurs at 0.1-1 1x (Y. Mukai, unpublished data). These varied visual thresholds could be specific adaptations to the fishes' habitats. The thresholds of retinomotor responses of Asian seabass were not different during the larval stage. Although the response of *E. fuscoguttatus* at 28 days was not so clear, the thresholds seem to be not different in 28 and 42 days.

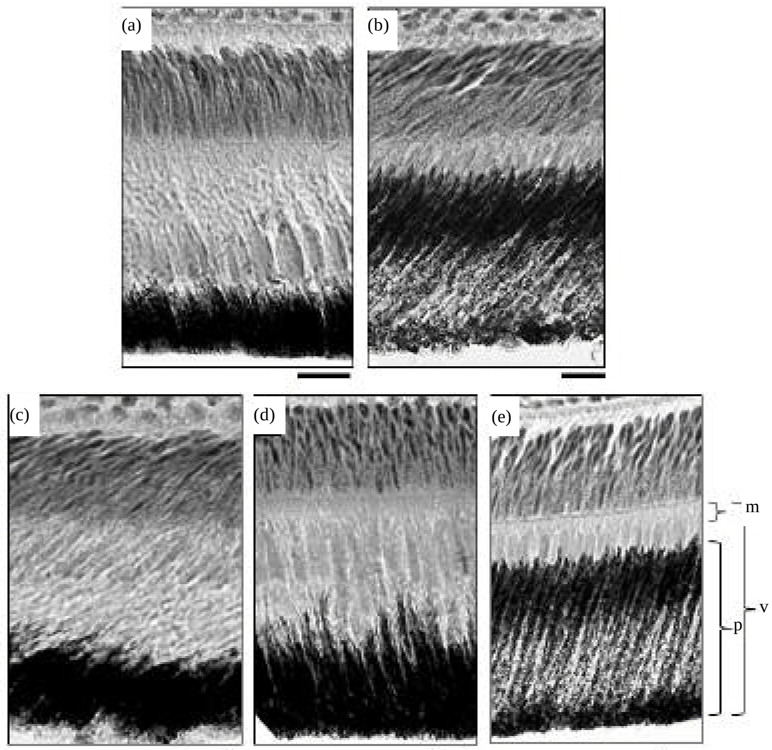

Fig. 3(a-e): Photomicrographs of retinomotor responses in 42 days old larvae of *Epinephelus fuscoguttatus*. a, 0 1x (dark-adapted); b, 100 1x (light-adapted); c, 0.1 1x; d, 1 1x; e, 10 1x. v, distance from the outer edge of the pigment epithelium to the external limiting membrane; p: width of the pigment epithelium; m: length of the cone myoids. Scale bars, 10 µm

Larvae of *E. fuscoguttatus* from age 3 days could catch rotifers in the hatchery tank under daylight. However, under dim light or at night, before the larvae develop rod cells and retinomotor response, could they catch rotifers at all and how do they do so? Larvae of willow shiner (Mukai, 2006) and Asian seabass under dark conditions could catch rotifers by means of free neuromasts. The early larvae of *E. fuscoguttatus* have many free neuromasts on the head and trunk and presumably also catch zooplankton in dim light (<0.1 1x).

In hatchery tanks, *E. fuscoguttatus* became demersal at 45- 50 days, just after they developed fully functional rod cells and retinomotor response at 42 days. Other fish species also show clear relationships between the retinal development and habitat change (Kawamura *et al.*, 1984). Full retinal function is part of the adaptation strategy for changes in habitats during the life cycle.

In this study it was seen that 10-100 1x is necessary for cone vision of *E. fuscoguttatus* larvae. Yoseda *et al.* (2008) suggested that 1000-3000 1x at the water surface is required for grouper larval rearing in 60 m^3 tanks. Thus there is considerably difference between the visual threshold determined from physiological study and the light intensity used in the hatchery. Further study is needed to determine the optimum light intensity for grouper rearing in the hatchery based on retinal physiology and visual requirements.

CONCLUSION

The retinae of 42 days old grouper showed a clear retinomotor response during 0.1-10 1x and were completely light-adapted at 10-100 1x. It was seen that 10-100 1x is necessary for cone vision of *E. fuscoguttatus* larvae.

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