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Brief Report

Visual Cells of the Introduced Bluegill *Lepomis macrochirus* (Pisces; Centropomidae) of Korea

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The bluegill *Lepomis macrochirus* is an invasive species, not native to Korea, introduced for aquaculture. This species is ranked as a new top predator due to its massive aquatic carnivorous and herbivorous nature by acute vision and the absence of a natural enemy. The visual cells of the retina of *L. macrochirus* are composed of short single cones and equal double cones and long and bulky rods by light and electron microscopes. In particular, the cones show a regular square mosaic arrangement. This pattern is widely considered as a strong predator. With regard to the visual system, this mosaic pattern may closely be related to a dynamic visual acuity to track and hunt prey.

Key Words: Bluegill, Retina, Cone cell, Rod cell, Square mosaic pattern

INTRODUCTION

The bluegill *Lepomis macrochirus* is an invasive species from Japan for aquaculture in 1968 and released into Hangang River (Kim & Park, 2002; Ko et al., 2008; Song et al., 2013). Since then, due to the absence of a natural enemy and its massive consumption of aquatic fishes and invertebrate animals as well as aquatic plants, they were dominated throughout the country's streams, rivers, and ponds. Consequently, the fish may lead to the deterioration of the aquatic ecosystem as this species has been known to have an acute vision for a strong predation (Kim et al., 2014). In teleost fishes, the general retinal structure has both rod cells (scotopic vision) and cone cells (photopic vision) and shows a variety of morphology fishes (Bowmaker, 1995; Engström, 1963; Fernald, 1988; Nicol, 1989). Sometimes, such structural differences in some fishes are considered as ecological and environmental surroundings including feeding habits and photic habitats (Collins & MacNichol, 1978; Fernald, 1982; Kunz, 1980; Lyall, 1957). Therefore, this paper will aim at the relation between its visual cells and predation by a histological approach.

MATERIALS AND METHODS

For this species, four males and two females were used to investigate the structure of the visual cells and during the non-spawning season, they were obtained from Deokjin-dong, Deokjin-gu, Jeonju-si, Jeollabuk-do on the Mankyeong River in Korea (35°50′N; 127°07′E). The eyes were extracted after being anaesthetized with MS-222 (200 mg/L^{-1}; Woojin B&G, Korea) and fixed in 10% neutral buffered formalin. Fragments were dehydrated through a standard ethanol series to 100%, cleared in xylene, and embedded in wax (Paraplast; Leica, Germany). Five-micrometer sections were deparaffinized and then stained with Harris hematoxylin and counter-stained with eosin (Gurr, 1956) for general histology. For photographs and evaluation of the eyes, Carl Zeiss Vision was used (LE REL 4.4; Carl Zeiss, Germany). For scanning electron microscopy (SEM), the fragments were prefixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.4. Postfixation was performed in 1.0% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series and drying to a critical point with liquid CO_2, the dried samples were coated with O_SO_4 by ion sputtering and then examined with a SEM

(S-450; Hitachi, Japan). Serial semithin sections (0.5~1.0 μm thick) were stained with toluidine blue and examined with the light microscope (LM) for gross morphology. Both radial and tangential sections were examined at right angles and parallel to the plane of the retina, respectively.

RESULTS

In external morphology of eyes, the bluegill *L. macrochirus* has large eyes compared to its head length, horizontal diameter of mean 34.3% head length and each transparent eye is oval with a long horizontal length and a short perpendicular length.
The visual cells are composed of types of cones (short single cones and equal double cones) and long and bulky rods. Such visual cells are located between a retinal pigment epithelial layer and an outer nuclear layer (Fig. 1A). The both single and equal double cones consist of two segments with a small and conical outer segment, and a larger and bulbous inner segment (Fig. 1B and C, Fig. 2). The single cones are smaller than double cones (a mean of 31.4±3.0 μm in length and 3.3±0.3 μm in diameter vs 34.3±2.1 μm and 7.5±0.4 μm each). Situated closed to the single cones, the double cone cells are eosinophilic, unlike acidophilic single cones. They consist of two elements showing the same size as twin cones, and are put side by side. Their cell extensions reach the outer plexiform layer (Fig. 1A and B). In SEM, it can make it that the outer segments are linked to the inner segment by the calyceal process (Fig. 1C). Seemingly, the both single and double cones appearance form a flower-petal arrangement, which is a regular mosaic pattern that contains quadrilateral units by four double cones surrounding a central single cone (Fig. 2B-D).
The long and bulky rods typically consist of a single layer with two segments: a long and rod-shaped outer segment and a shorter inner segment. The outer segment surrounded by a large amount of pigment epithelial cells is positive with toluidine blue and its ellipsoid is somewhat positive, whereas its myoid region is negative (Fig. 2A). Compared to the cones in size, the rods are thin and long, a mean of 74.7±5.7 μm in length and 2.7±0.2 μm in diameter. Unlike the cones, they have no any calyceal process between the inner segment and the outer segment. The inner segment consists of a separate ellipsoid and myoid region (Fig. 1B and C).

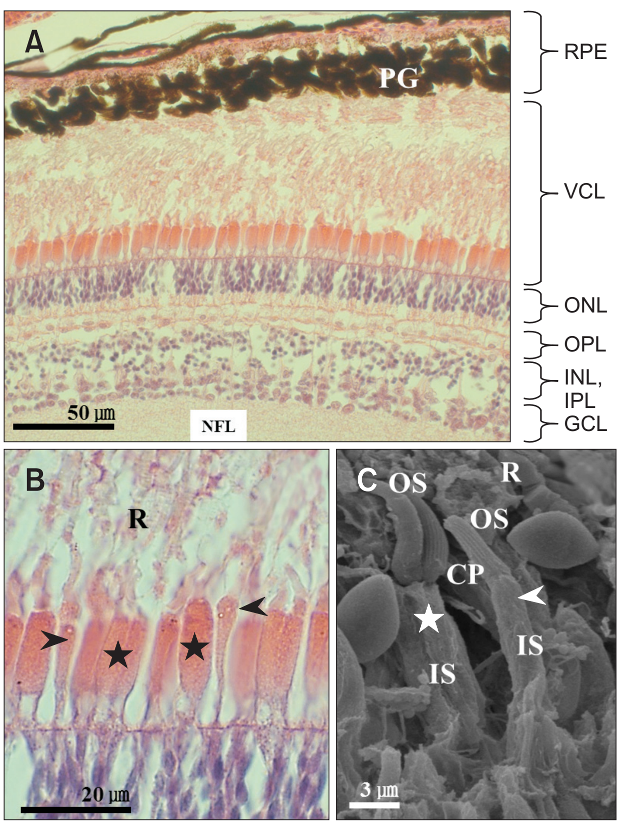

Fig. 1. Gross sections of the retina in a Harris hematoxylin and counter-stained with eosin used by light microscope and scanning electron microscope of *Lepomis macrochirus*. (A, B) Longitudinal sections of the retina. (C) Scanning electron micrograph of visual cells. PG, pigment granules; NFL, nerve fiver layer; RPE, retina pigment epithelium; VCL, visual cell layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; R, rod cell; OS, outer segment; CP, calyceal process; IS, inner segment; asterisks, double cones; arrowheads, single cone cells.

DISCUSSION

The visual cells in teleost fishes' retina generally form a mosaic-like arrangement that has cones being the dominating element with rods randomly interspersed (Ali & Anctil, 1976; Frank et al., 2001; Kunz, 1980; Thomas & Craig, 2010). Typically cone cells are distributed in a regular manner with an arrangement of four equal, double cones surrounding a single cone that may lies centrally or additionally or both, or may be absent (Engström, 1963; van deer Meer, 1992). There are three typical cone mosaic patterns: row, square, and triangular mosaic, which are sensitive to different wavelengths of light. Such different patterns are found in different species (Collins & MacNichol, 1978; Fernald, 1988; Lyall, 1957; Monica, 2001). For the cone mosaic pattern, two contradictory hypotheses exist: playing important role for detection in luminous environments (Kunz, 1980) but for hunting activity of predatory fish rather than the environmental luminosity (Rossetto et al., 1992).
The visual cells of *L. macrochirus* are composed of short single cones and equal double cones and long and bulky rods. In particular, the cones show a regular square mosaic pattern that four double cones surround a central single cone cell, with the axes of the doubles cones at right angles to one another. Such cone arrangement has been known in shallow-water diurnal teleosts that are greatly dependent on vision (Engström, 1963;

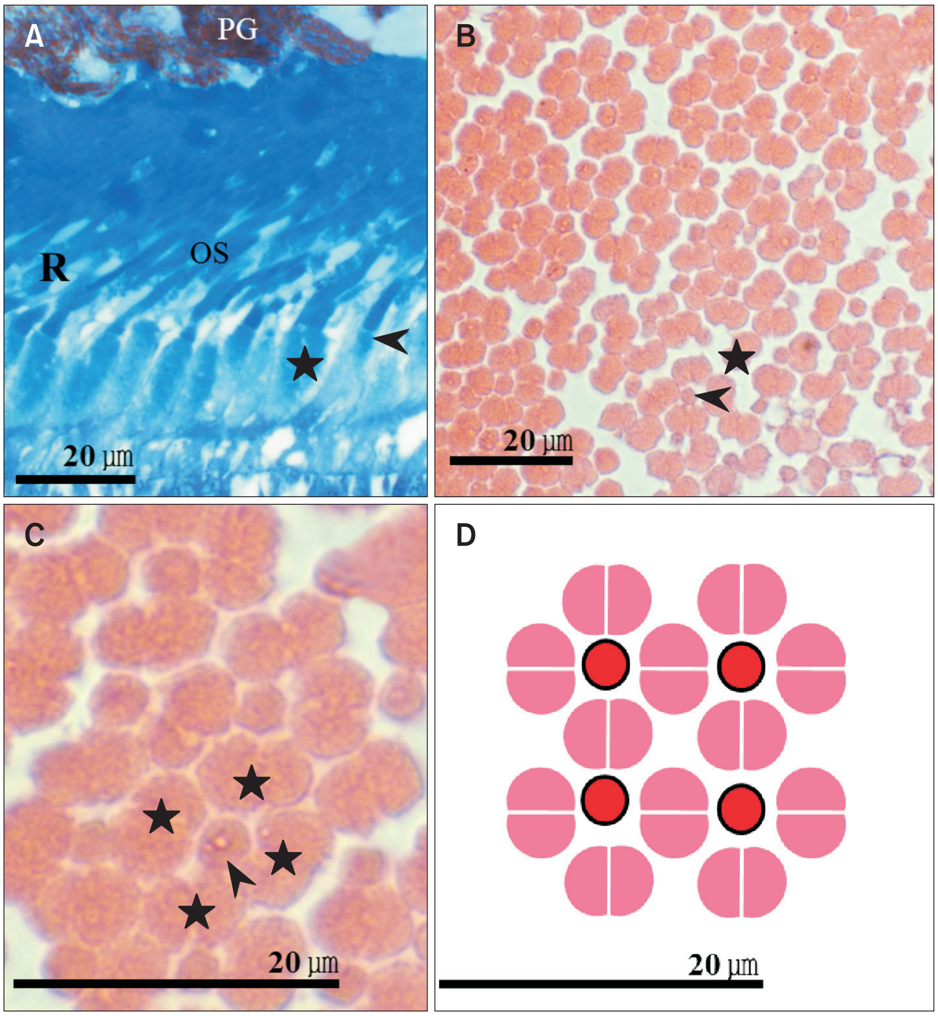

Fig. 2. Visual cell layer of the *Lepomis macrochirus*. (A) Longitudinal sections of the visual cell layer stained by toluidine blue. (B, C) transverse sections showing a mosaic model in the visual cells by H&E stain. (D) Diagram based on the left micrograph. PG, pigment granules; OS, outer segment; R, rod cell; asterisks, double cones; arrowheads, single cone cells.

Lyall, 1956; Nag & Bhattacharjee, 1993; Nicol, 1989). Of the same order as *L. macrochirus, Coreoperca herzi*, an endemic Korean freshwater fish, has nearly similar characters in having that is predatory and diurnal, and carnivorous (Kim & Park, 2002; Song et al., 2013). Moreover, the above two species coexist sympatrically. According to Kim et al. (2014), *C. herzi* showed the same pattern as *L. macrochirus* owned, a regular square mosaic arrangement, and suggested that it seems to be closely related to a carnivorous predator in nature. Two Indian cobitid fishes, *Nemacheilus beavani* and *N. devdevi*, have such pattern (Nag & Bhattacharjee, 2002).

CONCLUSIONS

Through this study, the square mosaic pattern of *L. macrochirus* is likely to be related to its ecological factors including microhabitat and the hunting activity of the predatory fish. With regard to the visual system of this specie, it is likely to be possible to give an answer to reasons why they species is rapidly dominated and outnumber indigenous fishes throughout Korean aquatic ecosystem.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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