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MICROSCOPIC OBSERVATION OF THE RETINAL PHOTORECEPTOR LAYER OF THE COMMON BARBEL (TELEOSTEI: CYPRINIDAE)

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Summary. Light and electron microscopic observations show that cones belonging to four types are present in the retina of the common barbel *Barbus barbus* (L.): short single cones, long single cones, twin cones, and unequal double cones. They do not exhibit any particular arrangement. Estimates of cone density suggest that the common barbel has multichromatic vision but of low acuity and that no difference exists between the lower and the upper parts of the retina. Both cone density (approximately 3000 units/mm^2) and the proportion of double cones to single cones (approximately 1:3) are low. The view that the barbel has an inferior colour vision is in good agreement with the fact that this species is active mainly at twilight, but with diurnal activity during spawning.

Key words: *Barbus barbus*, Teleostei, Cyprinidae, retina, photoreceptors, colour perception.

INTRODUCTION

From most studies concerning the visual perception of fish (see for review WAGNER, 1990), the retinal morphology of these animals appears mainly to reflect the functional requirements imposed upon the visual system by ecological and ethological factors. Photoreceptors are regarded as key determinants of the visual performance of a species. Visual communication, considered an important factor controlling fish behaviour (LEVINE *et al.*, 1980; WAGNER *et al.*, 1992), often involves colour patterns and hence a well-developed colour vision depending on the abundance and distribution of cones.

In barbels, reproductive behaviour is diurnal, though the animals tend to be active at dusk and at dawn (BARAS & CHERRY, 1990; BARAS, 1992; PONCIN *et al.*, 1994). During the spawning act - sometimes preceded by "forehead swim" in which two fishes swim together, at high speed, head against head - the female and some accompanying males (approximately 8 to 30 fishes) rise from the gravel and move their genital papillae in the gravel while releasing ova or sperm. Such spawning behaviour implies visual and vibrational cues which may be regarded as important factors controlling barbel reproduction as reported in streamwaters species, e.g. *Oncorhynchus nerka* (Walbaum, 1792)(SATOU *et al.*, 1987). The influence of such stimuli is of great interest when considering the mating pro-

cess in fish, particularly in the genus *Barbus* where hybridisation is possible between fishes of different size and colour (e.g. *Barbus barbus* (L., 1758) and *Barbus meridionalis* (Risso, 1826)(PONCIN *et al.,* 1994).

The aim of the present study was to observe the retinal photoreceptors of the common barbel by light and electron microscopy, with special emphasis on cone variety and distribution. This preliminary approach will allow determination of the visual capabilities of the barbel. Hypotheses thus formed could be tested by an experimental approach using dummies of different sizes and colours.

MATERIALS AND METHODS

Preparation of the material

Barbus barbus (L., 1758) individuals were taken from a stock originated from the River Ourthe (Belgium). They had been reared in tanks from the egg stage to maturity (PHILIPPART *et al.,* 1989), and kept in an aquarium for 2 years. One male (118 g) and two females (177 and 263 g) were chosen for microscopic observation of the retina. These fish were kept for 48 hours in the dark to limit the retino-motor reflex and anaesthetised by addition of up to 0.4‰ 2-phenoxyethanol to the water. From this time on, all handling took place under low-intensity red light. The eyes were excised from the orbits and opened by removal of the iris and the crystalline lens. Then, the detachment of the retina was induced by incubation for 2 hours in Ca^{2+}-free Ringer solution at pH 7.4 (composition: 113.5 mM NaCl, 11.9 mM $NaHCO_3$, 3.3 mM NaH_2PO_4, 3.4 mM KCl, 21 mM $MgCl_2$ and 11.1 mM glucose) according to LEVINE *et al.* (1980). The eyes were then fixed by immersion for 45 min in the dark at room temperature in 2.5% glutaraldehyde in the Ringer solution. Back in the daylight, one sector from the upper and one from the lower part of the detached retina were isolated. The fragments were fixed again for 30 min in glutaraldehyde-Ringer solution, then postfixed for 1 hour at 4°C in 1% osmium tetroxide in Ringer solution. After washing in distilled water and dehydration in a graded ethanol series and in propylene oxide, they were embedded in epoxy resin (Glycidether 100, Serva); flat rubber moulds or Beem capsules were used, respectively, for producing transverse or tangential sections. Semithin and ultrathin sections were obtained with a Reichert-Jung (Ultracut E) ultramicrotome equipped with a diamond knife. Semithin sections were stained with a 1% toluidine blue solution at pH 9.0 and observed under the light microscope. Ultrathin sections contrasted with uranyl acetate and lead citrate were observed in a Jeol JEM 100-SX transmission electron microscope under an accelerating voltage of 80 kV.

Photoreceptor cell count

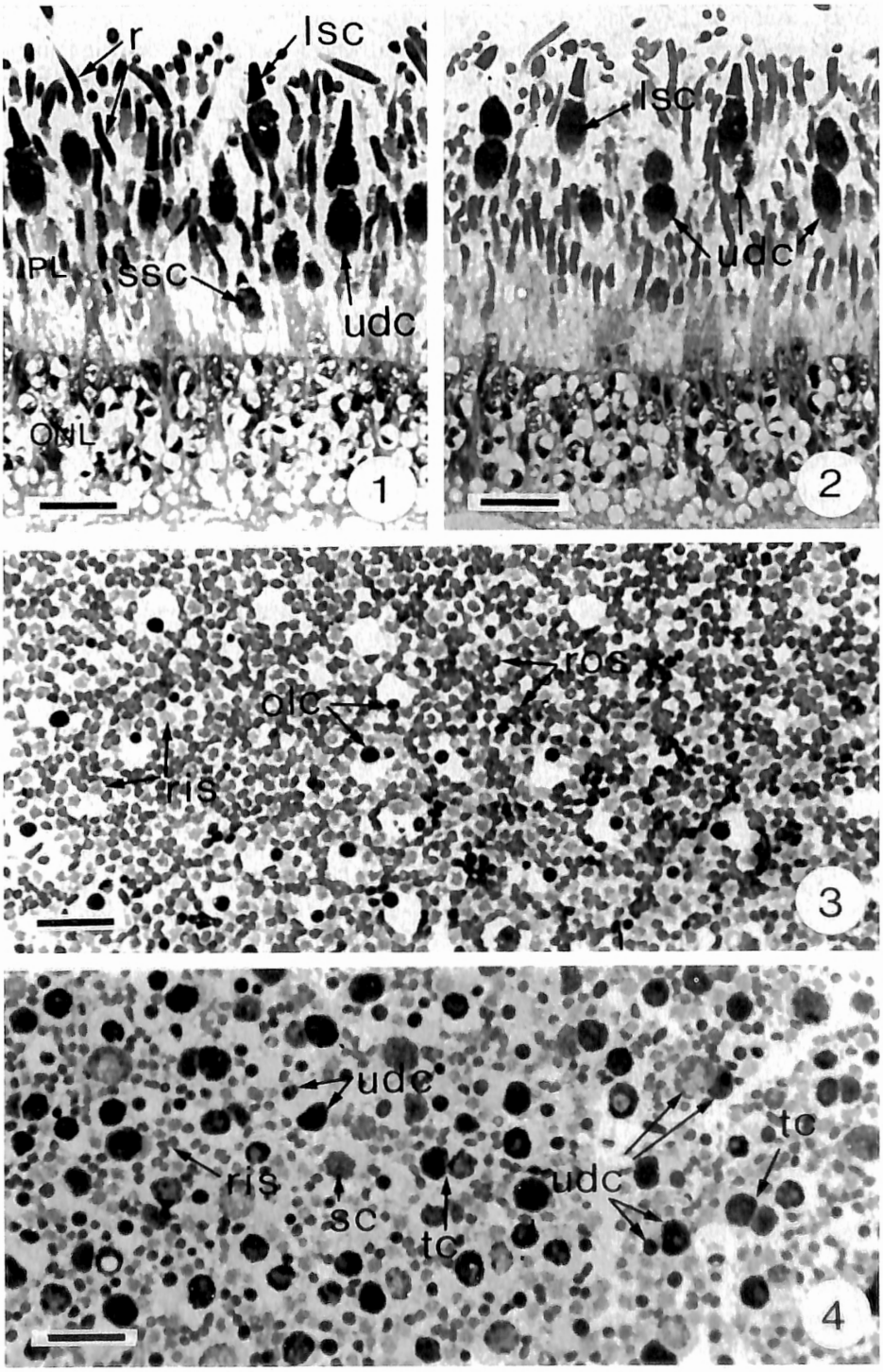
The cones were counted on serial semithin sections cut as parallel as possible to the retina surface. According to the section level, two counting zones were determined to count separately the outer segments of the long cones (long single cones and long partners of unequal double cones) and the inner segments of single cones, equal double cones, and unequal double cones. In each zone, the cone segments were counted in three to eight areas using a

clear chamber on an Olympus BH2 microscope. The mean number of cones of each type per surface unit (mm^2) was calculated for all three fish, separately for the sectors in the upper and lower parts of the eye. No distinction was made between the left and right eyes. The proportion of short and long single cones was calculated first for each fish before calculation of the mean values. For this evaluation, we considered the number of long single cones to be equal to the number of long cone segments minus the number of unequal double cones observed on the sections, and the number of short single cones to be equal to the number of single cones observed on the sections minus the calculated number of long single cones.

RESULTS

Microscopic observation of semithin (Figs 1-4) and ultrathin (Figs 5-6) sections of the common barbel retina shows that, in addition to numerous rods, many cones are present in the retina of both the upper and lower parts of the eye. In transverse sections (Figs 1, 2), the photoreceptor layer is approximately 100 mm thick, representing about half of the overall thickness of the retina. Cones are easy to distinguish from rods by the larger size of their inner segment. Four types of cones are observed : short single cones, long single cones, twin cones, and unequal double cones. Tangential semithin sections cut parallel to the retina surface show that the cones are randomly distributed; different types can be distinguished according to the section level. At the first level toward the outer side of the retina, the outer segment tops of long cones are easily distinguishable among those of the rods (Fig. 3). They appear more intensely toluidine blue-stained, their diameter is usually greater, and they are surrounded by a small rod-free area. They most likely belong to long single cones or to the long partners of unequal double cones. At the second level, deeper toward the external limiting membrane (Fig. 4), single cones appear as large, round, dark blue sections mostly corresponding to the sclerad pole of the inner segment also known as the "ellipsoid". These inner segments of cones are readily identified in transmission electron micrographs by the abundance of large mitochondria (Figs 5, 6). Similarly, double cones with identical partners *(i.e.* "twin cones") appear as two closely juxtaposed round or crescent-shaped sections of similar size and/or staining intensity. In contrast, the unequal double cones exhibit two sections of different size. Both partners of double cones are always located in the same surrounding rod-free area.

As reported in Table I, the estimates of the number of cones of the different types per surface unit do not reveal any significant difference between retina sectors in the upper and the lower parts of the eyes (Mann-Whitney U test values > 0,05). In both parts, the total cone density reaches approximately 3000 units per mm^2 and the relative proportion of the different cone types appears to be quite similar. Single cones constitute the great majority. The proportion of double cones to single cones reaches only about 1:3. Moreover, with a density between 1600 and 1700 units per mm^2, short single cones amount to over half of the total number of cones and over two-thirds of the number of single cones. The calculated results given for each fish do not show obvious differences between individuals. The large standard deviations on the mean values of cone densities may be due to the very low number of cones in the counting areas, e.g. the number of twin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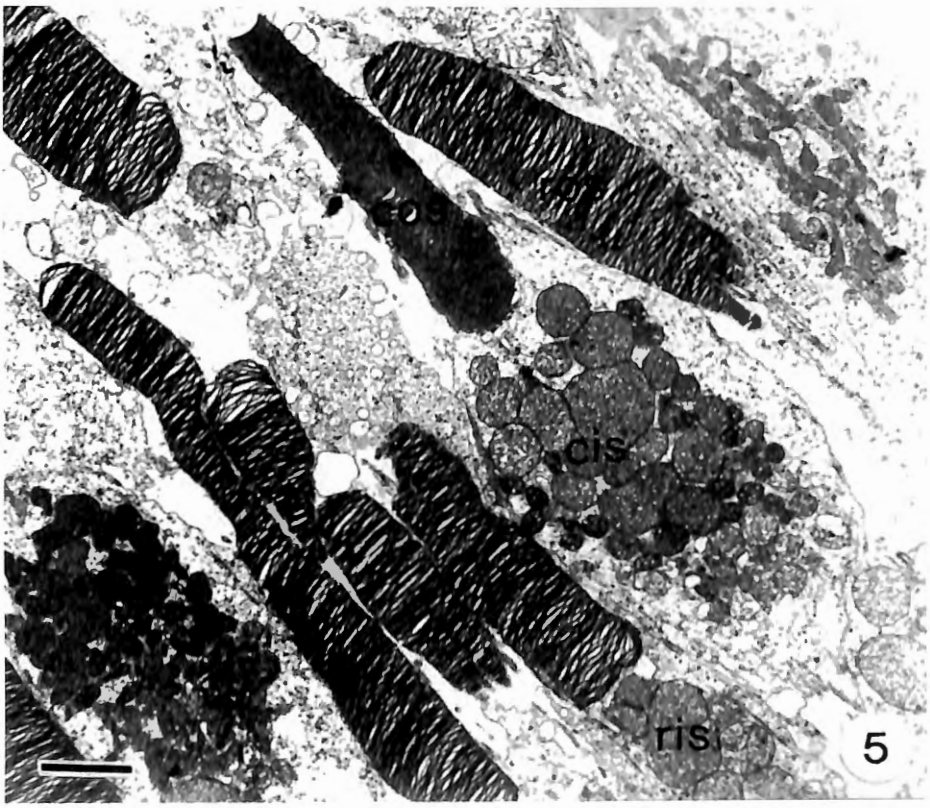
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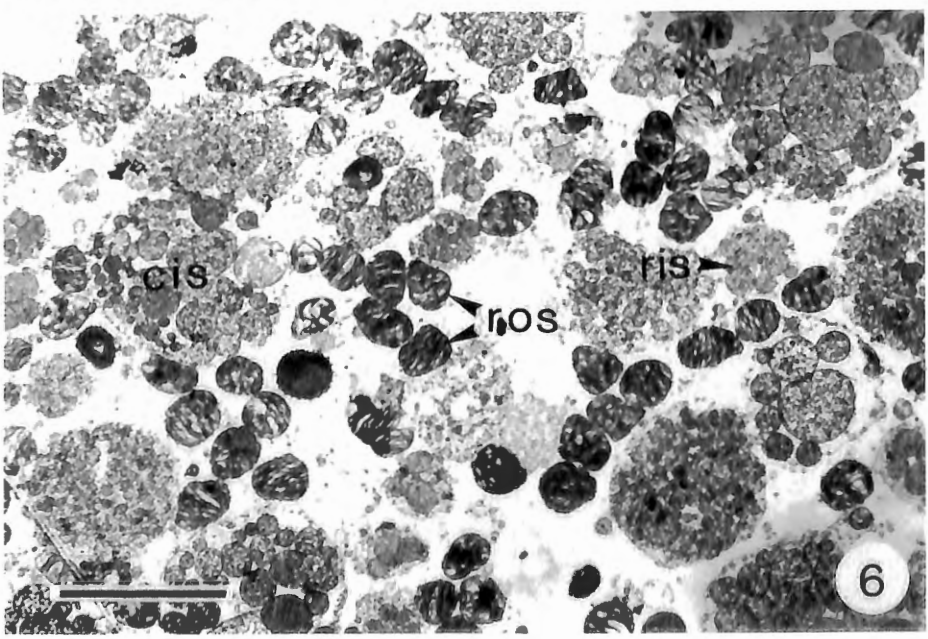
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cones or unequal double cones is often below 10 units and sometimes below 5 units in a microscopic field covering approximately 0.015 mm².

TABLE I

Density of cones of the different types in the upper and lower sectors of the retina of the common barbel, Barbus barbus. The results are reported for each fish as cone numbers per unit (mm2) of retinal surface (fish n°1: female of 177 g; fish n°2: female of 263 g; fish n°3: male of 155 g). The mean numbers in the retina sectors are calculated with standard deviation (n=3) and statistically compared by the Mann-Whitney U test.

	Upper sector of the retina				Lower sector of the retina				
Cone types	Fish n° 1	Fish n° 2	Fish n° 3	Mean ±SD	Fish n° 1	Fish n° 2	Fish n° 3	Mean ±SD	U test
Cones (total number)	2997	3200	3625	3274±320	3568	3215	3233	3339±199	24
Long cones	1240	1227	1200	1222±20	1200	800	2000	1333±611	3.5
Single cones	2133	2167	2750	2350±347	3048	2615	2289	2651±381	2
Long single cones	673	360	975	669±307	936	538	1644	1040±560	3
Short single cones	1460	1806	1775	1680±191	2112	2077	644	1611±837	3
Double cones	864	1033	875	924±95	520	600	944	688±226	2
Twin cones	297	167	650	371±250	256	338	589	394±173	4
Unequal doubles cones	567	867	225	553±321	264	262	356	294±54	3

DISCUSSION

Concerning the visual capabilities of *B. barbus*, the present microscopic observations of the retinal photoreceptor layer and our cone density estimates suggest that this species perceives colours rather poorly; this tallies with the crepuscular activity of the species (BARAS, 1992). Given the thickness of the photoreceptor layer, the size of the outer seg-

Figs 1 - 4. – Vertical semithin sections of the common barbel retina as observed by light microscopy after staining with toluidine blue – Figs 1-4: vertical sections.lsc, long single cones; ONL, outer nuclear layer; PL, photoreceptor layer; r, rod outer segments; ssc, short single cones; udc, unequal double cones. – Figs 3-4: Horizontal sections at the outer (Fig. 3) and inner (Fig. 4) levels in the photoreceptor layer; olc, outer segment of long cones including long single cones and the long patner of unequal double cones; ris, rod inner segments; ros, rod outer segments; tc, outer or inner segments of twin cones; udc, outer and/or inner segments of unequal double cones. (Scale bars : 20 mm). Figs 5 - 6 – Vertical (Fig. 5) and horizontal (Fig. 6) thin sections of the common barbel retina observed by transmission electron microscopy after staining in uranyl acetate and lead citrate; cis, cone inner segments or "ellipsoids"; cos, cone outer segments; ris, rod inner segments; ros, rod outer segments. (Scale bars in 5 : 2 mm; in 6 : 10 mm).

ments of rods, and the abundance of cones of four different types, the barbel retina seems to be half way between that of diurnal species and that of species living in low-light-level habitats (see for review WAGNER, 1990). The most obvious difference between the retina of the common barbel and that of fish living in low-light habitats is the presence of abundant cones of different types and lengths, as mostly develop in the retina of diurnal species. Since cones of different types are known to contain visual pigments sensitive to light of different wave-lengths (for review, see MARC & SPERLING, 1976; BOUWMAKER, 1990), the four types of cones in the barbel retina can be assumed to ensure multichromatic perception. A more detailed comparison reveals that the barbel possesses less developed rods with shorter outer segments and a thicker neural retina than fish living with a low light level. The relative thickness of the photoreceptor layer is approximately 50% of the overall retinal thickness, whereas it reaches over 80% in deep-sea fishes (MUNK, 1964, 1966) and in the glass catfish *Kryptopterus bicirrhis* (Cuvier & Valenciennes, 1839)(WAGNER, 1990). By comparison with diurnal species, however, the barbel retina appears more simply organised and can be assumed to have a colour vision of lower acuity. It contrasts with that of species with superior visual capabilities such as *Aequidens pulcher*(Gill, 1858), a diurnal cichlid predator, the roach *Rutilus rutilus* L. 1758, which has tetrachromatic colour vision, or the Salmonidae (AHLBERT, 1976; WAGNER, 1990): the cone density is lower, the proportion of single cones is higher, the cone densities are similar in different parts of the retina, and there is no regular or mosaic arrangement of cones. The total cone density of approximately 3000 units/mm2 in the barbel retina can be compared, for instance with values recorded by AHLBERT (1976) in the retina of the sea trout *Salmo trutta* L. 1758: lower value: 6,750 units/mm^2, *i.e.* twice as much; higher value: 28,500 units/mm^2, or twelve times the barbel value). Similarly, double cones are the most abundant type of cone in true diurnal species, whilst in the barbel they are but poorly represented. Double cones are 4 to 18 times less numerous in the barbel retina (approximately 700-800 units/mm^2) than in the retina of the sea trout (163 to 686 units/0.05 mm^2: AHLBERT, 1976) and 4 to 16 times less numerous than in salmon retina (153 to 598 units/0.05 mm^2: AHLBERT, 1976). Thus, the ratio of double to single cones is about 0.3 in the barbel, which is much lower than in diurnal salmonidae (1.07 in the sea trout and up to 1.80 in salmon).

In conclusion, the organisation of the retina of the common barbel suggests that the multichromatic perception of this fish is most probably poor, in keeping with the mainly crepuscular activity of this species. The barbel is most probably able to discriminate colours due to the presence of four different types of cones in its retina, but the rather large cone spacing suggests that it could have a low colour vision acuity. Nevertheless, the presence of cones suggests that the barbel retina can perceive colours; this may be related to the barbel's diurnal spawning behaviour, described by BARAS (1992).

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