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INTRARETINAL DISTRIBUTION OF CONE PIGMENTS IN BLACK PORGY, *ACANTHOPAGRUS SCHLEGELI*

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Bao-Quey Huang (1989) Intraretinal distribution of cone pigments in black porgy, *Acanthopagrus schlegeli*. *Bull. Inst. Zool., Academia Sinica* **28**(3): 175-182. In order to investigate the spatial properties and the spectral sensitivities of cone pigments in the black porgy (*Acanthopagrus schlegeli*) retina, a histochemical study with NBT (nitro-blue tetrazolium chloride) vital stainning was applied by stimulating with 8 narrow-band wavelength lights.

Response magnitudes were evaluated by the percentage of the responsive cones to the total of the single or paired cones. The results were indicated that single cones had sensitive peak at 420 nm. Paired cones had two responsive types: (1) both members were responsive, i. e. twin cones; (2) one member was responsive, i. e. double cones. Double cones had sensitive peak at 500 nm and 580 nm and twin cones had sensitive peak at 620-660 nm.

The chromatic organization of the retinae reveals that black porgy may be trichromatic with three sensitive spectral bands: the shorter (420 nm), the middle (500-580 nm) and the longer (620-660 nm) wavelength and likely obtain a good colour vision.

Key words: Cone pigments, Black porgy retina, NBT vital staining, Spectral characteristics.

Very few species of vertebrates have been investigated for their capacity of discriminating spectral lights. In the aquatic environment, the cone pigment were proved to present a better correlation with the spectral quality of the medium (Loew and Lythgoe, 1978). In the different light-sensitive pigments of the retinal cones, one should be more sensitive than the others to a narrow-band monochromatic wavelength (Munz and McFarland, 1975). Microspectrophotometry (MSP) is well known as a widely and accurately used method to measure the absorbance curve of the visual pigments located solely in the outer segment of each photoreceptors. This technique is unfortunately not readily lend itself to analyze the spatial characteristics of the pigment distribution among the cone mosaic intraretinally. MSP is carried out on the macerated retinae, so the cone arrangements are broken and sometimes double cones are easily separated into two members confused with single cones.

Marc and Sperling (1976a, b) applied the mechanism of the light-dependent reduction of nitro-blue tetrazolium chloride (NBT) to study of the intraretinal distribution of goldfish cone pigments. The photosensitive pigments in the outer segment of the cones, could capture photons and pass the energy to the electron transfer system of mitochondrion in the neightboring ellipsoid. NBT then is reduced into its diformazan (NBT-DF) which is insoluble and forms blue-violet crystals in the ellipsoid, the mitochondrial region of the cone cells.

A preliminary work on cone morphological classification and their possible chromatic sensitivity was done and demonstrated that black porgy, *Acanthopagrus schlegeli*, may be trichromate (Huang and Huang, 1988). By applying the chemical vital stainning of NBT reduction, the present studies were analyzed the different responsivities to the spectral lights in the various cones. The major goals are:

(1) to correlate the cone types with spectral sensitivity.

(2) to mark a possible intraretinal distribution of cone pigments.

MATERIALS AND METHODS

Retinal Preparation

The experiments were carried out on the black porgy (*Acanthopagrus schlegeli*) retinae for NBT (nitro-blue tetrazolium chloride, 2, 2′-di-p-nitro-phenyl-3, 3′-tetrazolium chloride) vital stain to investigate the spectral characteristics of their cone mosaic.

The fish was obtained at the west coast of Mid-Taiwan by hooking of netting. The body lengths ranged from 12 cm to 18 cm. A fully dark-adapted fish, kept in a well-aerated tank overnight, was killed and then its retinae were peeled off. The isolated retina with the adhering vitreous body was placed with its receptor side upward in a transparent stimulation chamber. To keep a viable condition for the stimulation retina, a ring of moist tissue paper was set around the retina. Such a preparation could remain responsive to light of several hours (Djamgoz and Downing, 1983). Therefore there is no doubt for the whole process to finish.

NBT Histochemical Reaction

The stimulus light used to identify and classify cone types were derived from an optical system served by a 250 W quartz tungsten-halogen lamp (ORIEL, No. 6334) connected to a 24 volt D.C. power supply. A light spot was focused on the retina from underneath as the physiological pathway. The stimulus intensity was constant and the wavelength was able to be changed by inserting 8 different filters. Their central wavelengths ranged from 420 nm to 700 nm (40 nm steps) with each half-band widths of 10.0 ± 0.4 nm and side-band blocking (ORIEL, U. S. A.).

The retinae were stimulated from the corneal side for 15 minutes. The stimulation was longer than commonly employed (Marc and Sperling, 1976a, b), because the light pathway was following physiological pathway rather than directly on receptor side. All stimulus conditions should be specified in terms of wavelength. After the stimulation, the moist tissue paper was discarded and the stimulation chamber was filled with NBT incubation medium (Marc and Sperling, 1976 a, b). Retinae were incubated for 5 minutes in darkness and then transferred to a chamber filled with 10% formalin in isosmotic phosphate buffer for another 10 minutes stabilization response in dark condition as well.

Well responsive retinae could be maintained in this buffer solution for several mouths until the wholemount analysis or histological studies were finished. The responsive percentage could be calculated from the unclear and unsectioned wholemount retinae. In addition, 4 μm paraffin sections (tangential and radial sections) could also provide the responsive cones. No further stainning is neccessary.

RESULTS

NBT vital staining technique could

deposit the precipitation of blue-violet NBT-DF in the mitochondria-filled ellipsoids of responsive cones (plate A-E) which have been metabolically altered by the irradiating visual pigments. The specific visual pigments in a studied retina, are selectively activated (and hence stainned) by the chosen wavelength for irradiation of the retina.

Plate A-E were representative photomicrographs of retinal histological sections to illustrate spectral sensitivity and spatial arrangements of the various cones in black porgy. Note the square mosaic of paired cones with one central single cone (plate A) and these single cones were regularly arranged and conspicuously responsive to the shorter wavelength, particularly at 420 nm (plate B). It was well demonstrated that black porgy had blue-sensitive single cone.

Paired cones had two responsive types: (1) both members were responsive, i. e. twin cones; (2) one member was responsive, i. e. double cones. The longer members of the double cones (i. e. long double cones) were responsive to 580 nm spectral light (plate C, D), the shorter ones, i. e. short double cones, to 500 nm light. Plate E presented some paired cones with both member (i. e. twin cones) or either member responsive to 620 nm spectral light.

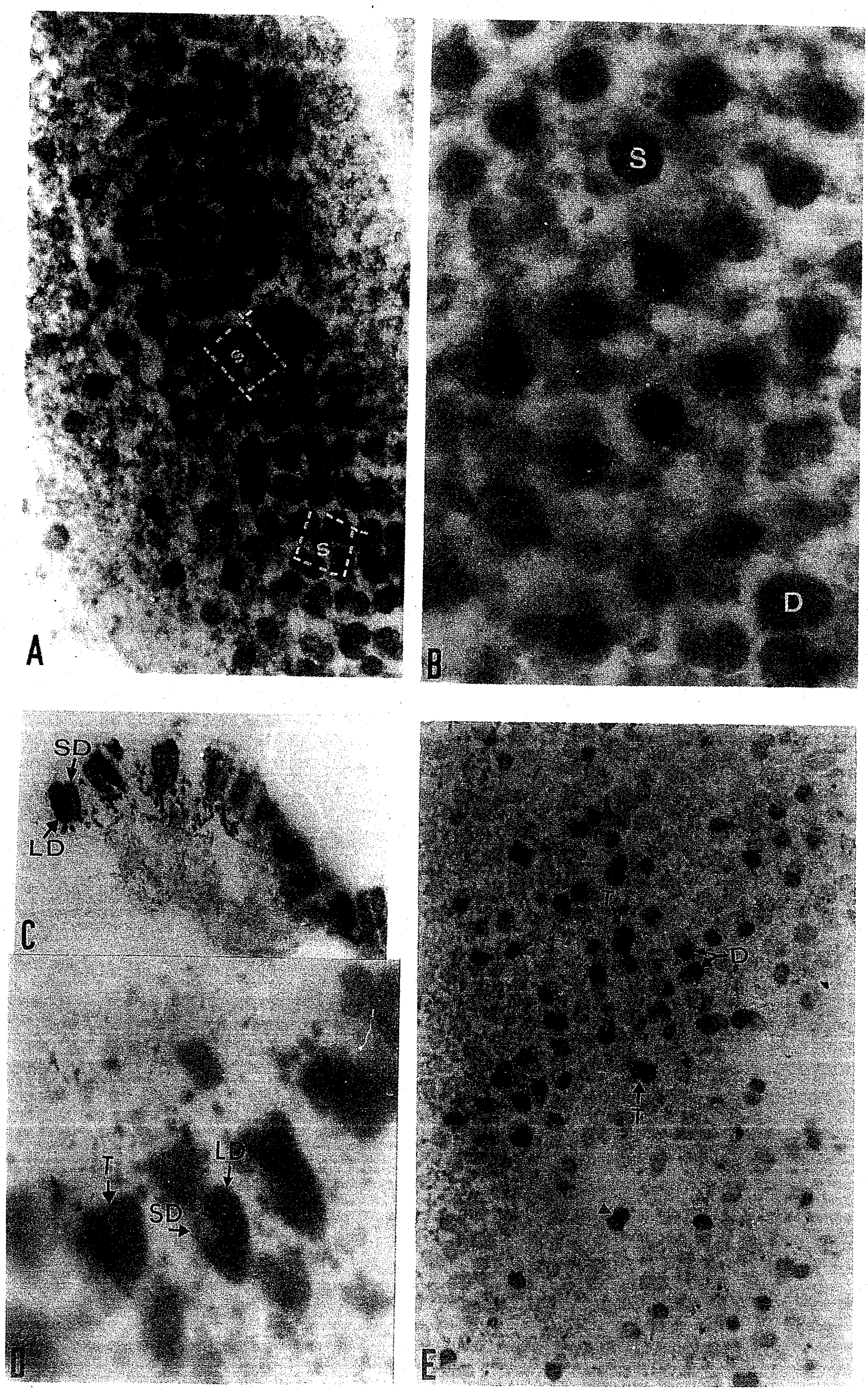
Response magnitudes were evaluated by the percentage of the responsive cones to the total of the single or paired cones in the measured area. Table 1 showed the responsive percentages of the single cone to the eight spectral light (420–700, 40 nm steps), which were obtained from the 16 studied retinae. The spectral sensitivity curve of the single cone to the selective wavelength irradiation was expressed by each responsive percentage, shown in Fig. 1. The results indicated that single cone possessed sensitive peak at 420 nm, i. e. single cone was blue-sensitive in black porgy retinae.

By the similar evalution, Table 2 provided the responsivity of the paired cones to the stimulus light. To each selective light, paired cones had three responsive patterns: (1) neither members was responsive, (2) one member was responsive, (3) both members were responsive. The responsive percentages were obtained by these criteria and presented as the spectral sensitivity curve in Fig. 2. The group with one member responsive had two sensitive peaks at 500 nm and 580 nm. The group with both members responsive had one flat peak at 620–660 nm, i. e. red-sensitive paired cones.

In summary, the black porgy is likely to have the single cone sensitives to

Table 1
Responsive percentages of the single cone to the eight stimulus spectral lights

Wavelength (nm)	Retina numbers	Total measured single cone (no.)	Total response single cone (no.)	Percent (%)
420	2	651	561	86.18
460	2	896	630	70.31
500	1	523	183	34.99
540	2	712	214	30.06
580	2	432	148	34.26
620	1	216	67	31.02
660	4	1045	294	28.13
700	2	852	304	35.68



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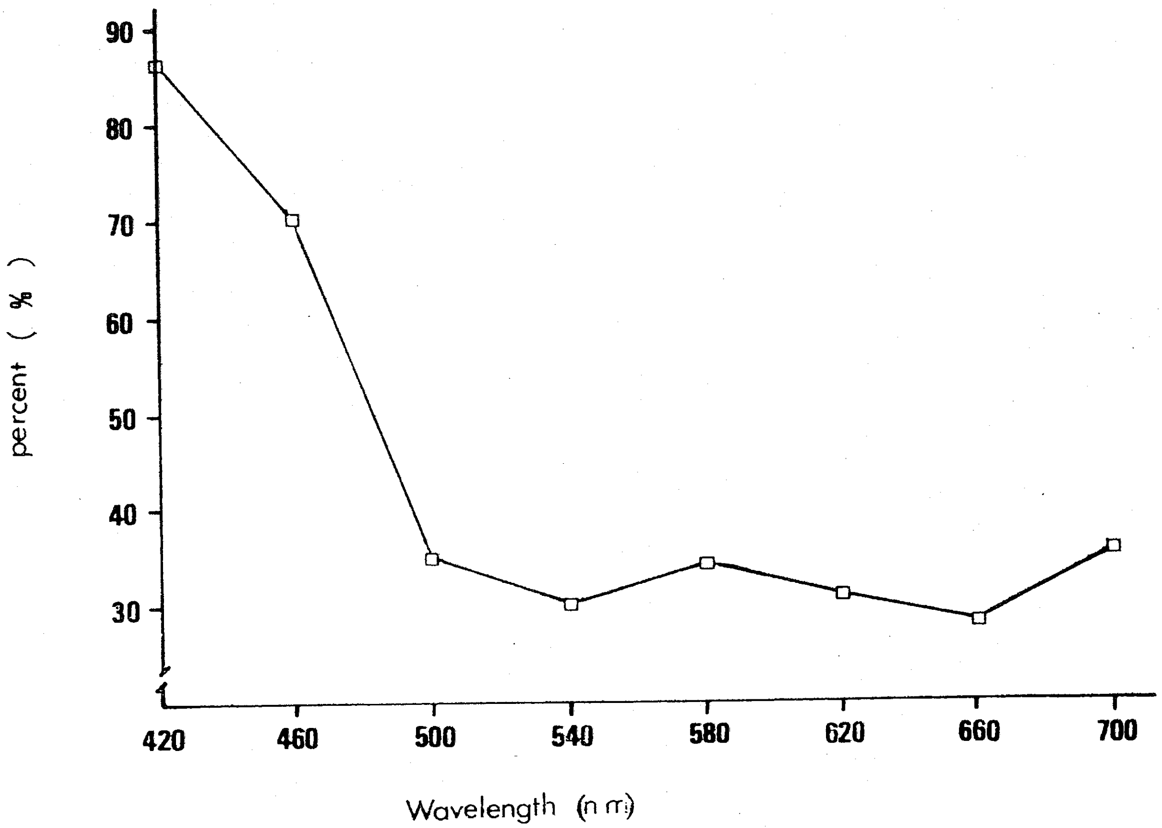

Fig. 1. Spectral sensitivity curve of the single cone shown by each responsive percentages. Note a peak at the wavelength 420 nm.

Table 2
Responsive percentages of the paired cones to the eight stimulus spectral lights

Wavelength (nm)	Retina numbers	Total measured pair cones (no.)	Total response twin cones (no.)	Total response double cones (no.)	Percent (%) (twin)	Percent (%) (double)
420	2	652	82	110	12.58	16.87
460	2	415	72	109	17.35	26.27
500	2	790	175	317	22.15	40.13
540	1	877	278	138	31.70	15.74
580	2	570	168	210	29.47	36.84
620	2	1352	471	354	34.84	26.18
660	1	228	79	56	34.65	24.56
700	2	981	166	270	16.92	27.52

Plate: Photomicrographs of retinal sections to show spatial and spectral characteristics of intraretinal distribution. 4 μm paraffin sections. A, B: stimulus with 420 nm light, ×600(A), ×1500(B) oil-immersion. C, D: stimulus with 580 nm light, ×600(C), ×1500(D) oil-immersion. E: stimulus with 620 nm light, ×600. S, single cone; D, double cones; T, twin cones; SD, short double cones; LD, long double cones.

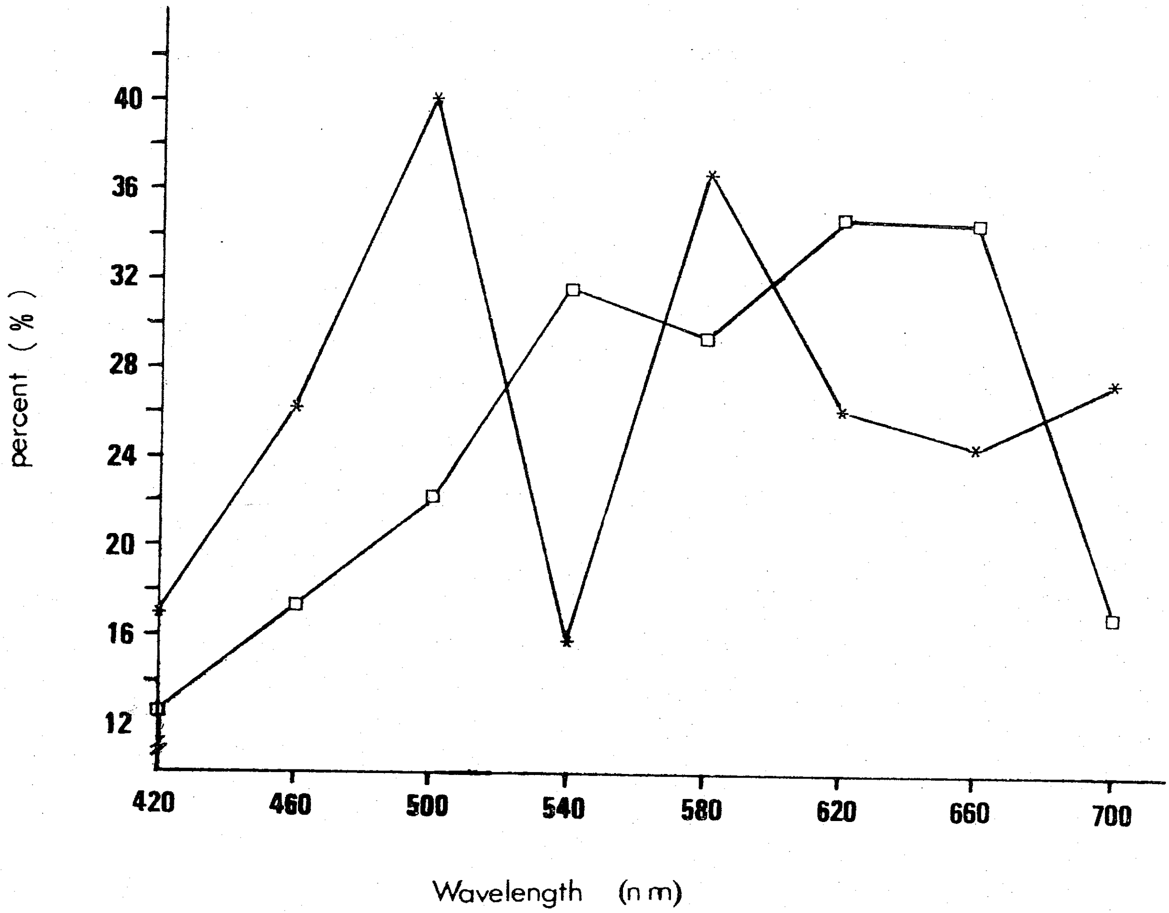

Fig. 2. Spectral sensitivity curve of the paired cones shown by each responsive percentages.
□: twin cones, i. e. both members are responsive. Note a peak at 620–660 nm.
*: double cones, i. e. one member is responsive. Note a peak at 500 nm and 580 nm.

blue light (peak at 420 nm), the double cones sensitive to green-yellow light (peaks at 500 nm and 580 nm respectively) and the twin cones sensitive to red light (peak at 620–660 ñm). In other words, this species might be trichromatic with three sensitive spectral bands: the shorter (420 nm), the middle (500–580 nm) and the longer (620–660 nm) wavelengths.

DISCUSSION

A preliminary work has been reported that the black porgy had two types of structural different cones: single and paired cones. The morphological and chromatic correlation also showed blue-sensitive in single cone and red-red sensitive, and red-green sensitive in paired cones (Huang and Huang, 1988). These three spectrally distinct types of cones are essentially provided that the black porgy was the potential of trichromatic colour vision over a range from blue to red. Although most of surface feeders have this characteristic, they still may have various sensitivity from species to species and also the colour discriminating capacity is closely associated with their own habitual mode of life (Jana and Manna, 1985).

The black porgy has been proved to be omnivorous (Nakagawa *et al.*, 1987). It is known that most omnivorous teleosts are able to adapt the poor photopic environment. Jana and Manna (1985) mentioned that one of the adaptions was its reduced eye size. The black porgy has a high value of relative ocular length approaching 0.1 (unpublished data) which was categorized into a daytime feeder (Aleev, 1969). In addition, their cones were proved to arrange in square mosaic (Huang and Huang, 1988) which was a characteristic of the highly visual feeders (Ahlbert, 1976; Bowmaker and Kunz, 1985). The present data indicated that the species had trichromatic colour visions and appeared to cope with a better photopic environment.

From a review of the ecology of cone pigments in teleosts, Loew and Lythgoe (1978) pointed out that the size order of the outer segments is the same as the spectral order in the species having three or more visual pigments. The single and paired cones in the black porgy were shown this characteristic. Particularly, the longer members of the paired cones were sensitive to the longer wavelength at 580 nm, the shorter members were the shorter wavelength at 500 nm. The black porgy has another paired cones with sensitive to 620-660 nm. It has been suggested that these paired cones with equal pigments, i.e. twin cones could function as "macroreceptors" and are associated with vision under the dim intensities attainable for photopic vision (Lythgoe, 1979). These twin cones in black porgy may act during twilight period on this mechanism. In addition, square mosaics register movement from all directions and are found in most teleosts feeding on fast-moving prey (Ahlbert, 1976). It is likely that the black porgy could obtain more excellent vision at dusk and dawn by the assistance of both this pattern of cone mosaic and the red sensitive macroreceptors.

However, the present evidences are not sufficient to judge the species as a visual feeder or as a typical crepuscular, or even a night feeder. Further information of their daily circadian and of the feeding periodicity from wild captured individuals would be helpful. The present results would provide the evidence that the black porgy may execute improved vision by their trichromasy, square cone mosaic and red-sensitive "macroreceptors", if they are obliged to adapt the poor photic environment.

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黑鯛網膜錐細胞色素之鑲嵌分佈

黃 寶 貴

為探討黑鯛 (*Acanthopagrus schlegeli*) 視網膜之色覺細胞 (卽錐狀細胞，cone) 所含之視覺色素 (visual pigment) 在視網膜之空間分佈，本實驗利用 NBT (nitro-blue tetrazolium) 還原反應之生化技術，對離體網膜進行不同波長色光之刺激，並配合 NBT 之活體培養 (incubation)，以各種視覺色素對單一波長之不同反應性 (responsivity)，評估黑鯛網膜內視覺色素之分佈。結果顯示單一錐細胞 (single cone) 之敏感波長為 420 nm，雙重錐細胞 (paired cone) 有兩種反應型，一為僅單一細胞反應者 (卽 double cone)，其敏感波長分別為 500 nm 及 580 nm，另一為兩者均反應者，特稱孿生錐狀細胞 (twin cone) 其共同之敏感波長為 620～660 nm。

黑鯛錐狀細胞之空間鑲嵌呈方形排列 (square mosaic)，以雙重錐細胞構成四邊，而以單一錐細胞填充中央，此結果顯示黑鯛具極佳之視覺以感受各種不同方向之料餌運動，且其視色素分別對長、中、短波長光敏感，顯示其與一般表層性魚種相同，可能具有色覺之敏感性。