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SHORT NOTE

THE CHROMATIC ORGANIZATION OF THE CONE MOSAIC IN THE SILVER SEA BREAM, *SPARUS SARBA*

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Bao-Quey Huang (1989) The chromatic organization of the cone mosaic in the silver sea bream, *Sparus sarba*. *Bull. Inst. Zool., Academia Sinica* **28**(4): 293-300. To elucidate the relationships between the spectral characteristics and the spatial properties of cone pigments in the silver sea bream (*Sparus sarba*), morphological identity with dissociating retinae and histochemical studies with NBT (nitro-blue tetrazolium chloride) vital stainning technique by stimulation with narrow-band spectral light were applied in the present experiments. Response magnitudes were evaluated by the relative proportions of the specific cones responded to the stimulation with each wavelength light. The results indicated that cones had two responsive types: (1) two members had the same responsivity peak at 580 nm wavelength, i.e. twin cones; (2) two members had the different responsivity peak at 460 nm and 660 nm wavelengths, i.e. double cones. Four paired (twin and/or double) cones framed the square array surrounding a central single cone. The extremely scarce single cones were found at the corners of some mosaic units.

The chromatic organization of the square mosaic reveals that the silver sea bream could sense the fast moving preys from various orientations and also could obtain the wide range of spectral light.

Key words: Silver sea bream, Cone mosaic, NBT vital stain, Chromatic organization.

The silver sea bream (*Sparus sarba*), is a shallow marine, rock-dwelling fish. It is widely distributed along the waters from southern Japan to Taiwan. Because of its fast growth, testy flesh and easy culture, the fish was considered as a promising species for fish farming (Kinoshita, 1986).

The black porgy (*Acanthopagrus schlegeli*), a closely related species with the same family Sparidae (Shen, 1984), occupies in a similar environment. In general, species living in the similar waters are expected to be sensitive to the same range of spectral light (Loew and Lythgoe, 1978). NBT (nitro-blue tetrazolium chloride) vital stain of the cone pigments of the black porgy demonstrated the presence of single cones, double cones and twin cones with peak sensitivity at 420 nm, 500 nm and 580 nm, and 640 nm respectively (Huang, 1989). Bowmaker (1984) reported that the weever fish (*Trachinus vipera*) and the dragonet (*Callionymus lyra*) live in a similar environment but have diverse potential for chromatic vision. Bowmaker and Kunz (1985) discussed that the weever fish, an ambush feeder, adapted a particular mode of vision for especially effective feeding under twilight conditions.

However, the spatial and the spectral characteristics of the cone pigments varies apparently related to not only the photic environment but also the important behaviour of the fish. The purpose of the present work is to study the spectral location of the cone pigment of the silver sea bream by the application of NBT vital stain in order to know their living photic environment and their possible feeding behaviour.

MATERIALS AND METHODS

Retinal preparation

The present study is carried out on the silver sea bream (*Sparus sarba*) retinae for vital staining of NBT (nitro-blue tetrazolium chloride, 2, 2′-di-p-nitrophenyl-3, 3′-tetrazolium chloride) to investigate the spectral characteristics of their cone mosaic.

Fish was obtained at the Tao-Yan coast of North-Taiwan by netting. The standard lengths of the breams ranged from 10.8 cm to 18.1 cm. A fully dark-adapted fish, kept in a well-aerated tank at least 12 hours, was killed and then its retinae were isolated. The retina, with the adhering vitreous body, was placed with its receptor side upward in a transparent stimulation chamber (Downing, 1983). In order to keep a retina viable, a ring of moist tissue paper was set around the sample inside of the chamber. Such a preparation could keep the retinae responsive to light for several hours to finish the whole experimental process (Djamgoz and Downing, 1983; Huang, 1986).

Retinal stimulation and NBT reaction

The stimulus light used to identify and classify cone types were derived form 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 kept constant and the wavelength was able to 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, 1976 a, b; Levine *et al.*, 1979), because the light pathway was following physiological pathway rather than directly on receptor side. All stimulating conditions were specified in terms of wavelength. After the stimulation, the retinae was immersed with NBT containing incubation medium (Marc and Sperling, 1976a) 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 months until the wholemount analysis was finished. The responsive percentage could be calculated from the uncleared

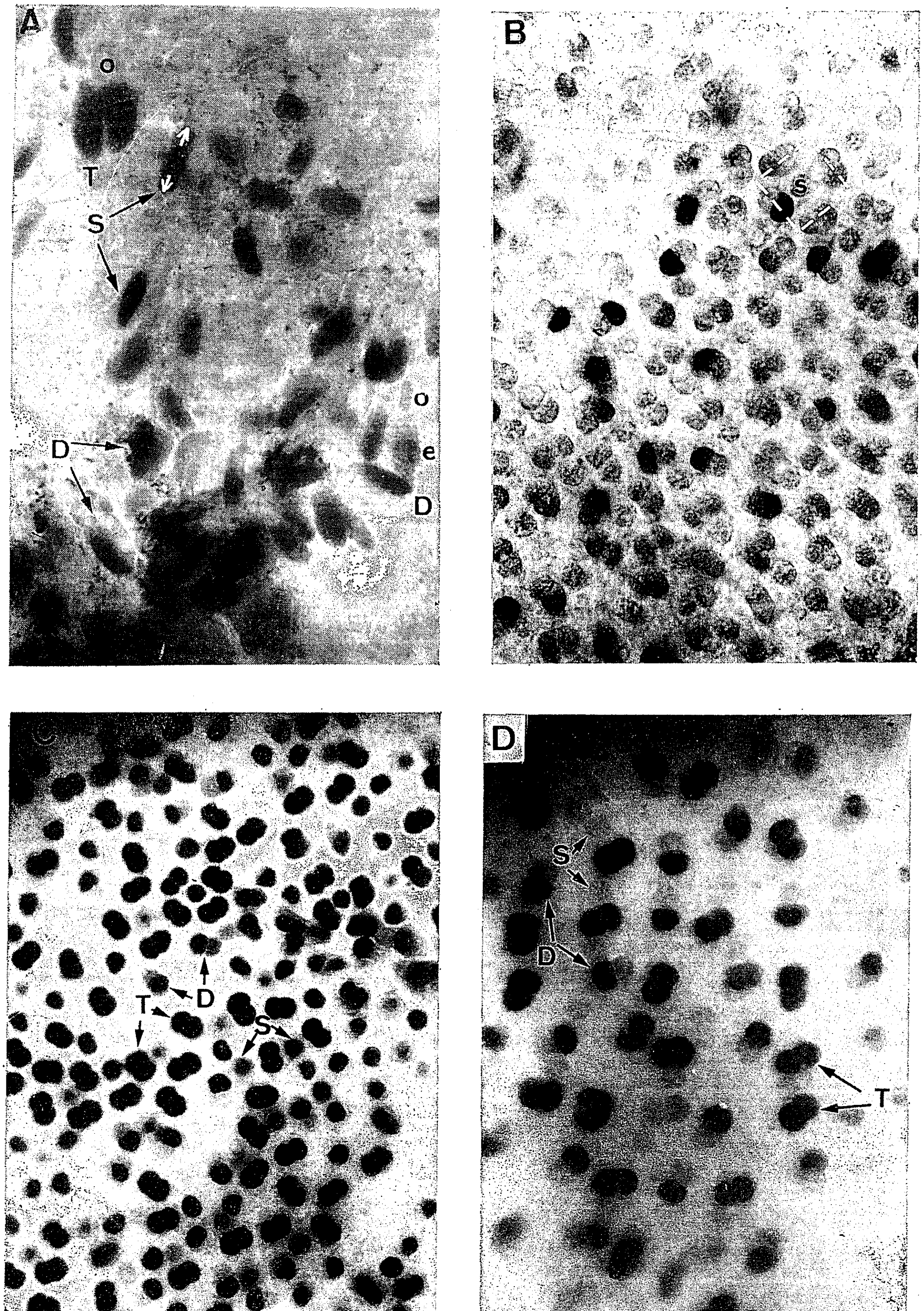
Plate: Photomicrographs of wholemount retinae to show the spatial and the spectral organization of the intraretinal cones mosaics.

A: stimulus with 620 nm light, ×600. Note the NBT-DF deposited in the ellipsoids(e), not in the outer segments (o) and two white arrowheads to show the height of the ellipsoid

B: stimulus with 420 nm light, ×600. Note the square mosaic frames by 4 paired cones surrounding a single cones (s).

C, D: stimulus with 580 nm and 660 nm light, respectively. ×600.

S: single cones, D: double cones. T: twin cones.


A
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and unsectioned wholemount retinae. No further stainning is essential.

Response magnitudes were evaluated by the percentage of the responsive cones to the total of the single or paired cones in the measured area. Because the single cones did not lend themselves to the analysis of response magnitudes to the eight experimental wavelengths, the present results were focussed on the paired cones.

RESULTS

By irradiating an isolated retinae from the silver sea bream, NBT accepts electrons from mitochondrial dehydrogenase to form blue-violet insoluble precipitation (Diformazan, NBT-DF) in the mitochondria-filled ellipsoids of the responsive cones (Plate A). A chosen wavelength light selectively activated a specific cone pigment by different response magnitudes. Note the NBT-DF presented conspicuously in the ellipsoid (the mitochondrial region of the cones), not in the outer segment in which cone pigments were existed (Plate A).

From the photomicrograph of a wholemount retina presented by NBT vital stain technique, two morphologically distincted cone types were clearly identified: single cones and paired cones (Plate A–D). The paired cones further had two responsive types: (1) two members had the same responsivity, (2) two members had different responsivity. They were named as twin cones and double cones

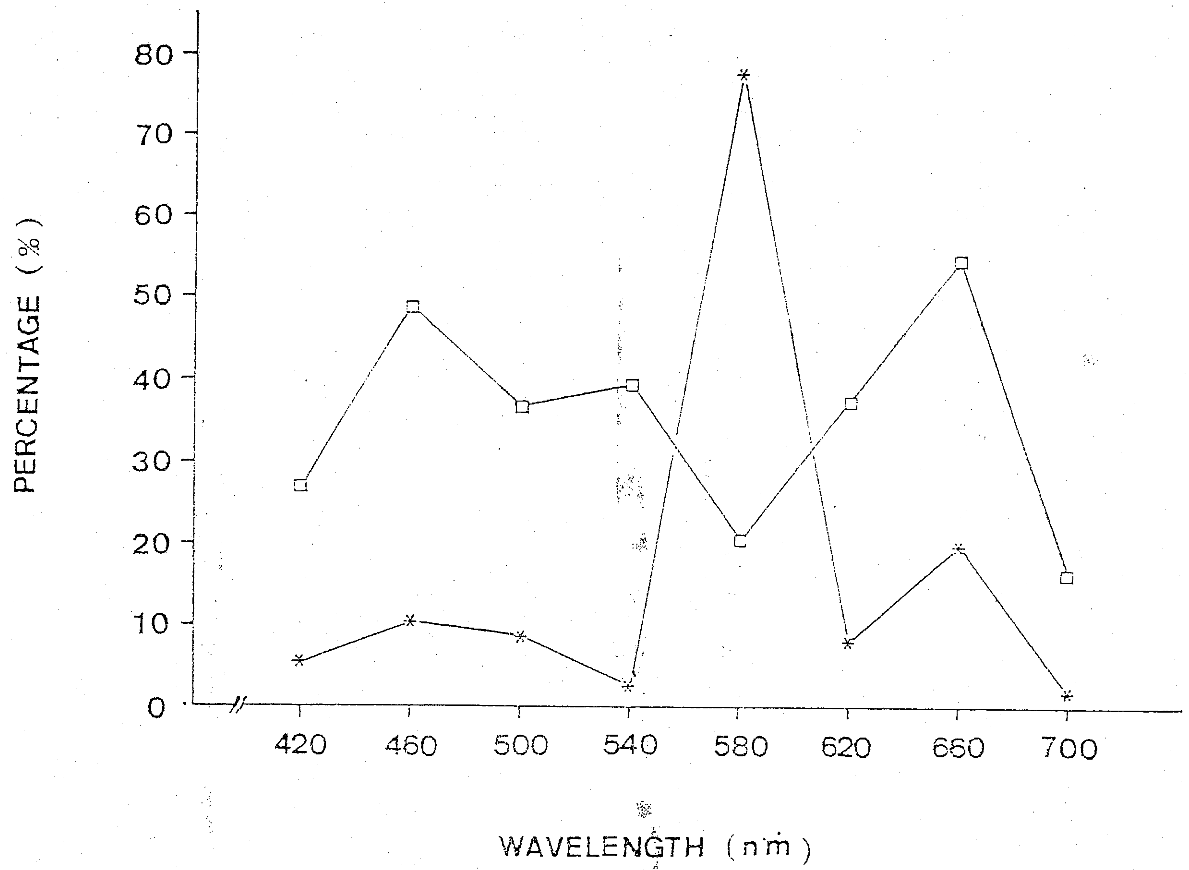

Fig. 1. Spectral sensitivity curve of the paired cones shown by each responsive percentages.
*: twin cones, i.e. both members are responsive. Note a peak at 580 nm.
□: double cones, i.e. one member is responsive. Note a peak at 460 nm and 660 nm.

respectively (Loew and Lythgoe, 1978).

These single and paired cones were organized into the square array with four binary cones (paired cones) surrounding a single cones (Plate B), i.e. central single cones. Very few single cones were found at the corners of the mosaic unit having highly square pattern and were called additional cones.

The responsive percentages of the paired cones to the eight spectral light (420–700 nm, 40 nm steps), were obtained from the 14 studied retinae. The spectral sensitivity curve of the double and twin cones to the selective wavelength irradiation was obtained by the criteria of each responsive percentage (Fig. 1). The former presented two sensitive peaks at 460 nm and 660 nm, i.e. blue and red sensitive (Plate B, D). The latter possessed a sharp sensitive peak at 580 nm, i.e. green sensitive (Plate C).

In summary, the silver sea bream might be trichromatic with three sensitive spectral bands; the shorter (460 nm), the middle (580 nm) and the longer (660 nm) wavelengths, i.e. blue, green and red sensitive.

DISCUSSION

The silver sea bream inhabits quite coastal and shallower waters. It is the usual components of the surf zone ichthyofauna (Kinoshita, 1986). It has paired cones, as the other studied marine species, with wide range of sensitive spectrum from 460 nm to 660 nm. Daylight has broad spectral distributions which is strongly effective on the teleost vision during staying near the surface (Lythgoe, 1979; Bowmaker, 1984; Bowmaker and Kunz, 1985). The fish might be a day feeder living in the shallow waters by its wide range sensitive cones.

The square mosaics are the most frequently encountered in marine teleosts (Loew and Lythgoe, 1978; Bowmaker, 1984). The cone arrangements in silver sea bream indicated this common property and this mosaic types were thought to register movement from all directions for feeding on fast-moving prey (Ahlbert, 1976). Having square cone mosaic with wide range of sensitive spectrum, the bream is very likely to be a visual feeder.

The yellow colouration at the fin tips was a significantly morphological evidence in silver sea bream. Therefore, their local name is "yellow-fin bream". They had twin cones with sensitive spectrum at 580 nm, i.e. yellow-sensitive cones. Twin cones have been suggested to function as "macroreceptors" attainable for photopic vision under the dim illumination (Lythgoe, 1979; Bowmaker and Kunz, 1985). It is likely that the fish could gain a better vision for intraspecific recognition even at twilight by using the yellow sensitive twin cones.

The silver sea bream and the black porgy have the close relation in taxonomy, in similar living habitat, and in having the same cone mosaics. The spectral sensitivity of their cone pigments were different. The bream has paired cones with sensitive spectrum ranged between 460 nm and 660 nm, but the porgy has sensitive spectrum ranged between 500 nm and 640 nm. The wider sensitive spectrum indicated that the present species might have better day life-mode. However, it is difficult to state whether they adopt different life modes to adapt the different optical environment. Further studies are needed to understand the differences of their spectral sensitivity.

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黃錫鯛錐細胞鑲嵌之色譜特徵

黃 寶 貴

爲瞭解黃錫鯛 (*Sparus sarba*) 錐細胞在視網膜上之色譜特性 (spectral characteristic) 及其空間排列 (spatial organization)，本實驗利用 分離之網膜 經窄波譜光刺激及 NBT (nitro-blue tetrazolium chloride) 活體染色技術之組織生化學測定，以評估該魚種之色覺特徵。

形態學分析顯示黃錫鯛具有 兩種錐細胞 ： 單一錐細胞 (single cones) 和 配對 錐 細胞 (paired cones)，不同波長刺激錐細胞之反應率顯示後者呈現兩種反應型式：(1) 兩者反應一致者對 580 nm 光較敏感，此爲孿生錐細胞 (twin cones)；(2) 兩者反應不一致者，分別對 460 nm 及 660 nm 光較敏感，此爲雙重錐細胞 (double cones)。四個配對錐細胞 (孿生或雙重者) 架構成正方形鑲嵌 (square mosaics) 之四邊，中央填以單一錐細胞，極爲罕見有單一錐細胞出現在方形鑲嵌之角落。

因此，黃錫鯛網膜之錐細胞之正方形鑲嵌四周爲黃-黃及藍-紅之配對錐細胞架構而成 ， 中央填以黃光敏感之單一錐細胞，此種空間排列顯示該魚種可能具極佳之視覺以感受不同方向之運動餌料 ， 其色覺特性亦呈現其具一般表層性魚種相似之色覺功能。