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SPECTRAL PROPERTIES OF ELECTRORETINOGRAMS IN TWO DICHROMATIC TELEOSTS

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Bao-Quey Huang and Mustafa B. A. Djamgoz (1989) Spectral Properties of Electroretinograms in Two Dichromatic Teleosts. *Bull. Inst. Zool. Academia Sinica* **28**(1): 41-48. Intra-retinal electroretinograms (ERG) were recorded extracellularly from the photoreceptor layers of perch and cod retina. The results show that dichromates of both species can sustain positive wave responses with spectral sensitivity peaks at 580-620 nm in perch and 500-540 nm in cod. The orange sensitive retinas in perch and green sensitive retinas in cod, were coincided well with the spectral sensitivity of their own twin cones (λ_{max}=615 nm and 517 nm, respectively) measured by direct microspectrophotometric surveys (Loew and Lythgoe, 1978; Huang, 1986). Therefore it was strongly suggested both species had dominant twin cones in their photoreceptor layers. The recordings also showed a better agreement with MSP measurement than the ordinary ERG with MSP measurement (cited in Huang, 1986).

Key words: Dichromates, Spectral sensitivity, Intra-retinal ERG, Colour coding.

Fish vision studies are concerned with many optical properties. Of these, spectral sensitivities were shown to be closely correlated to the natural habitats and determine some behavioural patterns. It is well known that vertebrate retina response can convey different spectral information from the photoreceptors via bipolar cells, and ganglion cells, via the optic nerve to the central nervous system. In addition, amacrine cells (the final order interneurones in the retina) could function in visual processing and integration located in the inner nuclear layer (Djamgoz *et al.* 1985).

At the photoreceptor level, the microspectrophotometric (MSP) data show that cod (*Gadus morhua*) have two spectrally distinct cones: green sensitive and blue sensitive (Huang, 1986). The data also indicate that the morphologically identical twin cones consist of spectrally-identical members: both members are green sensitive. The dichromatic characteristics do not appear to be very general in teleosts and give the fish spectral discrimination over a range of about 446 nm to 517 nm. Perch (*Perca fluviatilis, L.*) is representative of the percoid fishes, which are from an evolutionary point of view quite distinct from the other common and well studied freshwater cyprinids. Perch also possess two spectrally distinct cone pigments which are green and red sensitive. They have the same potential of dichromatic colour vision, but with wavelength discrimination over a different range: 535 nm to 615 nm (Loew and Lythgoe, 1978).

Because of the difficulties in intracellular recording from rods or cones, the extracellular potential could be directly recorded when the microelectrode runs through the epithelium pigment side to the vitreous side. These mass potentials evoked by a light stimulus could be assessed as intra-retinal electroretinogram rather than the ordinary electroretinograms recorded from corneal or other eye sites. The electrode tip is located at the photoreceptor level, not at the external plexiform layer where the horizontal cells are, so that light-evoked responses, particularly the spectral properties, are at the photoreceptor level.

In order to investigate the characteristics of the spectral sensitivities of the dichromatic teleost, the electrophysiological recordings from the photoreceptors were performed on both cod and perch in the present studies. Comparison of retinal spectral sensitivity as measured by electrophysiological recordings with the MSP data which had been done (Loew and Lythgoe, 1978; Huang, 1986) is expected to be made.

METHODS AND MATERIALS

The present experiments were carried out on isolated retinae of Atlantic cod (*Gadus morhua*) and perch (*Perca fluviatilis*). Recordings were made from the photoreceptor layers.

A fully dark-adpted fish placed in a well aerated tank, was sacrificed and the retina peeled off. The isolated retina with the adhering vitreous body was placed with its receptor side upward in a transparent recording chamber. The isolated retina was surrounded by a ring of moist tissue paper and supplied with moist oxygen. Such a preparation could remain responsive to light for several hours. During the electrophysiological recordings, the retina was maintained in a lab with an illumination level at about the cone threshold for the experimenters (Djamgoz, 1978).

Micropipettes were drawn from 1 mm o. d. borosilicate glass with an internal fibre on a modified Livingstone-type puller. They were filled with 2.5 M KCl and had tip resistance within the range of 50–80 MΩ. Due to the layered structure of the retinal neurones, it was possible to estimate the types of the recorded cells by the positions of the electrode tips (Downing, 1983).

The stimulus light used to identify and classify responses were derived from an optical system served by a 250 W tungsten-halogen lamp connected to a d. c. power supply. A light spot of about 0.5 mm diameter was focused on the retina from underneath and, during recording it was flashed with an "on" duration of 200 ms. The intensity and wavelength could be changed by inserting different interference filters (Huang and Djamgoz, 1988).

RESULTS

I. Electroretinograms Recorded from Cod Photoreceptors

In cod retina, light-evoked responses consisted of an initial sustained positive wave, and a negative off-transient. Fig. 1 shows the responses evoked by different wavelengths (420 nm to 660 nm, 40 nm steps) in the cod. The responses were about 0.5 mV at 660 nm and maximal amplitude 3–3.5 mV at 500–580 nm. Fig. 2 presents three complete sets of responses with peaks at 500–580 nm. Due to the higher relative energy (about 10% higher) at 580 nm (Huang, 1986), the electroretinograms of cod had peak responses at 500–540 nm. Because of the difficulties of getting stable intracellular recording from photoreceptors, these extracellular measurements were still very useful for evaluating the spectral sensitivity of the photoreceptors.

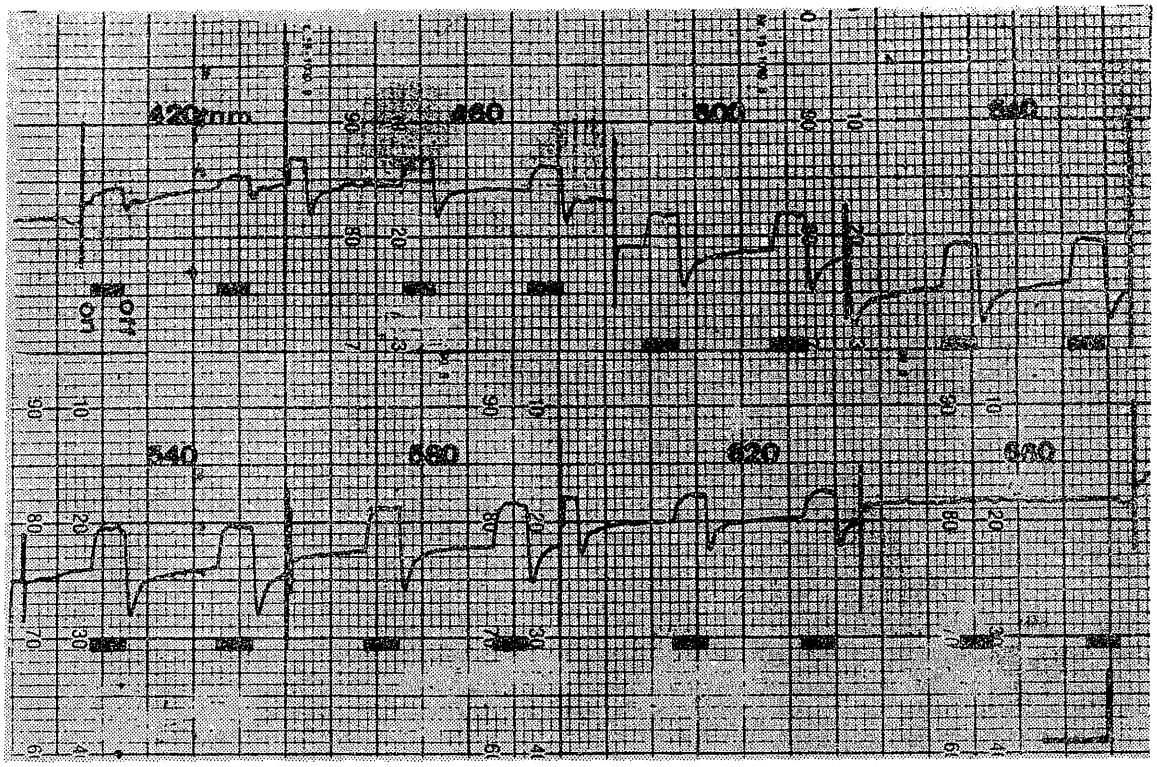

Fig. 1. Electroretinograms from extracellular recordings of photoreceptors to show the spectral properties of cod retina. Each record shows the response to two 200 msec flashes of light. calibration: 2.5 mV; 0.4 sec.

II. Electroretinograms Recorded from Perch Photoreceptors

Perch electroretinograms also consisted of an sustained positive wave and a negative off-transient but a slow off potential (Fig. 3A) at the stimulus wavelengths 617 and 674 nm. These slow off-responses were not shown in the shorter wavelength (454 and 534 nm) and disappeared in the presence of a red background illumination. From the result of electroretinograms, an organge-sensitive retina in the perch was clearly observed and it was resonable to interpret the absorbance peak of the twin cones at 615 nm. The electroretinograms were evoked in perch by stimulating a series of wavelengths (420-700 nm, 40 nm steps) and intensities (log I=−2.8 to 0.0) (Fig. 3B). It was shown that the maximal response at the longer wavelengths peaked at 580-620 nm (Fig. 4).

In summary, the intra-retinal recording electroretinograms showed the spectral sensitivity peak at 500-540 nm, i. e. a green sensitive retina in the cod and at 580-620 nm, i. e. an orange sensitive retina in the perch.

DISCUSSION

The studies on the spectral sensitivity at various surveys on photoreceptors (e. g. visual pigments, intracellular recording), recorded in different levels of visual system (e. g. S-potentials, electroretinograms, tectal evoked responses) and the visual behaviours, showed some disparity in some studied teleosts (Guthrie, 1983; Cameron 1982). In the MSP studies, perch and cod were proved

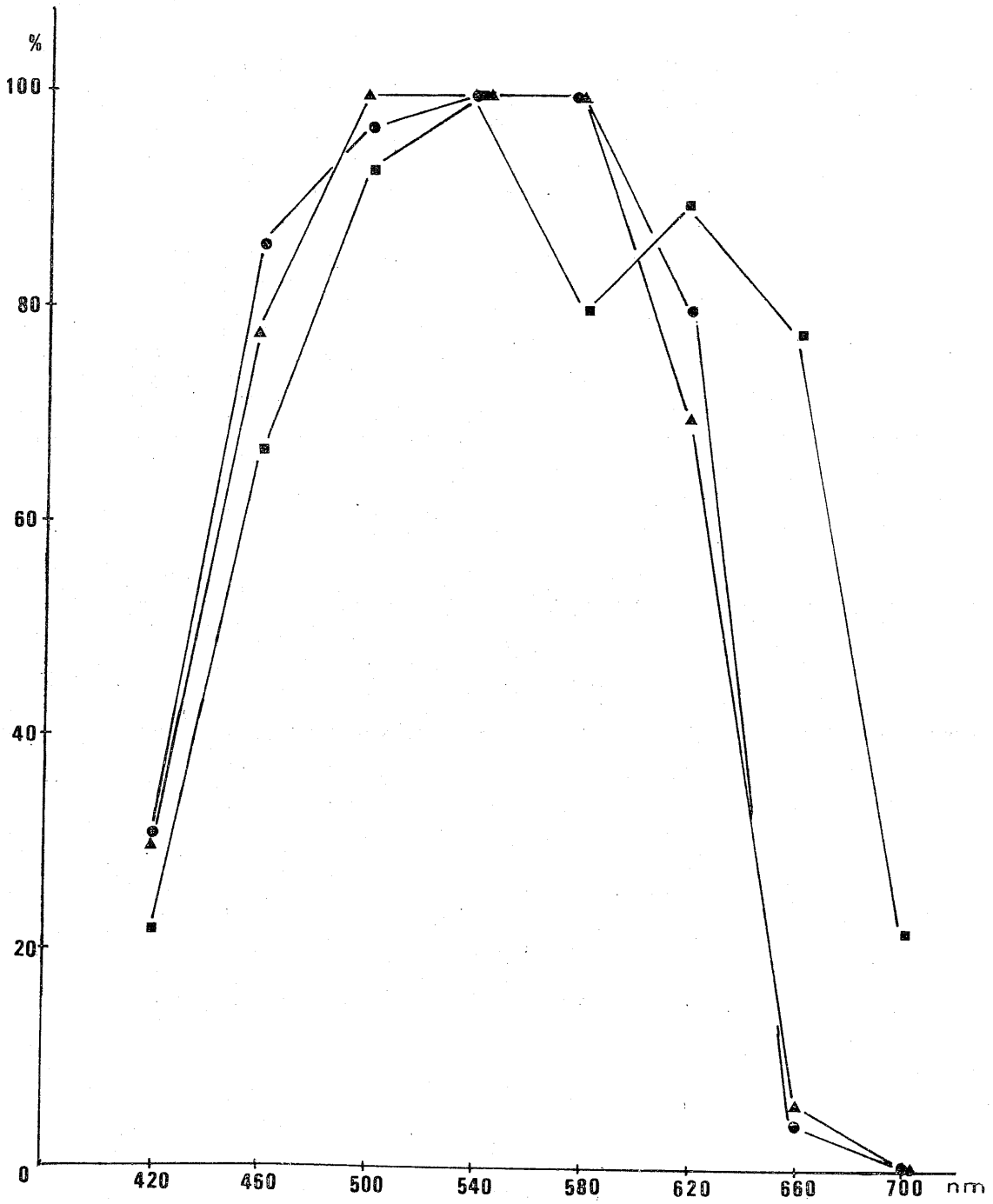

Fig. 2. Three extracellular electroretinographic responses to eight different wavelengths (420-700 nm, 40 nm steps) recorded from cod retina. All responses are relative to the largest response as 100%.

to be dichromatic: twin cones with λ_{max}=615 nm and 517 nm, single cones λ_{max}=535nm and 446 nm, respectively (Loew and Lythgoe, 1978; Huang, 1986). Due to the difficulties of intracellular recording and the importances of electrophysiological studies, the present work on recordings from the extracellular

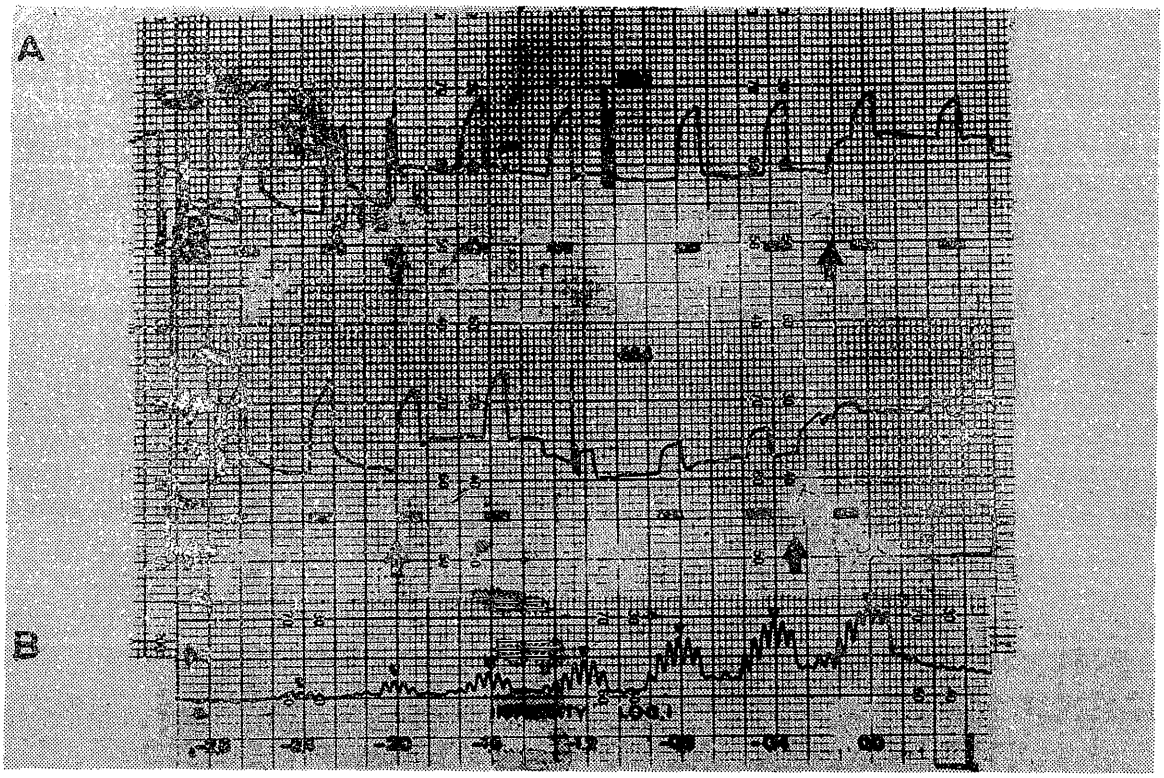

Fig. 3. The chromatic properties of perch photoreceptors extracellular recording.
A. Series of extracellular responses from Perch photoreceptor to 200 msec flash of light (lower trace) at different wavelengths showing typical "on" responses and slow "off" responses. A red light background is switched on at the arrow.
B. The responses to wavelengths 420-700 nm, in 40 nm steps at 8 intensities of light. The triangles points to 620 nm wavelengths.
Calibration: 0.4 sec and 2.5 mV.

photoreceptor response of both perch and cod retina were extraordinary. The obtained results were reasonably agreeing with the results from visual pigments measured from MSP surveys; i. e. systems are fitted to their own dominant photoreceptors (twin cones) spectral sensitivity. In other words, perch has its peak at a longer wavelength, cod at a shorter wavelength. The results suggested the responses of the cod electroretinograms were evoked more from twin cones (λ_{max}=517 nm) than from single cones (λ_{max}=446 nm) if considering the spectral sensitivity of the cod photoreceptors measured from the MSP survey (Huang, 1986).

The spectrograms of perch electroretinograms by intra-retinal recordings were also fitted the results from MSP studies on the spectral sensitivity of photoreceptors. The spectral sensitivity obtained from the present studies was clearly shown that perch photoreceptor layer was sensitive to the wavelength of 580-620 nm. The absorption curve of perch twin cones was measured by MSP surveys with peak at 615 nm (Loew and Lythgoe, 1978). In the mosaic pattern of the perch retina, the area occupied by twin cones was measured to be about twelve times that of single cones (Guthrie, 1983). The system appeared to be very strongly biased towards the longer wavelength of

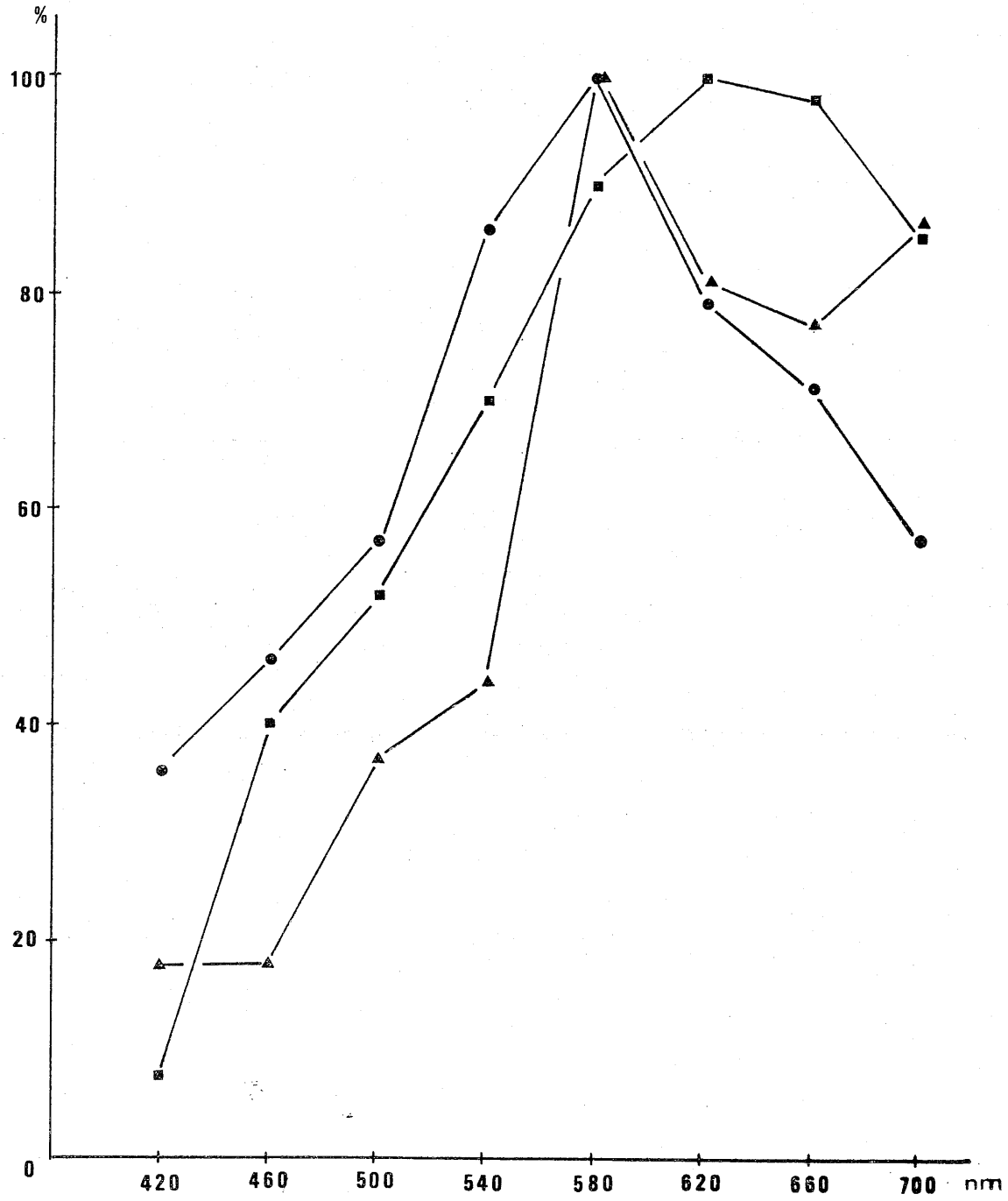

Fig. 4. Three extracellular electroretinographic responses to eight different wavelengths (420-700 nm, 40 nm steps) recorded from perch retina. All responses are relative to the largest response as 100%.

the twin cones rather than that of the shorter wavelength of the single cones with λ_{max}=535 nm. It is clearly indicated a twin cone dominate retina in perch.

In the present studies on both cod and perch, it is clearly shown that their

photoreceptor cells have reasonably similar spectral sensitivities to the ERGs and the horizontal cell potentials (Huang, 1986). The present results were not sufficient to understand all of the functional significance of these electrophysiological characteristics, but they showed some properties analogous to those of goldfish and roach (kaneko, 1970; Djamgoz *et al.*, 1985). One of the properties is the spectral-dependent response.

The response which is main characteristic of the photoreceptor layers shows more sensitivity to longer wavelength stimuli in perch and to shorter wavelength stimuli in cod. In general, the spectral distribution of the underwater light influences the visual pigments of aquatic animals. Roach (*Rutilus rutilus*) which is commonly found with perch, has the spectral sensitivity range more than 250 nm between the absorption peaks of the two extreme pigments (356-619 nm). Perch has the range less than 100 nm (535-617 nm) which is much narrower (Djamgoz, 1978).

It is also worth noting that spectral sensitivity of photoreceptors measured by MSP and by extracellular electroretinograms or S-potentials measured by intracellular recording were not demonstrated any spectral sensitivity peak at blue end in perch. On the contrary, the behavioural studies were showed a high sensitivity around 400 nm (Cameron, 1982; Guthrie, 1983) as the intracellular recordings from the amacrine cells, the final interneurones of a retina (Huang, 1986). It is likely that the visual processing and integration are operated with a complicated synaptic connections in the visual system (Werblin, 1974; Cameron, 1982), not only in any retinal neurones or any level of the visual system. Although the present results were not sufficient to understand all of the functional significance of the electroretinogram in these two dichromates, the extracellular recordings from the photoreceptor layers were proved to be a good and working technique to study spectral sensitivity of retinas.

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雙色視硬骨魚之網膜電位之色覺特徵

黃寶貴　詹果士

以顯微光譜分析儀 (Microspectrophotometry, MSP) 直接測定鱸魚 (*Perca fluviatilis*) 與鱈魚 (*Gadus morhua*) 之視細胞之吸收光譜已被證實兩魚種雙色視 (dichromatic) (Loew & Lythgoe, 1978; Huang, 1986)。由於視細胞不易記錄其胞內電位，本實驗利用顯微電極記錄分離網膜之視覺細胞層之胞外電位，結果顯示兩魚種均具持續性正電位反應 (sustained positive wave)，最敏感波長 (λ_{max}) 則分別爲 580～620 nm 和 500～540 nm，此與 MSP 顯示之雙生錐細胞之敏感波長 (分別爲 615 nm 和 517 nm) 極爲吻合，故推測兩魚種之視細胞層以此種雙生錐細胞爲主要組成。本結果亦較一般記錄之網膜電位圖 (Electroretinograms) 所得之光學特性與 MSP 有較佳之符合 (cited in Huang, 1986)。