Stud. Mar., 23(1): 107-114

UDC 597:612.842

VISUAL PIGMENTS AND SPECTRAL SENSITIVITY OF FISHES WITH CHANGEABLE CORNEAL COLORATION

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ABSTRACT

The photoreceptors, visual pigments and corneas of three species of family Hexagrammidae were studied using microspectrophotometry and light microscopy. The single cones in *Hexagrammos octogrammus*, the most shallow water species among the fish studied, contain visual pigments of λ_{max} at 510 nm, while in two other species, *H. stelleri* and *Pleurogrammus azonus,* they peaked at 474 and 472 nm, consequently. The non-identical double cones in *H. octogrammus* have λ_{max} at 523/545 nm, and identical double cones in semipelagic *Pl. azonus* - at 523/523 nm. The colour discrimination in *H. octogrammus* having dense orange corneas is possible only at dim light despite red shifts of λ_{max} of its pigments.

Key words: microspectrophotometry, cone photoreceptors, colour vision, Hexagrammidae

VIDNI PIGMENTI I SPEKTRALNA OSETLJIVOST RIBA SA PROMENLJIVOM BOJOM ROŽNJAČE

REZIME

Fotoreceptori, vidni pigmenti i rožnjače tri vrste riba iz famlije Hexagrammidae sutudirani su upotebom mikrospekrtofotometrije i svetlosne mikroskopije. Jednostruki pehari vrste *Hexagrammos octogrammus*, koja naseljava najpliće vode, sadrže vidne pigmente sa λ_{max} na 510 nm, dok je kod ostale dve vrste, *H. stelleri* i *Pleurogrammus azonus* ta vrednost na 474, odnosno 472 nm. Neidentični dvostruki pehari vrste *H. octogrammus* imaju λ_{max} na 510 nm, a identični pehari semipelagične vrste *P. azonus* na 523/523 nm. Razlikovanje boja kod *H. octogrammus*, vrste koja ima gustu naradžastu rožnjaču, moguće je samo na prigušenoj svetlosti, iako postoji crveni pomak λ_{max} njenih pigmenata.

Ključne riječi: mikrospektrofotometrija, fotoreceptori, razlikovanje boja, Hexagrammidae

INTRODUCTION

The presence of a cornea pigmented in yellow or orange colour was described for a long time for freshwater and marine fishes. At present, there are a considerable number of papers reviewing this phenomenon (Walls & Judd, 1933; Muntz, 1973; Heinermann, 1984; Kondrashev *et al.*, 1986; Orlov & Kondrashev, 1998; Douglas & Marshall, 2000). The biological role of such filters is still obscure but it is evident, that they can modify the spectral sensitivity of photoreceptors and thus influence on the colour discrimination of the whole animal. The problem looks more intriguing as in many fishes the corneal coloration is changeable (Orlov & Gamburtzeva, 1976; Kondrashev *et al.*, 1986; www.iitp.ru/projects/cornea/html). It appears as the dependence of the optical density of a cornea on ambient illumination, so on a bright light the cornea looks pigmented, and after dark adaptation within one-two hours it becomes colorless. It was shown, that the coloring of the cornea is made by chromatophores of the specialized type - with a big body and single, primary huge process, directed to a central part of a cornea. The reallocation of carotenoid pigments in the light and darkness also frames effect of the variable filter of different colour and optical density. In the masked greenling, *Hexagrammos octogrammus*, and some other fishes the corneal filter is very prominent - with large cell bodies, thick processes and very intensive coloring reaching several log units of density in a maximum of absorbance in the day (Kondrashev *et al.*, 1986). Thus, spectral and light intensity changes introduced by the alterations of the corneal coloration could be sufficient complicate factors superimposing other natural light changes. Generally, a photoreceptor system in fishes corresponds well to the spectral properties of the environment, and the existence of such a bright optic filters raise the question as to whether there is any matching between spectral properties of the photoreceptors and properties of the cornea. Some evaluations on the interrelation of the corneal density dynamics and visual discrimination have been made earlier (Appleby & Muntz, 1979; Gnyubkin, 1989; Shukolyukov & Tyurin, 1975), but without exact data about the spectral sensitivity of individual photoreceptors. On the other hand, in some fish species which possess colored corneas, the visual pigments were already studied by microspectrophotometry but their visual ecology was discussed without reference to the corneal filtering (Collins & McNichol, 1984; Bowmaker *et al.*, 1994).

In a following study, we set out to measure the spectral sensitivity of the retinal photoreceptors of different fish species having changeable corneal coloration. Our aim was to reveal the modification of the spectral sensitivity of photoreceptors by the colored cornea and look for correlations between the spectral properties of photoreceptors and spectral shifts of ambient illumination due to corneal re-coloration.

MATERIAL AND METHODS

The fish species selected for this study belong to the family Hexagrammidae which, according to the morphological data, have the most colorful corneas, so this should increase our chance to find out a correlation between spectral properties of cornea and photoreceptors. Among the three species studied, *Hexagrammos octogrammus, H. stelleri* and *Pleurogrammus azonus,* the first one have the most dense corneal filter ever found (Orlov & Gamburtzeva, 1976; Kondrashev *et al.*, 1986). Most of them inhabit shallow depths and follow diurnal benthic mode of life (except semipelagic *P. azonus*). The fish were caught with cage nets and stationary net in the Vostok Bay (Sea of Japan) at depth of 1-20 m in June-July 1999-2001. They were kept in aquaria of the Vostok Biological Station (Institute of Marine Biology FEB RAS) before being prepared for measurements. Prior to taking measurements of visual pigments and corneal media, fish were anaesthetized with MS222 and decapitated.

1. *Microspectrophotometry.* Microspectrophotometry (MSP) was carried out from the fresh tissue using a single-beam device with a "jumping stage" (Govardovskii & Zueva, 1988a) with spectra recorded on a X-Y plotter or under computer control with AD converter. This was the same installation used earlier for measurements on Baikalian fishes (Bowmaker *et al.*, 1994). To avoid bleaching only one scan was used over the range 700-400 nm at scanning speed about 20 nm/sec. The methodological details for MSP analysis of individual photoreceptors were as described previously in many papers (Collins & MacNichol, 1984; Govardovskii & Zueva, 1988b; Bowmaker *et al.*, 1994; (Sillman *et al.*, 1990). All measurements were conducted under dim red or IR light using CCD camera.

Spectra recorded with a plotter were digitized with a digitizing tablet (Summagraphics II) and converted into computer data files using program DIDGER (Golden Software). A computerized version of the same MSP allowed getting data files directly at the measurements. The absorbance values where than fitted with a template curves using equations proposed by V.V. Maximov (1988) and curve fitting program (TABLECURVE 2D, Jandel Scientific). The raw data, without smoothing, were normalized and the best fit was found for the long wave limb between 30% and 70% of maximum. Same program showed λ_{max} as an argument value when the 2nd derivative of the resulted template curve equals zero.

2. *Cornea transmission measurements*. The cornea was isolated and was mounted between two coverglasses in glycerol-gelatin after 2 h fixation in 2% glutharaldehyde for longer storage, or mounted fresh in a few drops of 0.9% saline for immediate measurements. Spectral transmission spectra of the whole cornea (except its peripheral part where the cell bodies of corneal chromatophores are located) were obtained using Perkin-Elmer 555 spectrophotometer. Spectral absorbance of individual corneal chromatophores was measured using the same MSP device, as was used for visual pigment measurements.

3. *Retinal morphology*. For histological examination, the eyes were incised, the cornea removed and the eye cup immersed in fixative (2.5% glutaraldehyde in 0.1M PSB, pH 7.4) for several hours. Fixed retinas were then processed and embedded for purposes of conventional light microscopy in paraffin and semi thin EPON sections.

RESULTS AND DISCUSSION

Histological observations show that in all the species studied cone photoreceptors form a regular mosaic pattern. As found in many teleosts, four double cones form a square with a single cone in a center (Fig. 1). In greenlings (Hexagrammidae) double cones looked symmetrical, only several cells had the two halves of slightly different size. Some other properties, including differential staining of ellipsoids by the haematoxylin-eozyn reagent (our unpublished observation) prove that at least in two species, *H. octogrammus* and *H. stelleri*, double cones are unequal.

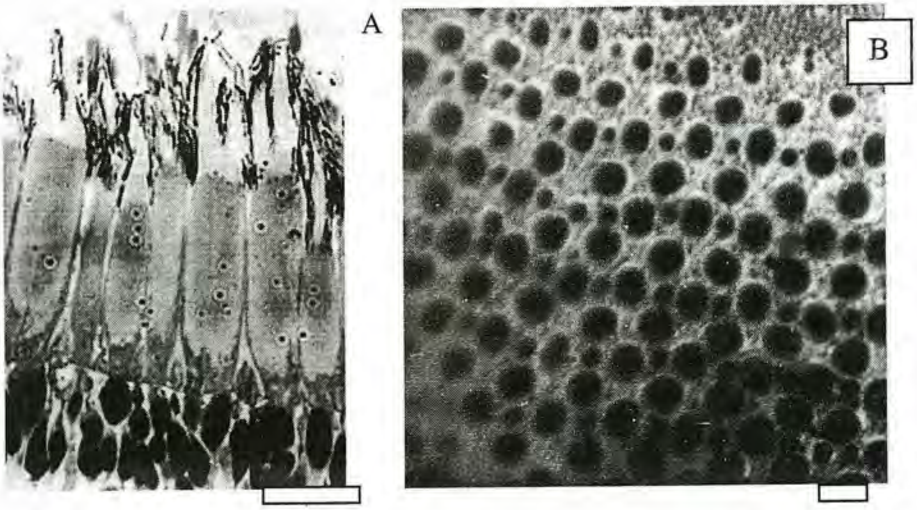

Fig. 1. Light micrographs of the retina of *H. octogrammus*. (A) Radial section, objective x100; (B) tangential section at the level of ellipsoids showing the square mosaic of cones, objective x40. DC – double cone, SC – single cone. Calibration bars: 10 mkm.

This observation was confirmed during MSP-measurements when different visual pigments in the two members of the double cones where found in both above species: 523/545 nm in *H. octogrammus* (Fig. 2) and 530/555 in *H. stelleri.* We cannot explain the difference between very close related species dwelling almost the same habitat, because more MSP-data from *H. stelleri* are needed (only 4 members of the same double cone were measured). The double cones in another greenling species, *Pleurogrammus azonus*, look quite equal both in morphology and pigment content. The spectral absorbance of both outer members of double cones peaked at 523/523 nm; close value was revealed earlier on the same setup by Dr. V.I. Govardovskii (pers. comm.) (Fig. 2).

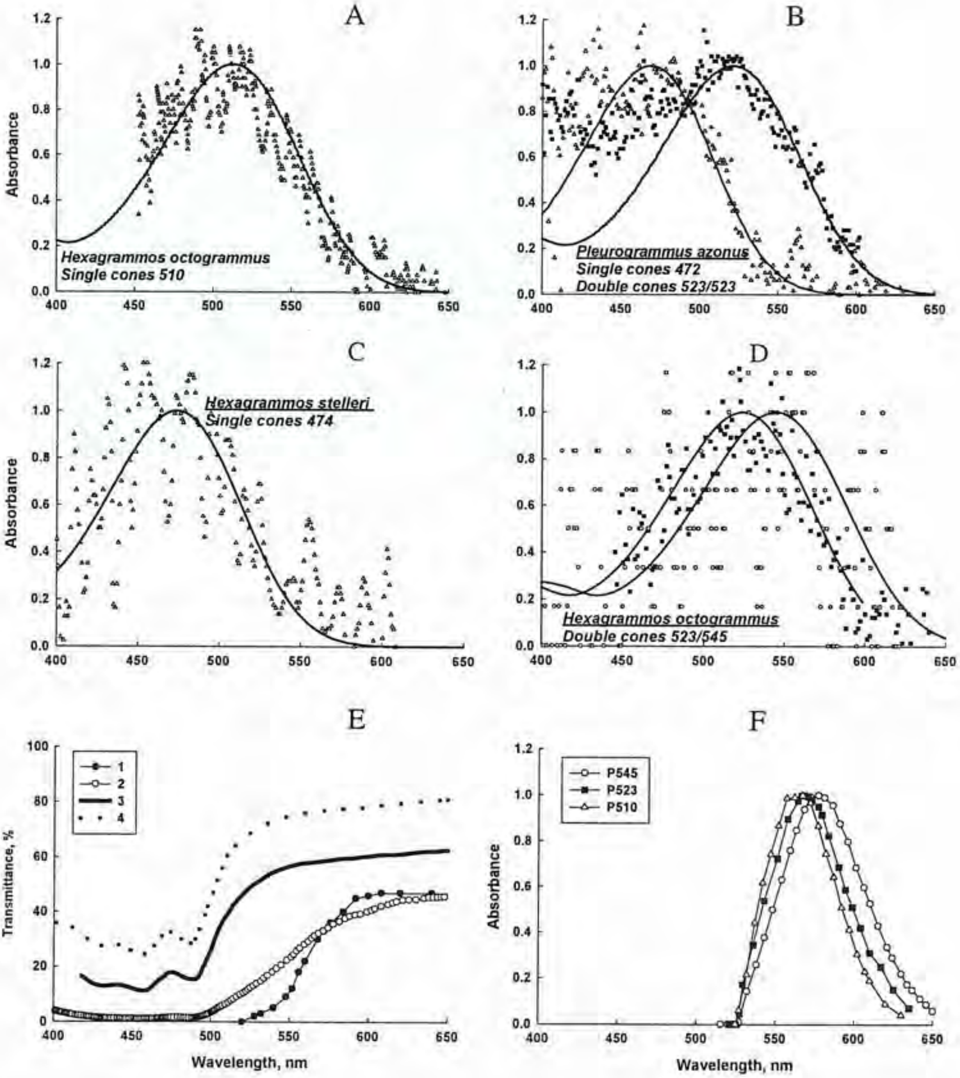

Fig. 2. MSP absorbance spectra from cone outer segments (A-D, normalized), transmittance spectra of whole corneas (E) of three Hexagrammid fishes and cone spectra of *H. octogrammus* modified by orange cornea in full colour (F, normalized). E – (1) *H. octogrammus*, full color; (2) *H. octogrammus*, at 1000 lux; (3) *H. stelleri*, full color; (4) *P. azonus*, full color. The cone spectra are raw date from the single cell with λ_{max} close to the mean values. The full curves are rhodopsin template curves best fitted to the long-wave limbs of spectra.

Like in most marine teleosts, single cones in Hexagrammid fishes revealed more shortwave λ_{max} (Fig. 2). It is pertinent to note, that in *H. octogrammus* their λ_{max} was significantly more red-shifted in comparison to single cells from the related species having (510 nm vs. appr. 470 nm). As we know from our previous studies, fishes of the family Hexagrammidae possess the mostly colored corneas (Fig. 2E). Their optic density at λ>520 nm can reach more than 3 log. units. Such dense filters could drastically alter the light spectra perceived by fish, and we tried to match some spectral characteristics of corneas and visual pigments sensitivity. As it is seen on the Fig. 2F, the spectral sensitivity shifts of the cone visual pigments modified by deep orange cornea in *H. octogrammus* are so significant that colour vision is, probably, impossible. We suppose that only in the dim light (in this fish), or in other species, when less dense changeable yellow corneal filters are used, the perceived colour range could be much broader.

The studied fishes (although a limited number) represent benthic littoral, sublittoral and semipelagic (*Pleurogrammus*) species, which demonstrate a known correlation between their depth of habitat and λ_{max} of the cone pigments. Another correlation exists between the habitat and the density of corneal coloration. Both this correlations "work" in the same "direction": the more shallow is the habitat the more red-sensitive visual pigments and densely orange corneas fishes have. Thus, there may not be a direct interrelation between visual pigment's and corneal properties.

The "red-shift" of single cones in *H. octogrammus* (Fig. 2) is remarkable because the presence of single cone pigments with λ_{max}>510 nm in "true marine" fishes is rather unusual (Loew & Lythgoe, 1978; V.V. Maximov, pers. comm.). According to our preliminary data, another shallow water fish, *Stichaeus punctatus* (Stichaeidae) also has single cones with λ_{max} about 510 nm and, as in *H. octogrammus*, this is combined with the densest orange cornea among other Stichaeids. Almost nothing is known about the interrelation between cone visual pigments and properties of the corneal light filters in Labrid fishes, but, again, the single cone pigment with λ_{max}=555 nm was found in Mediterranean corkwing wrasse, *Crenilabrus melops*, wich is known to have a constant yellow corneal filter (Gnyubkina & Gamburtzeva, 1981). To put more light on the scene, more comparative studies of visual pigments, eye media and visual ecology of the related species are needed.

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