

SPECTRAL SENSITIVITY OF THE PERCH (*PERCA FLUVIATILIS*) FROM THE DANUBEZ. GAČIĆ<sup>1</sup>, A. BAJIĆ<sup>2</sup>, MILENA MILOŠEVIĆ<sup>2</sup>, M. NIKČEVIĆ<sup>1</sup>, B. MIĆKOVIĆ<sup>1</sup> and I. DAMJANOVIĆ<sup>3</sup><sup>1</sup>Center for Multidisciplinary Studies, University of Belgrade, 11000 Belgrade, Serbia<sup>2</sup>Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia<sup>3</sup>Institute for Problems of Information Transmission, Russian Academy of Sciences, 119071 Moscow, Russia

**Abstract** — In spectral sensitivity studies, electroretinograms (ERG) were recorded from the in situ eyecup of immobilized perch (*Perca fluviatilis*) electrofished in the floodplain zone of the Danube River. It is shown that the ERG b-wave is a good indicator of spectral sensitivity, although it reflects the activity of photoreceptors indirectly. With a maximum around 542 nm, the scotopic spectral sensitivity of perch obtained using fitted amplitude-log intensity functions for threshold calculation and two models developed in our laboratory for computer-assisted fitting of spectral sensitivity curves did not differ from the sensitivity determined using the microspectrophotometric and isolated pigment methods (maximum around 541 nm).

**Key words:** Perch, ERG, spectral sensitivity, the Danube, Serbia

UDC 597.331.1:577.3:591.484.6.08

## INTRODUCTION

Perch (*Perca fluviatilis*), like most teleosts, possesses a duplex retina, containing both rod and cone photoreceptors. The cones are of moderate size and oriented to increase visual perception, with most of the cones situated near the optic nerve. The rods are large and regularly distributed over the retina (Sandstrom, 1999). According to Bridgess (1961), perch have A2 visual pigment porphyropsin (P) with maximum value of the spectral sensitivity curve ( $\lambda_{max}$ ) at about 541 nm and two cone pigments, P530<sub>2</sub> and P617<sub>2</sub> (Cameron, 1982). Measurements of the b-wave of the electroretinogram (the complex extracellular field potential caused by light stimulation, ERG) were obtained in perch caught in the floodplain zone of the Danube River. The b-wave, the most prominent wave of the ERG, although it indirectly reflects the responsiveness of photoreceptors, is a good indicator of spectral sensitivity in fishes (Andjus et al., 1998a; 1998b; Nusdorff and Powers, 1988; Saszik and Bilotta, 1999; Gačić et al. 2007). This is generally accepted opinion with some exceptions: carp and goldfish possess rod photopigments with  $\lambda_{max}$  at around 520 nm, but the

peak spectral sensitivity based on b-wave measurements is broader and shifts to the red part of the spectrum (Nusdorff and Powers, 1988).

In searching for the best-fitting peak value of the spectral sensitivity curve based on the b-wave measurements in perch, we made parallel use of two procedures. The first consisted of applying formula proposed by Lamb (1995), which uses Mansfield's normalization (absorbance spectra plotted on normalized frequency; Mansfield 1985), to prove the Dartnall's fundamental hypothesis that the absorbance spectral curve of any visual pigments has same basic shape when plotted on a frequency scale ( $c/\lambda$ ), depending only on one parameter(s) as the basis of his nomogram (Dartnall, 1953). In our work, we used the modification of Lamb's parameters proposed by Govardovskii et al. (2000) for the  $\alpha$ - and  $\beta$ -band (Stavenga et al., 1993) of A2 pigments. The second procedure, use of a three-parameter curve, was developed in our laboratory (Gačić et al. 2007). However, the design and universality of a valid template depend only on empirical curve fitting to recorded data because there is no comprehensive physical theory that

explains the relation between molecular structure and absorbance characteristics of visual pigments.

## MATERIALS AND METHODS

### *Animals*

Perch were electrofished in the floodplain zone of the Danube River (km 1136). Fish were kept in captivity for at least 15 days (water temperature 15–17°C) in order to acclimatize them to experimental conditions. Animals were maintained under conditions of a 14<sup>h</sup> / 10<sup>h</sup> dark/ light cycle.

### *In situ eyecup preparation*

Fish were anesthetized (with phenobarbital sodium) and curarized (with tubocurarine) following procedures recommended by Hamasaki et al. (1967), adjusting the dosage to induce the arrest of respiratory movements. Artificial respiration was provided continuously by forcing aerated and temperature-controlled water through the gills. The immobilized fish were positioned laterally on a plastic platform inside a light-proof Faraday cage. The preparations were surgically deprived of cornea, lens, and most of the vitreous; filled with teleost Ringer (in mM): 145 NaCl, 20 NaHCO<sub>3</sub>, 2.5 KCl, 0.7 CaCl<sub>2</sub>, 1 Mg Cl<sub>2</sub>; and maintained at 15°C. Experiments were performed in the late afternoon or evening during the autumn to prevent any influence of diurnal rhythms on spectral sensitivity (Halstenberg et al., 2005).

### *Recordings and stimulation*

ERG potentials were detected with non-polarizable chlorided silver electrodes (Ag-AgCl, World Precision Instruments, Inc., model EP2), the active one being introduced into the interior of the saline-filled eyecup. The reference electrode was in the retro-orbital space. The electrodes were connected to the input stage of a directly coupled differential preamplifier, and responses were recorded by transferring from the preamplifier to a computer with the aid of an AD-converter. The data acquisition rate was 130 Hz. Original software was developed for data acquisition and analysis. Photoc stimuli were delivered by a single-beam optical system using an 8

V 50 W Tungsten-halogen lamp as the light source and providing independent control of intensity (neutral density filters), duration (electromagnetic shutter, UniBlitz model T132), and spectral composition (interference filters) of the test flashes. The stimuli consisted of single flashes guided through an optic fiber positioned normal to the surface of the eyecup and casting a circular patch of light that covered the external surface of the preparation. Light intensities were calibrated and checked by placing the active surface of the custom-made radiometer probe in the position usually occupied by the eyecup preparation. The maximum unattenuated light intensity of the beam from the 50 W Tungsten-halogen lamp was 282 μW/cm<sup>2</sup>. When comparing intensity/amplitude relations in different preparations, relative intensity (I<sub>R</sub>) scales were used, plotting ERG amplitude voltage against attenuation extent in log units.

### *Fitting procedures*

Two procedures were simultaneously used for fitting ERG-based spectral sensitivity data. The first consisted of applying the Lamb model (Lamb, 1995) with the parameters proposed by Govardovskii (Govardovskii et al., 2000). The second one was use of our three-parameter model for the α-band of A1- (Gačić et al., 2007) and A2-based pigments. It is a three-parameter (a-c) equation of the form:

$$(1) \quad S_{(\lambda)} = a \cdot (1+n)^{-(b+1)/b} \cdot n \cdot (b+1)^{-(b+1)/b}$$

with

$$n = e^{\frac{[\lambda + c \cdot \ln(b) - \lambda_{\max}]}{c}}$$

where the set of parameters in Eq. (1), which provided a good fit to the full range of our A1 data, was as follows: a=27.5749, b=0.3809 and c=35.5. For A2-based pigments, the parameters' values were a=32.8, b=0.2132 and c=46.42.

The short-wave peak remaining after subtraction of the α-band template (1) was fitted with the Gaussian equation:

$$(2) \quad S_{\beta}(\lambda) = A_{\beta} \cdot e^{\{-[\lambda - \lambda_{m\beta}]^2/d\}}$$

where:

$A_\beta$  is amplitude of the  $\beta$ -band relative to the  $\alpha$ -band

$\lambda_{m\beta}$  is position of the  $\beta$ -maximum

and  $d$  is a band-width parameter.

$A_\beta$  was fixed at a value of 0.26 for A1-based pigments because of its best fit with Dartnall's frog spectral sensitivity data (Dartnall, 1953). The relationships between  $\lambda_{m\beta}$  and position of the  $\beta$ -maximum ( $\lambda_{m\beta}$ ) and between  $\lambda_{m\beta}$  and  $d$  could be approximated as straight lines:

$$(3) \quad \lambda_{m\beta} = 170.1 + 0.339 \cdot \lambda_{\max}$$

$$(4) \quad d = 41.63 + 0.0086 \lambda_{\max}$$

In the same way as for A1 pigments, the full absorbance spectrum of A2-based pigments was decomposed into  $\alpha$ - and  $\beta$ -bands. We fitted the  $\beta$ -bands with equation (2).  $A_\beta$  was fixed at a value of 0.2043 for A2-based pigments because of its best fit with Bridges's carp spectral sensitivity data (Bridges, 1967). The relationships between  $\lambda_{m\beta}$  and position of  $\beta$ -maximum ( $\lambda_{m\beta}$ ) could be approximated with a straight line (5), but that between  $\lambda_{m\beta}$  and  $d$  required a second-order approximation (6), similar to the model of Govardovskii (Govardovskii *et al.*, 2000).

$$(5) \quad \lambda_{m\beta} = 217.6 + 0.277 \lambda_{\max}$$

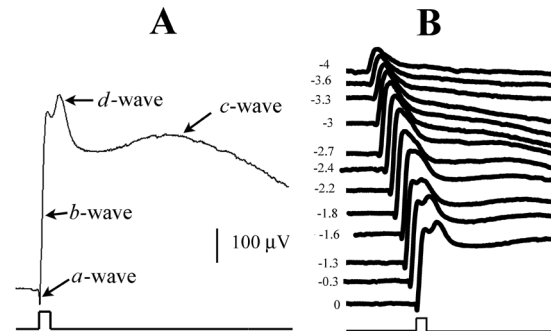
$$(6) \quad d = 419 - 1.538 \lambda_{\max} + 0.001583 \lambda_{\max}^2$$

A complete description of the absorbance spectra of A1- and A2-based visual pigments between 400 nm to far red was provided by equations (1)-(6).

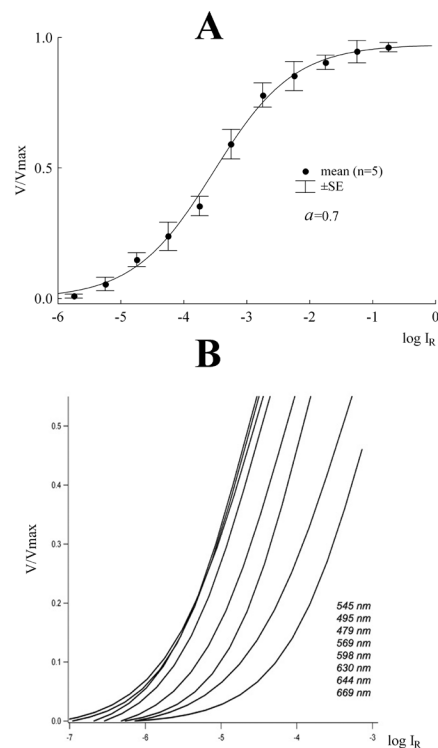
## RESULTS

### Waveforms

As Fig. 1A shows, the four principal components of the cone-dominated vertebrate retina were well

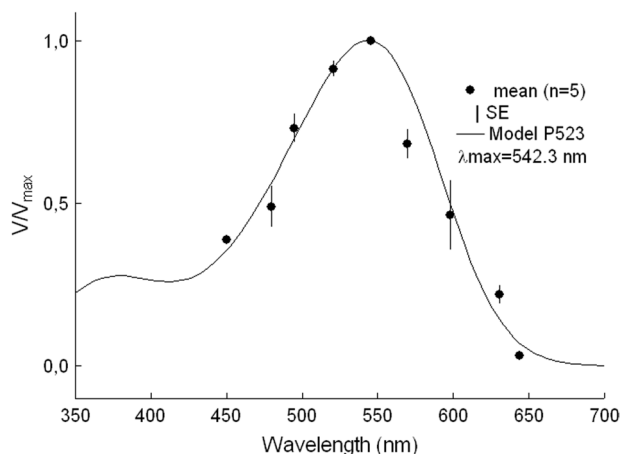


**Fig. 1.** Examples of ERG waveforms. A: normal ERG of perch obtained with saturating light intensity. B: series of ERGs obtained with incremental stimulation from the eye of perch (log attenuation units presented at each trace). Stimuli were white light (maximal intensity was 282 W/cm<sup>2</sup>); 0.1 s.



**Fig. 2.** Amplitude/intensity relations in perch. A: relationship of normalized amplitude of response  $V/V_{\max}$  (b-wave data) and log intensity of stimulation. Fitted curve according to  $V_o = I^a / (I^a + I_o^a)$ ;  $V_o$  is normalized voltage ( $V/V_{\max}$ ) of the ERG signal,  $I_o$  is the stimulating light intensity (corresponding to  $V_o = 1/2$ ), and exponent  $a$  is a constant.

B: averaged intensity/response function recorded in five fish at different wavelengths between 479 and 669 nm. Wavelength numbers corresponding to lines from left to right.



**Fig. 3.** Perch scotopic sensitivity curve. Simultaneously fitted are five sets of experimental scotopic b-wave data. Fitting according to model for A2 based pigments;  $\lambda_{\max} = 542.6$  nm. SE-standard error.

represented: an initial negative deflection (*a*-wave), a fast positive transient (*b*-wave), reaction to light offset (*d*-wave), and a slow positive potential (*c*-wave). Under scotopic conditions with near threshold stimuli, the perch ERG normally consists of only the *b*-wave (Fig. 1B, top record).

#### Intensity-amplitude relations

Threshold values were determined from intensity-response functions obtained by increasing intensity of the stimulus light for each wavelength (from 450 to 669 nm) by 0.3 log units. In no case were variations of parameter *a* in correlation with wavelength. An average value of  $0.81 \pm 0.05$  for parameter *a* was provided by a total of 80 b-wave  $V_o/\log I$  sigmoids obtained at eight wavelengths in perch. Although the difference for fitted log-sigmoids (parameter *a*) was small, we used the  $\log V/\log I$  curve to calculate threshold values on which to base spectral sensitivity determinations. A signal amplitude equal to 65  $\mu\text{V}$  was adopted as the threshold criterion; it is approximately 10% of the greatest response obtained with light stimuli of the most effective wavelength. The intensity-response series shown in Fig. 2A was obtained following dark-adaptation of a perch; it showed that the response amplitude of the *b*-wave to incremental photostimulation is in conformity with the basic model (Naka and Rushton, 1966; Dowling and Ripps, 1972).

Figure 2B shows the averaged intensity-response function recorded in five fish at different wavelengths between 450 and 669 nm. It can be seen that in the case of relatively short wavelength stimuli (479–545 nm), the curves are shifted to the left, where increased wavelengths require lower intensity of stimuli to evoke a response. In the case of relatively long wavelength flash stimuli (569–669 nm), increased wavelengths cause shifts to the right, to higher intensity of stimuli.

Figure 3 shows an example of averaged spectra obtained in five perch constructed by simultaneously fitting all *b*-wave spectral sensitivity data. The average value obtained using the proposed model (see Materials and Methods) for A2-based pigments was 542.3 nm.

Individual fitted perch exhibited values ranging from 534.6 to 548.5 nm (average  $542.1 \pm 2.2$  nm) and from 534.2 to 543 nm (average  $538.7 \pm 1.9$  nm) in the cases of fitting by our model and the Lamb-Govardovskii model, respectively.

#### DISCUSSION

The spectral sensitivity of photoreceptors of fish should be in accordance with the spectral composition of light in their environment. In general, for deep-sea fishes, this appears to be correct, but in many surface-dwelling fishes (including percids) it is not the case. The retina of percids absorbs maximally in regions of the spectra slightly deviating from the regions where water transmits maximally. To resolve this discrepancy Lythgoe (1975) emphasized the importance of visual contrast as the main selective agent for the evolution of different adaptations of the visual system in fish to their environment. Underwater objects reflect light that travels a shorter distance than the scattered light behind it before it reaches the eye of a fish giving light from the object a different chromatic composition than the background (Sandstrom, 1999). The wavelength maximum of light reflected from the object is closer to the spectral region dominant at the surface, while the wavelength maximum of the background is closer to the maximum transmission point of the water (Lythgoe, 1986). The maximal absorption

of freshwater and coastal fish eyes is therefore at a wavelength somewhat shifted from the transmission maximum of the surrounding water, in order to enhance the visual contrast between the object and the background (Lythgoe, 1986). This is believed to be advantageous when detecting objects under water. The fitted values (both models) are in general agreement with those obtained previously (Bridges, 1961).

## REFERENCES

- Andjus, R. K., Damjanović, I. Gačić, Z., Konjević Dj. and P. R. Andjus (1998a). Electroretinographic evaluation of spectral sensitivity in yellow and silver eels (*Anguilla anguilla*). *Vis. Neurosci.* **15**, 923-930.
- Andjus, R. K., Konjević, Dj., Damjanović, I., Gačić, Z., and P. R. Andjus (1998b). Dogfish sharks and eels as experimental models. II. Electroretinography: effects of temperature and light. *Iugosl. Physiol. Pharmacol. Acta* **34**, 381-399.
- Bridges, C. D. B. (1961). Studies on the flash photolysis of visual pigments. *Biochem. J.* **79**, 128-134.
- Bridges, D. (1967). Spectroscopic properties of porhyropsins. *Vis. Res.* **12**, 349-369.
- Cameron, N. E. (1982). The photopic spectral sensitivity of a dichromatic teleost fish (*Perca fluviatilis*). *Vis. Res.* **22**, 1341-1348.
- Dartnall, H. J. A. (1953). The interpretation of spectral sensitivity curves. *Brit. Med. Bull.* **9**, 24-30.
- Dowling, J. E., and H. Ripps (1972). Adaptation in skate receptors. *J. Gen. Physiol.* **60**, 698-719.
- Gačić, Z., Damjanović, I., Mičković, B., Hegediš, A. and M. Nikčević (2007). Spectral sensitivity of the dogfish shark (*Scyliorhinus canicula*). *Fish Physiol. Biochem.* **33**, 21-27.
- Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin D. G., and K. Donner (2000). In search of the visual pigment template. *Vis. Neurosci.* **17**, 509-528.
- Halstenberg, S., Lindgren, K. M. Samagh, S., Nadal-Vicens, M., Balt, S., and R. Fernald (2005). Diurnal rhythm of cone opsin expression in the teleost fish *Haplochromis burtoni*. *Vis. Neurosci.* **22**, 135-41.
- Hamasaki, D. I., Bridges, C. D. B. and K. A. Meneghini (1967). The electroretinogram of three species of elasmobranchs. In: *Sharks, Skates, and Rays* (Eds. P. W. Gilbert, R. F. Mathewson, and D. P. Rall), 447-463. Johns Hopkins Press, Baltimore.
- Lamb, T. D. (1995). Photoreceptor spectral sensitivities: Common shape in the long-wavelength region. *Vis. Res.* **35**, 3083-3091.
- Lythgoe, J. N. (1975). Problems of seeing colors under water, In: *Vision in Fishes*. (Ed. M. A. Ali), Plenum Press, London.
- Lythgoe, J. N. (1986). Light and vision in the aquatic environment, In: *Sensory Biology of Aquatic Animals* (Eds. R. R. Fag, A. A. Popper, and W. N. Tarologa), Springer Verlag, Hamburg.
- Mansfield, R. J. W. (1985). Primate photopigments and cone mechanisms, In: *The Visual System* (Eds. J. S. Levine and A. Fein), 89-106. Alan Liss, New York.
- Naka, K. I. and W. A. H. Rushton (1966). S-potentials from luminosity units in the retina of fish (Cyprinidae). *J. Physiol.* **185**, 587-599.
- Nussdorf, J. D. and Powers, M. K. (1988). Spectral sensitivity of the electroretinogram b-wave in dark-adapted goldfish. *Vis. Neurosci.* **1**, 159-168.
- Ren, J. and L. Li (2004). A circadian clock regulates the process of ERG b- and d-wave dominance transition in dark-adapted zebrafish. *Vis. Res.* **44**, 2147-2152.
- Sandstrom, A. (1999). Visual ecology of fish - a review with special reference to percids. *Fiskeriverket Rappt.* **2**, 45-80.
- Saszik, S., and J. Bilotta (1999). The effects of temperature on dark-adapted spectral sensitivity function of the adult zebrafish. *Vis. Res.* **39**, 1051-1058.
- Stavenga, D. G., Smits, R. P. and B. J. Hoenders (1993). Simple exponential functions describing the absorbance bands of visual pigment spectra. *Vis. Res.* **33**, 1011-1017.

**СПЕКТРАЛНА ОСЕТЉИВОСТ ГРГЕЧА (*PERCA FLUVIATILIS*) ИЗ ДУНАВА**

З. ГАЧИЋ<sup>1</sup>, А. БАЈИЋ<sup>2</sup>, МИЛЕНА МИЛОШЕВИЋ<sup>2</sup>, М. НИКЧЕВИЋ<sup>1</sup>, Б. МИЋКОВИЋ<sup>1</sup> и И. ДАМЈАНОВИЋ<sup>3</sup>

<sup>1</sup>Центар за мултидисциплинарне студије Универзитета у Београду, 11000 Београд, Србија

<sup>2</sup>Институт за физиологију и биохемију, Биолошки факултет, Универзитет у Београду, 11000 Београд, Србија

<sup>3</sup>Институт за проблеме преноса информација Руске академије наука, 119071 Москва, Русија

У одређивању спектралне осетљивости методом електроретинографије (ERG) коришћен је препарат *in situ* очног пехара гргеча (*Perca fluviatilis*) из плавне зоне Дунава. Показано је да *b*-талас, иако само посредно представља активност фоторецептора, може бити добар показатељ спектралне осетљивости код риба. У одређивању прага надражаја коришћене су прилагођене криве односа амплитуде одговора и јачине стимулуса. Сама

спектрална осетљивост гргеча адаптираног на таму израчунавана је на основу два модела који су развијени и усвојени у нашој лабораторији. Добијена максимална вредност спектралне осетљивости гргеча (*Perca fluviatilis*) на око 542 nm одговара вредностима добијеним микроспектрофотометријском методом као и вредностима добијеним методом изолације пигмента (максимум око 541 nm).