Fish Physiol Biochem (2014) 40:1899–1906
DOI 10.1007/s10695-014-9977-9

Spectral sensitivity of the electroretinogram b-wave in dark-adapted Prussian carp (*Carassius gibelio* Bloch, 1782)

Z. Gačić · A. Bajić · M. Milošević · M. Nikčević · B. Mićković · A. Hegediš · L. Gačić · I. Damjanović

Received: 10 April 2014 / Accepted: 15 August 2014 / Published online: 24 August 2014
© Springer Science+Business Media Dordrecht 2014

Abstract One of the purposes of this study was to examine whether b-wave measurements can be used in the evaluation of scotopic spectral sensitivity in Prussian carp measurements when the eyes were surgically deprived of cornea, lens, and most of the vitreous. Another goal was testing the new fitting procedure for A2-based photopigments. Using fitted amplitude-log intensity functions for threshold calculation, and two models for computer-assisted fitting of spectral sensitivity curves, no significant differences in λ_{max} were found between rod photopigments and b-wave-based spectral sensitivity.

Keywords Prussian carp · Spectral sensitivity · b-Wave

Z. Gačić (✉) · M. Nikčević · B. Mićković · A. Hegediš
Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, 11000 Belgrade, Serbia
e-mail: zorga@imsi.rs

A. Bajić · M. Milošević · L. Gačić
Faculty of Biology, University of Belgrade, Studentski trg 16, 11001 Belgrade, Serbia

I. Damjanović
Institute for Problems of Information Transmission, Russian Academy of Science, Ermolova Str.19, Moscow 101447, Russia

Introduction

The b-wave, the most prominent electroretinogram (ERG) wave, is a good indicator of spectral sensitivity in fish, although it only indirectly reflects responsiveness of photoreceptors (Andjus et al. 1983, 1998; Nussdorf and Powers 1988; Easter and Hamasaki 1973; Saszik and Bilotta 1999). Until this study, it was considered true for all fish species except carp and goldfish, which possess rod photopigments with λ_{max} around 520 nm, because their peak spectral sensitivity based on b-wave is broader and shifts toward to the red part of the spectrum (Nussdorf and Powers 1988; Easter and Hamasaki 1973; Saszik and Bilotta 1999). There are two possible explanations for broadening of spectral sensitivity curve and inadequate λ_{max} based on the b-wave; contribution of red-sensitive cones in overall spectral sensitivity sharing (Burkhardt 1966; Nussdorf and Powers 1988) or self-screening of porphyropsin (Yang et al. 1990).

Discrepancy between photopigments in solution and intact retina is well known, and the difference is <10 nm (Bowmaker 1973). Nussdorf and Powers (1988) reported, based on b-wave measurements on the dark-adapted eye of goldfish, that λ_{max} was around 550 nm indicating a difference more than 25 nm. We must point out that measurements in both cases were conducted on intact eyes of goldfish.

One of the purposes of this study is to reexamine the scotopic spectral sensitivity in Prussian carp (*Carassius gibelio* Bloch, 1782) based on b-wave measurement

when the eyes were surgically deprived of cornea, lens, and most of the vitreous and to show that b-wave measurements are a good indicator of spectral sensitivity in Prussian carp when the above method is used. Another goal is testing the new fitting procedure for A2-based photopigment.

Materials and methods

Animals

Prussian carp were electrofished in the floodplain zone of the Danube River 1,136 km. Fish were kept in captivity for at least 15 days in order to acclimatize to experimental conditions. Animals were maintained on a 14-h/10-h dark/light cycle. All experiments were performed in the late afternoon (between 4 and 7 pm) to prevent possible circadian effects on visual sensitivity. Water temperature was between 15 and 17 °C keeping in mind the fact that exposure to high temperatures rises the percent of rhodopsin in rods of goldfish (Saszik and Bilotta 1999).

In situ eyecup preparation

Fish were anesthetized (phenobarbital sodium) and curarized (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 (mgw Lauda PTP Regler R20 K, range from −50 to +50 °C, precision 0.02 °C) 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, and filled with teleosts Ringer (in mM): 145 NaCl, 20 $NaHCO_3$, 2.5 KCl, 0.7 $CaCl_2$, 1 $MgCl_2$, and were maintained on 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).

In experiments with iodate, the eyecup was filled with physiological solutions in which a given amount of NaIO3 was substituted for an equivalent amount of NaCl. A built-in dose-dispensing and sucking device (polyethylene tubing) allowed for the replacement of solutions and intermittent washing without causing mechanical disturbances or changes in illumination. After 20 min, the iodate solution was washed out and replaced with the physiological medium. After introducing $NaIO_3$-containing solutions into the in situ eyecup, the ERG underwent gross changes resulting in a complete disappearance of the b-wave and the transformation of the ERG into a single negative deflection, the isolated late receptor potential (LRP), which directly reflects the responsiveness of photoreceptors.

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 in 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 transferring from a preamplifier to a computer by means of an AD-converter. Data acquisition rate was 130 Hz. Original software was made for acquisition and data analysis. Photic 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. 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 50 W tungsten-halogen lamp was 282 μW/cm^2. When comparing intensity/amplitude relations in different preparations, relative intensity (IR) scales were used, plotting ERG amplitude voltage against attenuation extent in log units.

Fitting procedures

In fitting our ERG-based spectral sensitivity data, we used our 3-parameter model for α- band of A1- (Gacic et al. 2007a) and A2-based pigments (Gacic et al. 2007b). It is a 3-parameter (a–c) equation of the form:

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

with

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

where the set of parameters in Eq. (1) that provide a good fit to the full range of our A1 data is $a = 27.5749$, $b = 0.3809$, and $c = 35.5$ and for A2-based pigments data is $a = 32.8$, $b = 0.2132$, and $c = 46.42$.

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

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

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

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

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

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

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

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

Equations (1)–(6) provide complete description of the absorbance spectra of A1- and A2-based visual pigments, between 400 nm to far red. Bearing in mind the fact that eyes of Prussian carp possess mixture of A1- and A2-based visual pigment (Powers and Easter 1978), we combined both fitting procedure of our spectral sensitivity data to find the best fit that provide percents of A1-based photopigment.

Results and discussion

Intensity-amplitude relations

The results that were included in the evaluation of the characteristics of the goldfish retina were subsequently tested by constructing an overlapping canonical curve (van Roessel et al. 1997). ERG amplitudes were acquired by using rising stimulus with a wavelength of 520 nm (light stimuli of the most effective wavelength) on five specimens where the amplitude of the b-wave was measured and on two specimens from the LRP extraction experiment (Fig. 1). The normalization of the independent variable was performed by dividing the data for each fish with the appropriate Io values. The measured slope of the canonical curve was $a = 0.86$ with a standard sigmoid deviation of 0.02. For the b-wave data, the slope value was 0.81 ± 0.05, and for the LRP data, the slope of the canonical curve was 0.90 ± 0.12. These values are in accordance with the distribution of the measured slopes from individual data (Table 1). The measured mean value of the slope a according to these data is 0.84 ± 0.04, which is also comparable to the canonical curve slope. In Table 1, the minimal and maximal response values detected during stimulation with 520 nm light are presented. Table 1 contains the measurements for mean stimulus values that were used for normalization and slope calculating of the sigmoid. In the b-wave data group, the mean Io value was -2.516 ± 0.37, while in the LRP results, the mean Io value was -1.81 ± 0.13. The mean value of the a slope was $a = 0.84 \pm 0.04$. From the values of sigmoid asymptotes and its central point, the differences in individual specimen sensitivity can be seen. Therefore, these results could be compared during the evaluation of spectral sensitivity. Relative spectral sensitivity for each individual fish was done with a procedure described in the "Methods".

Figure 2 shows the V/log$I$ function distribution along the axis that represents the stimulus intensity. The curves represent the amplitude of b-waves acquired by different color light stimulus. The central point of every sigmoid also designate the spectral sensitivity of the retina to the applied light. RSS maximum sensitivity was recorded at 520 nm light, as shown in Fig. 2. The average slope of the regression lines in log$V$/log$I$ shape during the goldfish threshold RSS measurement was 0.326 ± 0.04.

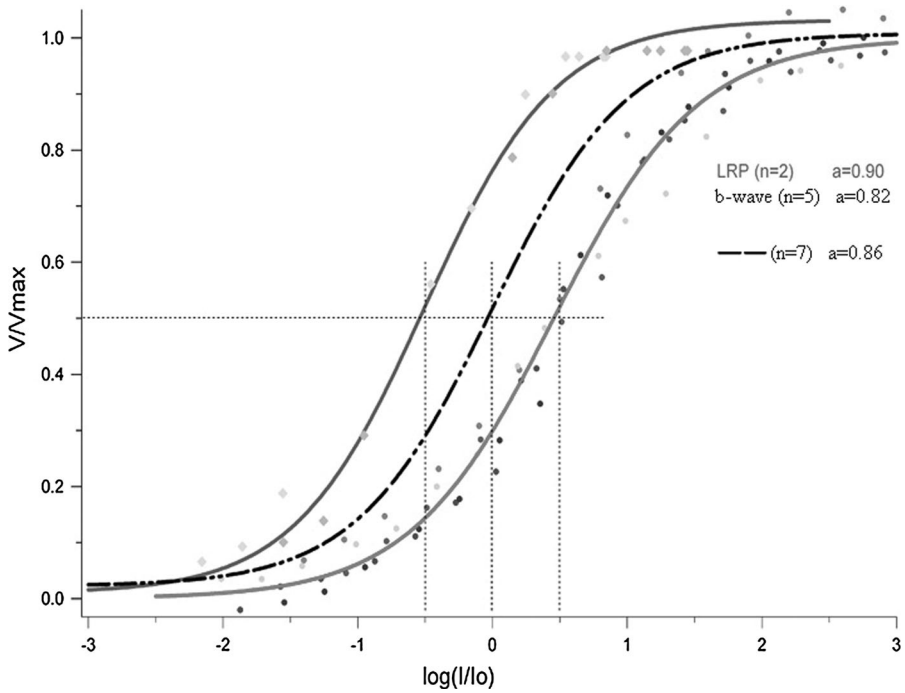

Fig. 1 Canonical curve shows an expected sigmoid dependency of the normalized amplitudes of b-waves and LRP in *C. gibelio* under stimulation by a rising stimulus with a wavelength of 520 nm. Points acquired from the same fish specimen share the same color. Curve and points for the b-wave data are shifted 0.5 log units to the right for a better overview, while the LRP values are shifted 0.5 log units in the opposite direction. Canonical curve gained by merging these two groups of data is shown with a *black dotted line*

Table 1 Basic parameters for normalized curve dependence to response in seven specimens of *C. gibelio*

Retina	V_{max} (μV)	V_{min} (μV)	$\log(I_0/I_{max})$	a
b-wave				
1	350	37	−2.23	0.9
2	627	100	−2.55	0.93
3	225	55	−3.01	0.75
4	634	100	−2.70	0.88
5	290	50	−2.09	0.64
LRP				
6	390	160	−1.94	0.88
7	350	84	−1.68	0.91

"Red shift" of the RSS curve was observed in a large number of Cyprinidae species (Burkhardt 1966; Witkovsky 1968; Nussdorf and Powers 1988). The possible reason for this is the existence of shared sensory routes for two or more types of receptors. It is known that in *Carassius auratus*, the cones and rods share the same bipolar cells (Ishida et al. 1980). Therefore, this receptor coupling could be the reason for the observed increase in RSS toward higher wavelengths. If that were true, we could expect difference in shape of the ERG signals caused by different wavelength stimuli. Figure 3 shows normalized responses at seven different wavelengths (a–e individual responses, f each trace represents average value of five individual responses) in five Prussian carp. It is evident that b-wave showed no convincing dependence of shape on wavelength similar to the observation of Yang et al. (1990) in arguing that in *Carassius* only rods are functioning in dark-adapted state. At the same time, significant differences were observed in the shape and duration of c-waves. Measurements from these fish specimens were included in the spectral sensitivity testing.

In the experiments performed after sodium iodate administration, out of all cell components that contribute to the shape and amplitude of ERG the only ones active are photoreceptors. Comparison of results from the b-wave amplitude with seven fish specimens

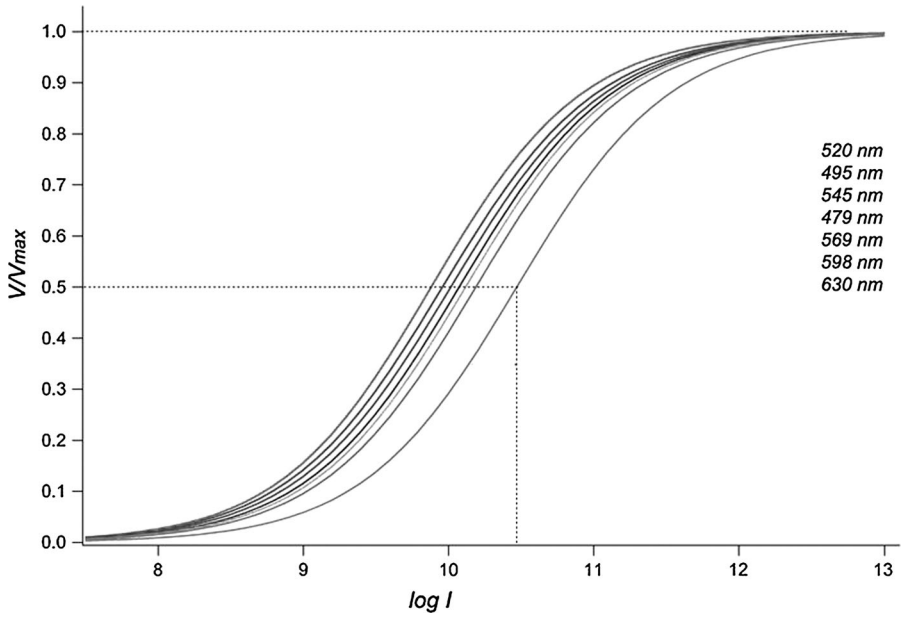

Fig. 2 Response dependence from stimuli strength in *C. gibelio* while stimulation with light of different wavelengths. The order from *left* to *right* responds to the column with the wavelengths. Stimulus strength is shown on the log abscise in photons/cm^2 s

and the LRP with two fish specimens is shown in Fig. 4. When using the Lamb–Govardovskii model for measuring the b-wave values, the mean maximum sensitivity and standard deviation equaled 529.9 ± 2.9, while the value for the LRP were 528.3 ± 3.6. While using our model, the values were 529.9 ± 2.7 and 529.7 ± 4.5, respectively (Fig. 4). The curve overlap into the experimental point was in the case of LRP statistically better than in the b-wave measuring. RSS acquired through LRP using either model do not show a difference in the major part of the curve when compared to the measured value gained through b-wave amplitude evaluation. Because of the curve overlap and close maximum sensitivity values, the data from these two experiments were subsequently processed together.

Figure 5 shows an example of averaged spectra obtained in seven Prussian carps constructed by simultaneously fitting all b-wave spectral sensitivity data. λ_{max} a value obtained by the model (see "Materials and methods") for A2-based pigments was 529.9 nm. When we fit simultaneously A1 and A2 models, the best result provides curve with 21 % of rhodopsin ($\lambda_{max} = 529.9$ nm). A significant feature of visual pigments is that their absorbance spectra varies, visual pigments with λ_{max} at longer wavelengths have broader spectra, while those at shorter wavelengths have narrower spectra (MacNichol 1986). There were no differences between peak values, but the curve obtained with 21 % of rhodopsin was 122.8 nm wide at 50 % relative spectral sensitivity which most closely resembles the half band width (HBW) results for fitted Bridges carp spectral sensitivity data (Bridges 1967). It is noticeable that our results although moved toward higher wavelengths have a much lower deviation from λ_{max} when compared to the other known RSS curves for closely related species. Individual fitted b-wave data provided λ_{max} values ranging from 515.7 to 531.0 nm (Table 2). It is evident that the variation in estimated A2/A1 pigment ratio in individual fish could be one of the reasons for discrepancy between rod photopigments λ_{max} and λ_{max} obtained by b-wave measurements. In our study, and in the literature (Nussdorf and Powers 1988; van Roessel et al. 1997; Saszik and Bilotta 1999), the models for RSS curves in Cyprinidae species were better matched to experimental points in short range wavelengths than to points in the yellow–red part of the spectra. In the aforementioned studies, different involvements of more types of receptors and pigments for better experimental point model fitting were postulated.

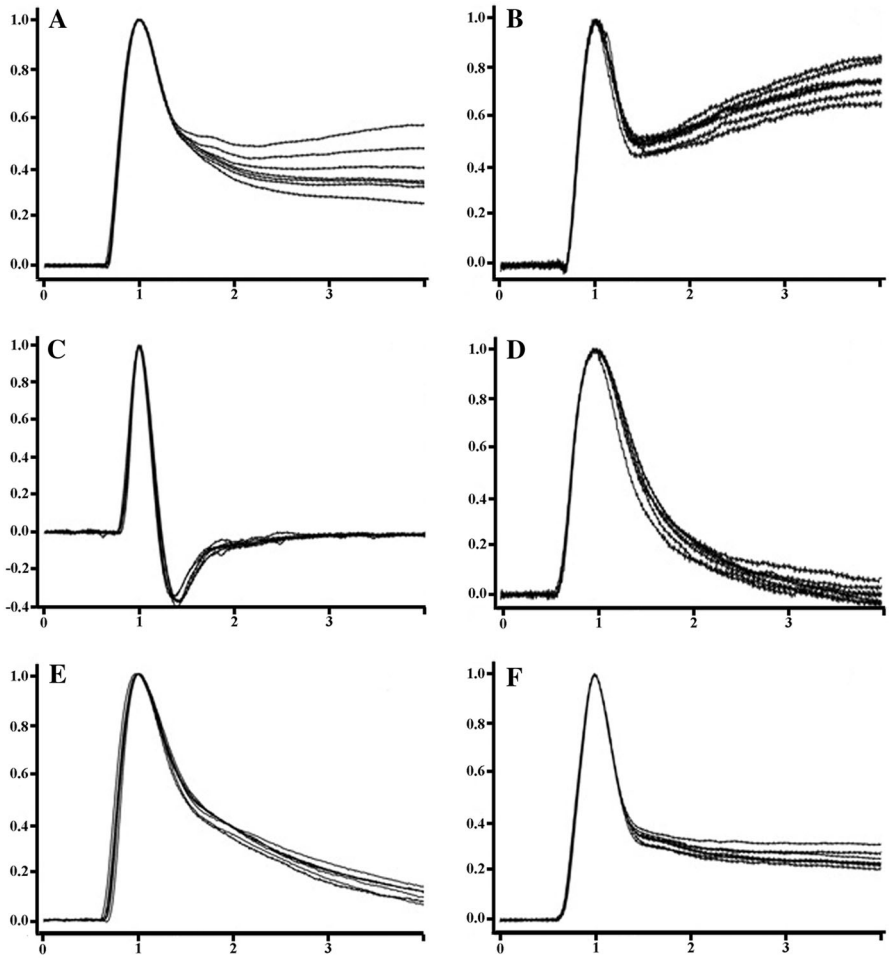

Fig. 3 Independence of shape of the normalized ERG from wavelength in *C. gibelio*. **a**–**d** Mediated ERG lines for five individual specimens calculated from normalized ERG lines which were acquired by individual stimulation by monochromatic filters 479, 495, 520, 545, 569, 598, 630 nm and with a stimulus intensity of 1.293 μmol/m^2 s. The duration of stimuli was 0.1 s. Time on the abscise is shown in seconds. **f** Mean values of normalized data for all five fish specimens for every wavelength ($n = 35$). Mediation was performed according to the b-wave amplitudes, and also the overlapping of time coordinates of the b-wave peaks was also performed

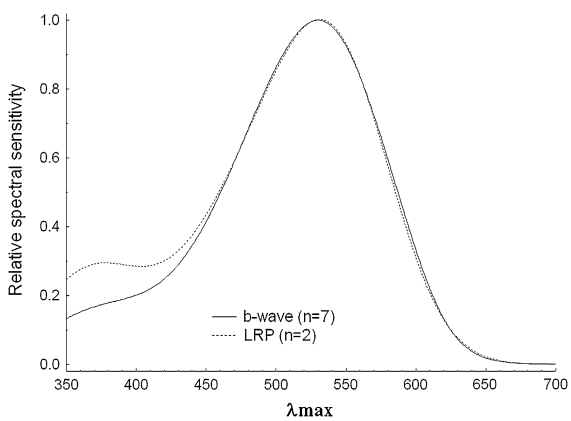

Fig. 4 Relative spectral sensitivity in *C. gibelio*—two group data comparison. The b-wave curve is shown with a *solid line*, which represents the results obtained from seven fish specimens. The cut curve was determined based on the LRP amplitudes. Both curves were attained by using the 523 model

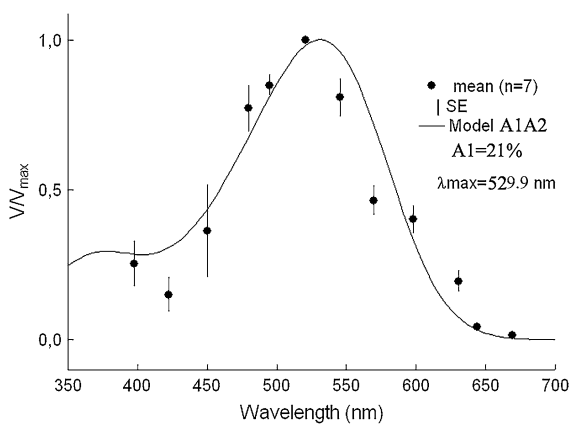

Fig. 5 Relative spectral sensitivity in *C. gibelio*. The full curve was calculated using the model A2/A1 with 21 % of rhodopsin. Figure also shows the mean and standard errors of this data

Table 2 λ_{max} values (nm) obtained in seven Prussian carp by fitting b-wave spectral sensitivity data separately in each individual or by fitting simultaneously all data points

Retina	Model 502 (λ_{max})	Model 523 (λ_{max})	Lamb-GovA1 (λ_{max})	Lamb-GovA2 (λ_{max})	Model A2/A1 (λ_{max})	Lamb-GovA2/A1 (λ_{max})
b-wave						
1	527.6	531	529.4	531.3	530.6	529.6
2	516.1	517.4	518.1	518.2	518	518.1
3	523.5	525.5	523.7	524.8	524.9	526.1
4	517.0	515.7	516.3	514.2	516.4	516
5	522.9	517.5	526.6	519.6	519.3	519.0
6	523.7	518.6	523.7	516.5	519.7	519.3
7	525.0	516.6	523.7	516.5	518.2	517.6
Fitted simultaneously	524.3	529.9	526.6	530.0	529.9	530.1

Results obtained by means of various fitting methods (spectral curve models specified in the table)

As already mentioned, the reason for the noticeable widening of the RSS curve and its significant deviation from its maximum of 520 nm in *C. gibelio* (main pigment being porphyropsin) could be the cones contribution to the rods response, which leads to considerable porphyropsin curve deviation. However, in his study on this species Nussdorf (1988) described the shift of the maximum and curve widening, but also concluded that the backlight from the red part of the spectrum does not contribute to the differences in RSS shape when compared to the one acquired in scotopic conditions.

While studying *C. auratus*, Yang et al. (1990) held the notion that in adaptations to dark conditions only rod activity is electroretinographically measurable. They contributed the referred shifting of porphyropsins sensitivity toward the red part of the spectrum to self-screening, the interaction of incoming light with optical and sensory elements within the eye. The problems with light characteristics that are the result of passing through the retina were resolved by using several correctional factors in spectral sensitivity equations. In our study, we rejected that approach for the following reasons: (a) The majority of the literature considers calculating the value of *self-screening* of little importance and entirely wrong even; (b) unlike most authors that did ERG testing on the intact eye of *C.auratus*, we removed a significant part of the eyes content before stimulation.

In *C. auratus*, influence of the temperature on the pigment pair level ratio was observed (Tsin and Beatty 1979). The temperature at which most of the testing on this species was performed was 20 °C and above, while in our study it was 15 °C. Under these conditions, the amount of rhodopsin in total pigment levels would have to be low, and as a result his influence on RSS should be minimal.

In our study, goldfish has shown significant individual deviations for the A_2/A_1 pigment mixture, which was already described by Govardovskii et al. (2000), and Parry and Bowmaker (2000). On the other hand, two models for computer-assisted fitting of spectral sensitivity curves used in our study did not reveal any significant differences in λ_{max} between rod photopigments and b-wave-based spectral sensitivity. Approaching the goldfish maximum sensitivity toward the porphyropsin curve, obtained in our study, could be explained with individual deviations of A2/A1 mixture and by the experimental design and applied analysis with more *V*/*log*I dependency checkpoints than in previous studies. The scotopic spectral sensitivity in Prussian carp (*C. gibelio* Bloch, 1782) based on b-wave measurement when the eyes were surgically deprived of cornea, lens, and most of the vitreous shows that b-wave measurements are a good indicator of spectral sensitivity in Prussian carp when the above method is used, which the results in our study confirm.

Acknowledgments This study represents a part of activities within the Project No. 173045, funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

References

Andjus PR, Dj Konjević, Andjus RK (1983) The electroretinogram of the dogfish shark: isolation of the late receptor component after low temperature aging of the eyecup preparation. Period Biol 85:181–183

Andjus RK, Damjanović I, Gačić Z, Konjević Dj, Andjus PR (1998) Electroretinographic evaluation of spectral sensitivity in yellow and silver eels (*Anguilla anguilla*). Vis Neurosci 15:923–930

Bowmaker J (1973) The photoproducts of retinal-based visual pigments in situ: a contrast between Rana pipiens and Gekko gecko. Vision Res 13:1227–1240

Bridges CDB (1967) Photopigments in the char of Lake Windermere (Salvelinus willughbii, forma autumnalis and forma vernalis). Nature 214:205–206

Burkhardt A (1966) The goldfish electroretinogram: relation between photopic spectral sensitivity functions and cone absorption spectra. Vision Res 6:517–532

Dartnall HJA (1953) The interpretation of spectral sensitivity curves. Br Med Bull 9:24–30

Easter SS, Hamasaki DI (1973) Electroretinographically-determined scotopic spectral sensitivities of some marine fish. Vision Res 13:1175–1181

Gačić Z, Damjanović I, Mićković B, Hegediš A, Nikčević M (2007a) Spectral sensitivity of the dogfish shark (*Scyliorhinus canicula*). Fish Physiol Biochem 33(1):21–27

Gačić Z, Bajić A, Milošević M, Nikčević M, Mićković B, Damjanović I (2007b) Spectral sensitivity of the perch (*Perca fluviatilis*) from the Danube. Arch Biol Sci Belgrad 59(4):335–340

Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K (2000) In search of the visual pigment template. Vis Neurosci 17:509–528

Halstenberg S, Lindgren KM, Samagh PS, Nadal-Vicens M, Balt S, Fernald RD (2005) Diurnal rhythm of cone opsin expression in the teleost fish Haplochromis burtoni. Vis Neurosci 22:135–141

Hamasaki DI, DB Bridges C, Meneghini KA (1967) The electroretinogram of three species of elasmobranchs. In: Gilbert PW, Mathewson RF, Rall DP (eds) Sharks Skates and Rays. Johns Hopkins Press, Baltimore, pp 447–463

Ishida AT, Stell WK, Lightfoot DO (1980) Rod and cone inputs to bipolar cells in goldfish retina. J Comp Neurol 191:313–335

MacNichol EF (1986) A unifying presentation of photopigment spectra. Vision Res 26:543–1556

Nussdorf JD, Powers MK (1988) Spectral sensitivity of the electroretinograms b-wave in dark–adapted goldfish. Vis Neurosci 1:159–168

Parry JWL, Bowmaker JK (2000) Visual pigment reconstitution in intact goldfish retina using synthetic retinaldehyde isomers. Vision Res 40:2241–2247

Powers MK, Easter SS (1978) Wavelength discrimination by the goldfish near absolute visual threshold. Vision Res 18:1149–1154

Saszik S, Bilotta J (1999) The effects of temperature on the dark-adapted spectral sensitivity function of the adult zebrafish. Vision Res 39:1051–1058

Tsin ATC, Beatty D (1979) Scotopic visual pigment composition in the retinas and vitamins A in the pigment epithelium of the goldfish. Exp Eye Res 29:15–26

van Roessel P, Palacios AG, Goldsmith TH (1997) Activity of long-wavelength cones under scotopic conditions in the cyprinid fish Danio aequipinnatus. J Comp Physiol 181:493–500

Witkovsky P (1968) The effect of chromatic adaptation on color sensitivity of the carp electroretinogram. Vision Res 8:823–837

Yang XL, Fan TX, Li JD (1990) Electroretinographic b-wave merely reflects the activity of the rod system in the dark-adapted carp retina. Vision Res 30:993–999