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The photoactivation energy of the visual pigment in two spectrally different populations of *Mysis relicta* (Crustacea, Mysida)

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Abstract We report the first study of the relation between the wavelength of maximum absorbance (λ_{max}) and the photoactivation energy (E_a) in invertebrate visual pigments. Two populations of the opossum shrimp *Mysis relicta* were compared. The two have been separated for 9,000 years and have adapted to different spectral environments (''Sea'' and ''Lake'') with porphyropsins peaking at $\lambda_{max} = 529$ nm and 554 nm, respectively. The estimation of E_a was based on measurement of temperature effects on the spectral sensitivity of the eye. In accordance with theory (Stiles in Transactions of the optical convention of the worshipful company of spectacle makers. Spectacle Makers' Co., London, 1948), relative sensitivity to long wavelengths increased with rising temperature. The estimates calculated from this effect are $E_{a,529} = 47.8 \pm 1.8$ kcal/mol and $E_{a,554} = 41.5 \pm 0.7$ kcal/mol (different at $P < 0.01$). Thus the red-shift of λ_{max} in the ''Lake'' population, correlating with the long-wavelength dominated light environment, is achieved by changes in the opsin that decrease the energy gap between the ground state and the first excited state of the chromophore. We propose that this will carry a cost in terms of increased thermal noise, and that evolutionary adaptation of the visual pigment to the light environment is directed towards maximizing the signal-to-noise ratio rather than the quantum catch.

Keywords Visual ecology · Rhodopsin · Porphyropsin
Spectral absorbance · Activation energy

Abbreviations C: Cold · E_a: Photoactivation energy · ERG: Electroretinogram · IR: Infrared · S: Relative fractional sensitivity · SNR: Signal-to-noise ratio · W: Warm

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Introduction

The first step in vision, 11-*cis* to all-*trans* isomerization of the visual pigment chromophore, occurs via transition to the first electronically excited state upon photon absorption. The energy gap between this state and the zero vibrational energy level of the ground state is here termed the photoactivation energy (E_a). In the absence of thermal energy, only photons with energy $hc/\lambda > E_a$ would be able to activate the pigment, and spectral sensitivity would drop abruptly to zero beyond a limiting wavelength $\lambda_0 = hc/E_a$. However, the thermal (vibrational) energy that visual pigment molecules possess at any temperature above absolute zero will decrease the gap to the first excited state. Therefore, even photons with energy $< E_a$ (wavelength $> \lambda_0$) can produce photoactivation and elicit a visual sensation with a probability that is proportional to the fraction of molecules having thermal energy $\geq E_a - hc/\lambda$ (Stiles 1948; Lewis 1955).

A seminal hypothesis developed by Barlow (1957) states that the participation of thermal energy in pigment activation inevitably carries a cost in the form of ''dark'' noise due to spontaneous activations caused by thermal energy alone. Under the reasonable assump-

tions that (1) the wavelength of peak absorbance (λ_{max}) is negatively correlated with E_a, and (2) the energy barrier for thermal activation does not differ too greatly from E_a, the probability for spontaneous photon-like events to occur will increase with increasing λ_{max} (cf. Ala-Laurila et al. 2004a, b). ''Red-sensitivity'' (a high value of λ_{max}) will be associated with high dark noise. Thus the photoactivation energy E_a appears as the entity that connects the two most important functional variables of visual pigments: the absorbance spectrum, characterized by λ_{max}, and the rate of spontaneous activations. These relations have been extensively studied in vertebrate visual pigments (Matthews 1984; Donner et al. 1990; Firsov and Govardovskii 1990; Koskelainen et al. 2000; Ala-Laurila et al. 2002, 2003; Rieke and Baylor 2000; Sampath and Baylor 2001). Here, we report the first measurements of the $E_a(\lambda_{max})$- relation for invertebrate visual pigments. It should be noted that phylogenetically all invertebrate opsins constitute an outgroup in relation to all vertebrate opsins (Yokoyama and Yokoyama 2000).

We use two closely related populations of the opossum shrimp *Mysis relicta*: one from the Baltic Sea at the SW coast of Finland, the other from the freshwater Lake Pääjärvi in southern Finland, where it occurs as an Ice Age relict. The two were separated only ca. 9,000 years ago when Lake Pääjärvi was formed (Ruuhijärvi 1974). Although the former species *M. relicta* Lovén has recently been split into four sibling species, the two populations studied here remain within *M. relicta sensu stricto* (Väinölä 1986; Väinölä et al. 1994; Audzijonytė and Väinölä 2005). Only one visual pigment has been found in microspectrophotometric studies of the rhabdoms of each population (Jokela-Määttä et al. 2005). Their absorbance maxima are offset by ca. 25 nm (λ_{max} = 529 nm for the ''Sea'' population vs. 554 nm for the ''Lake'' population), correlating with differences between their present light environments (Lindström and Nilsson 1988; Dontsov et al. 1999; Jokela-Määttä et al. 2005). Since both populations use the same A2 chromophore (3,4-dehydroretinal), the difference in spectral absorbance must be due to changes in the amino acid sequence of the opsin (Jokela-Määttä et al. 2005). Here, Nature provides a beautiful within-species model for studying adaptations and constraints in the evolution of visual pigments.

To estimate E_a, we measured temperature-dependent changes in relative spectral sensitivity at long wavelengths (Stiles 1948; Lewis 1955; Srebro 1966; Koskelainen et al. 2000; Ala-Laurila et al. 2002, 2003). We find a statistically significant difference in E_a consistent with the idea that the red-shift of λ_{max} in the Lake population compared with the Sea population is achieved by lowering the photoactivation energy of the visual pigment. The consequences in terms of the signal-to-noise ratio (SNR) of vision in the very dim light environments of the animals is discussed.

Materials and methods

Animals

Adult opossum shrimps (*M. relicta*) were obtained from two localities with different spectral light distributions (Lindström and Nilsson 1988; Lindström 2000b). Animals from Pojoviken Bay of the Baltic Sea were captured in January 2001 in daylight with an epibenthic sledge ending in a plastic cup serving to minimize water flow and damage to the animals. Animals from Lake Pääjärvi were captured from a depth of 80 m in June 2001 with a vertical net during the night, as their eyes are easily destroyed by excessive light (Lindström and Nilsson 1988; Lindström et al. 1988). The animals from both localities were immediately put in light-tight tanks. They were kept at Tvärminne Zoological Station in aerated aquariums in darkness with water temperatures of 3–8°C, corresponding to their natural habitats.

Preparation

The animal was decapitated and the head was mounted in the specimen chamber bathed in brackish water from the Baltic Sea (salinity 0.3 %). The temperature of the eye was held at a set value by means of a heat exchanger placed around the specimen chamber and continuously monitored with a thermistor in the bath. A hole was made with a microneedle through the cornea in the dorsal region of the eye and the recording glass pipette electrode was lowered through the hole. The perforation would destroy only a small proportion of the up to 2000 ommatidia of the eye. Preparation typically lasted 10–20 min and was performed in infrared (IR) light under an IR converter (Find-R-Scope, FWJ Industries). In view of their high sensitivity to light of very long wavelengths, the eyes were allowed to adapt in complete darkness for ca. 1 h after preparation before the experiment was started.

Recording and light stimulation

The temperature effects that allow determination of E_a appear at long wavelengths, where the absorbance of the visual pigment has fallen below 1% of maximum. Therefore they cannot be reliably studied by spectrophotometric methods, but have to be measured electrophysiologically as changes in spectral sensitivity (cf. Ala-Laurila et al. 2002). This of course requires that the changes in spectral sensitivity truly reflect changes in the spectral absorbance of the visual pigment (see below).

Spectral sensitivity was measured by the electroretinogram (ERG) technique as described by Lindström and Nilsson (1983, 1988). The recorded signal is the ohmic voltage due to changes in extracellular ra-

dial currents upon light stimulation, reflecting the summed response from a large number of ommatidia. The recording electrode was an extracellular glass micropipette with a fairly large tip diameter (about 10 μm) filled with 100 mM NaCl, advanced to a depth of 40–50 μm through the hole in the cornea, while an Ag/AgCl wire in the bath served as reference.

The light source was a 6 V, 15 W microscope lamp driven by a stabilized power source in constant-current mode. The seven wavelengths used in the experiments were produced by inserting one of the following interference filters: 528 nm, 549 nm, 569 nm, 591 nm, 720 nm, 752 nm and 777 nm. The four shorter-wavelength filters were Schott DIL, the three longer-wavelength filters were Melles-Griot (half-bandwidths 7–16 nm). The light intensity reaching the eye was controlled with neutral density filters and a wedge. The photon flux per mm^2 reaching the eye was measured for each of the interference filters with an OOS2000 spectrometer (OceanOptics Inc., FL, USA). The optical densities of the neutral filters and the 3-log unit neutral wedge (at 0.25 log unit intervals) were measured separately with each of the interference filters in place with a calibrated photodiode (EG&G HUV-1000B, calibration by the National Standards Laboratory of Finland). The purpose of this was to correct for minor deviations of the neutral filters from strict spectral neutrality. Light intensity in this paper is given in relative units, proportional to photon flux per unit area incident on the plane tangential to the eye at the entrance point of the recording electrode. The stimulus field was homogeneous over the projection area of the eye onto this plane, and the beam was perpendicular to it. Stimuli were brief (33 ms) light pulses or "flashes". By *flash intensity*, denoted *I*, we here mean the number of incident photons per mm^2 delivered by a stimulus flash. The interstimulus interval was 1 min for small responses, but was increased to 2 min if the response was >200 μV or ca. 20% of the maximum amplitude. The DC signal was low-pass filtered at 3 KHz and 0.3 KHz and further filtered by a 50-Hz notch. The amplitudes of responses were read from a Tektronix 5031 dual-beam storage oscilloscope.

In each experiment two spectra were recorded, one at a lower temperature (2–7°C, "cold", denoted C) and one at a higher temperature (15–20°C, "warm", denoted W). The exact temperatures varied between experiments within the ranges given, but the W − C difference in any single experiment was always 13°C. During recordings the temperature was stable within ± 0.1°C around the set point. The order of C and W measurements was varied between experiments. Sequences of stimulation with 5–7 flash intensities at the "reference" wavelength (549 nm) were interleaved with series of 2–3 dim flashes at "test" wavelengths. The intensities of "dim" flashes were chosen to fall in the (roughly) linear response range, eliciting ~10–20% of the maximum amplitude.

Spectral sensitivities

The stimulation protocol described above allowed continuous monitoring of the intensity-response (log *R* vs. log *I*) function and determination of the *relative fractional sensitivity*, denoted *S*, at each of the test wavelengths. We here define fractional sensitivity as the fraction of the saturating response amplitude elicited in the linear response range per unit flash intensity incident on the eye (R/R_{max} photon^{-1} mm^2), normalized by setting $S(549)=1$. This sensitivity measure eliminates effects of possible overall changes in response amplitude, due, e.g., to changes in responsivity of the eye or to changes in extracellular resistivity that could affect the size of the ERG field potential, effects that would compromise the correspondence between the measured spectral sensitivity of the eye and the spectral absorbance of the visual pigment.

Each set of log *I*–log *R* data for the reference wavelength (549 nm) was fitted with a modified Michaelis function. The lateral shift on the log *I* axis required to make the amplitude of a response to any other wavelength match the most recent reference Michaelis curve was taken as log *S* for that wavelength (see Koskelainen et al. 2000; Ala-Laurila et al. 2002). Log *S* as function of wavenumber ($1/\lambda$) for the whole set of experimental wavelengths constitutes the sensitivity spectrum (see Fig. 1).

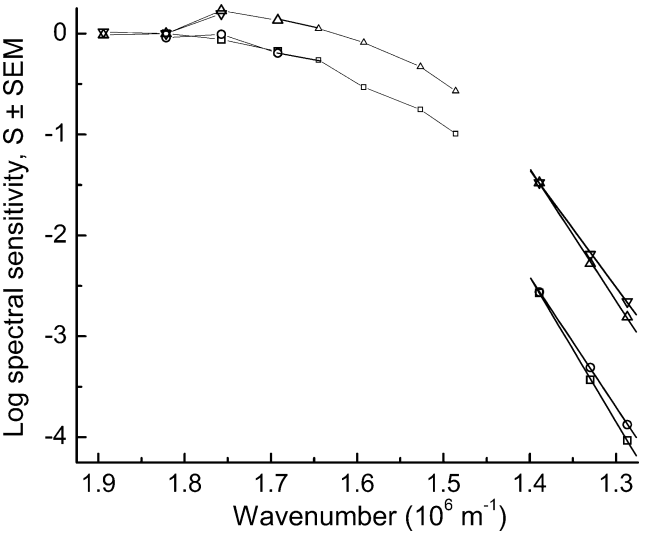

Fig. 1 Sensitivity spectra of one "Lake" animal (*top*, *triangles*: *upright*—cold, *inverted*—warm) and one "Sea" animal (*bottom*: *squares*—cold, *circles*—warm). *Large symbols* show data from the present study, *small symbols* "cold" data from Lindström (2000). The latter have been plotted only to give a visual impression of the shape of the spectra in the intermediate wavelength domain. The determination of E_a is based on the diverging final slopes of cold and warm spectra (at the three rightmost pairs of data points)

Determination of E_a from temperature effects in the long-wavelength range

The photoactivation energy E_a was estimated from temperature effects on log S in the long-wavelength range according to the simple physical theory by Stiles (1948) and Lewis (1955). The general idea is as follows. At any wavelength λ beyond $\lambda_0 = hc/E_a$ ($\geq \lambda_{max}$), the insufficient photon energy hc/λ needs to be supplemented by molecular thermal energy in order to activate the pigment. Warming of the eye will increase the proportion of pigment molecules that possess the required energy supplement ($\geq E_a - hc/\lambda$), which will be evident as an increase in relative sensitivity to such low-energy photons. The size of this effect allows estimation of the photoactivation energy, $E_a = hc/\lambda 0$. The procedures have been described in detail in Ala-Laurila et al. (2002) and only a brief account is given here.

Point estimates

The C and W sensitivity spectra were normalized to equal relative sensitivity around peak by least-square fitting in the domain assumed to be temperature-independent (data recorded at three or all of the "short" test wavelengths 528 nm, 549 nm, 569 nm and 591 nm). Small temperature effects around λ_{max} not envisaged by Stiles' theory are thereby neglected, but as shown by Ala-Laurila et al. (2002), these can have only minor effects on the E_a estimate. The W − C sensitivity difference at each of the "long" test wavelengths (720 nm, 752 nm and 777 nm) then yields an independent point estimate for E_a via a formula also involving the slope of the log $S(1/\lambda)$ spectrum at that wavelength (Koskelainen et al. 2000).

Difference spectra

Difference spectra calculated as $\Delta\log S = \log S_W - \log S_C$ are particularly helpful for visualizing the temperature effect and estimation of E_a in this case does not require knowledge of the shape of the original sensitivity spectra. A horizontal baseline was fitted by a least-square criterion to the $\Delta\log S$ data points for the short wavelengths (528 nm, 549 nm, 569 nm, and 591 nm). The temperature effect appears as a monotonic rise of $\Delta\log S$ from the baseline beyond λ_0. A second straight line, constrained to have slope b predicted by Stiles' (1948) theory, was used to describe these data points (720 nm, 755 nm and 777 nm):

$$b = \frac{hc(T_C - T_W)}{kT_W T_C \ln 10} \quad (1)$$

The line was positioned by a least-square criterion for best fit to the data. The wavelength where it crosses the baseline gives an upper bound for λ_0, thus a lower bound for E_a (Ala-Laurila et al. 2002).

Results

Spectra

Electroretinogram sensitivity spectra of the two populations of *M. relicta* considered here have been determined previously at a fixed temperature (ca. 4°C), but only up to the wavelength 673 nm (e.g. Lindström 2000). For our present purpose it was essential to obtain reliable spectral sensitivities from each preparation at two different temperatures and including wavelengths considerably longer than 673 nm. The number of data points to be determined had to be minimized in the interests of physiological stability, as the periods at high temperature are stressful to the eyes. Our choice of seven experimental wavelengths represented a minimum judged to afford sufficient accuracy for the estimation of E_a: 3 or 4 "temperature-independent" short wavelengths for matching W and C spectra around peak sensitivity and three long wavelengths for measuring the temperature effect.

Figure 1 shows representative cold and warm sensitivity spectra from one experiment on a "Sea" and one experiment on a "Lake" animal. Short- and long-wavelength points recorded in the present work are shown by large symbols, whereas the intermediate ranges shown by small symbols have been reproduced from Lindström (2000). Data from his Fig. 2 have been vertically positioned for best correspondence with ours in the short-wavelength range. The purpose is only to give a visual impression of the overall shape of the spectra. These whole-eye sensitivity spectra do not have the

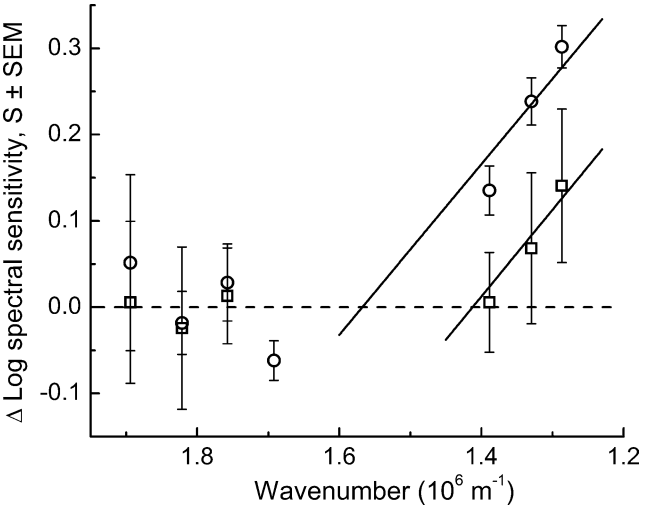

Fig. 2 Mean logarithmic W − C sensitivity differences (Δ log S) for the two populations: *circles*—Sea (five experiments), *squares*—Lake (six experiments). *Error bars* show SEMs. The *straight lines* are a horizontal baseline (*dashed*) vertically positioned for best fit to the short-wavelength points and two lines (*full-drawn*) constrained to have the slope predicted by Stiles' (1948) theory (Eq. 1) and positioned for best fit to the long-wavelength circles and squares, respectively (least-square criterion)

standard shape of visual-pigment absorbance spectra described e.g. by the template of Govardovskii et al. (2000). The discrepancy can be attributed to a combination of "self-screening" by the visual pigment itself and screening by protective pigments in the eye, which absorb light especially at shorter wavelengths before it can reach the visual pigment to initiate the electrical response (Goldsmith 1977; Dontsov et al. 1999; Cronin and Caldwell 2002; Jokela-Määttä et al. 2005). The resultant sensitivity spectra are flat over the mid-wavelength range, with a rather indistinct peak preceding the long-wavelength decline. Consequently, the eye sensitivity peaks of the two *M. relicta* populations studied here have been variably reported as 550 (Sea) and 570 nm (Lake) (Lindström and Nilsson 1988; Lindström 2000), or 569 nm (Sea) and 593 nm (Lake) Audzijonytė et al. (2005). Yet, the 20–25 nm offset between "Sea" and "Lake" spectra is robust against variable readings of the absolute positions of the peaks. The offset reflects that between the actual absorbance spectra of the visual pigments, which have λ_{max} at 529 nm and 554 nm, respectively (Jokela-Määttä et al. 2005).

At the very longest wavelengths, the ERG sensitivity spectra do converge with the visual-pigment templates that fit microspectrophotometric measurements of transverse absorbance in single rhabdoms of animals from the same populations. Importantly, the roughly linear decline at the longest wavelengths in a log *S* versus $1/\lambda$ plot (as in Fig. 1) is characteristic of visual pigments and an essential feature of Stiles' theory (even as modified by Lewis 1955). Moreover, the slope coefficients in this region are consistent with those expected for the respective visual pigments. For example, the slope between 720 nm and 777 nm of the C-spectrum of the "Sea" animal in Fig. 1 is 14.6×10^{-6} m, where the Govardovskii et al. (2000) template predicts 14.7×10^{-6} m over the same domain for an A2 visual pigment with $\lambda_{max} = 529$ nm. Thus, we are confident that relative long-wavelength sensitivities reflect the properties of the visual pigment alone.

While no systematic effect of temperature could be seen at short or intermediate wavelengths, higher temperature was always associated with higher relative sensitivity in the long-wavelength range. It should be noted that this effect involves a change in the long-wavelength *slopes* of spectra, excluding explanations in terms of translatory shifts, e.g. artifacts that could arise from improper vertical matching of W and C spectra. (See Ala-Laurila et al. (2002, 2003) for an analogous discussion concerning possible changes in self-screening of rods in the amphibian retina.)

Photoactivation energies

The W − C sensitivity difference at each long-wavelength gives one independent estimate of the photoactivation energy (see Methods and detailed descriptions in Koskelainen et al. (2000) and Ala-Laurila et al. (2002)). The point estimates were averaged within each experiment and means were then calculated across experiments for each of the two populations. The results are (mean ± SEM) $E_a = 47.8 \pm 1.8$ kcal/mol for the "Sea" animals ($n = 5$) and $E_a = 41.5 \pm 0.7$ kcal/mol for the "Lake" animals ($n = 6$). The difference, 6.3 kcal/mol, is statistically significant at the $P < 0.01$ level (two-sided probability for Student's *t*).

Figure 2 summarizes the results in another format, plotting the mean log sensitivity differences Δ logS as function of $1/\lambda$ (see Methods). The oblique lines are constrained to have the slopes predicted by Stiles' (1948) theory and have been positioned for best fit to data points that lie more than 0.07 log units above baseline. The intersection with the baseline defines the wavelength λ_0, where photon energy is equal to the photactivation energy E_a. This method gives $E_a = 44.8$ kcal/mol for the Pojoviken population and $E_a = 40.4$ kcal/mol for the Pääjärvi population. The values are somewhat lower than those calculated above from the point estimates and should be regarded as lower bounds, as discussed by Ala-Laurila et al. (2002).

Inferred signal-to-noise ratios

Convolution of A2 visual-pigment templates (Govardovskii et al. 2000) with the spectra of downwelling light in the respective environments of the two populations (Fig. 1 of Lindström 2000) indicates that quantum catch would be maximized if λ_{max} were red-shifted as far as 590 nm in the "Sea" and 650 nm in the "Lake" environment (Jokela-Määttä et al. 2005). In this sense, the λ_{max} values of the actual visual pigments, 529 nm and 554 nm, respectively, appear to be far from optimal. The further red-shifts of the peaks of ERG sensitivity spectra are achieved by selective light filtering, which can only decrease quantum catch, of course. Although there is no reason to assume that evolution should have had time to optimize spectral absorbance in these specific cases, we think it is useful to look beyond the simple idea that quantum catch is what should be maximized (the so-called "sensitivity hypothesis") and include the opposite selection pressure that might be associated with minimizing noise. The collected data from both rod and cone pigments in vertebrates clearly show that spectral red-shifts on average imply lowered activation energy and increased thermal noise (Barlow 1957; Ala-Laurila et al. 2004a, b). How do the *Mysis* pigments fare, if the SNR is what should be maximized, rather than quantum catch as such?

We have no direct measurements of rates of spontaneous thermal activations in the *Mysis* pigments. However, as it now seems likely that the ground-state energy barrier for thermal activation correlates closely with the energy gap to the first electronic excited state, E_a as measured here (Okada et al. 2001; Ala-Laurila et al. 2004a), the factor by which thermal noise will differ between pigments may be calculated on the basis

of some physical model. Fig. 3 shows SNR as function of λ_{max} in the "Sea" (Pojoviken Bay) and "Lake" (Pääjärvi) light environments for A2 pigments assumed to conform to the average relation between λ_{max}, E_a and thermal noise found in *vertebrate* pigments (Ala-Laurila et al. 2004a, b). The optimal λ_{max} in "Sea" and "Lake" according to this would be ca. 510 nm and 590 nm, respectively. The exact values depend on several assumptions, but the main point is that distinct optima emerge in a realistic range of λ_{max} (see Discussion).

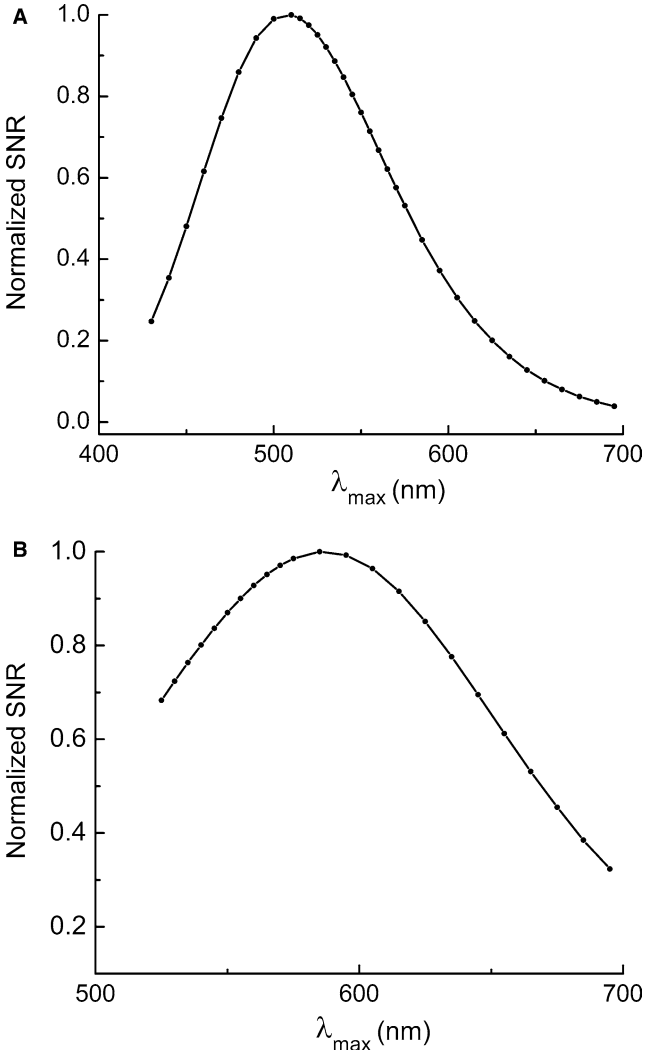

Fig. 3 Calculated SNRs attainable by A2 visual pigments with different λ_{max} in the "Sea" environment (**a**) and "Lake" environment (**b**). The assumed *signal* was obtained by convolution of A2 templates (Govardovskii et al. 2000) with the downwelling light spectra published by Lindström (2000) ("Sea" = Pojoviken Bay, spectrum B, "Lake" = Lake Pääjärvi, spectrum C in Lindström's Fig. 1). The assumed *noise* was the square root of values obtained from the average relation of λ_{max} and thermal activation rate among vertebrate visual pigments (Ala-Laurila et al. 2004a). Distinct SNR maxima emerge at $\lambda_{max} \approx 510$ nm for "Sea" and $\lambda_{max} \approx 590$ nm for "Lake"

Discussion

Photoactivation energy vs. spectral absorbance compared with vertebrate pigments

We measured effects of temperature on long-wavelength sensitivities in the eyes of two populations of *M. relicta* in order to determine the relation between λ_{max} and E_a in a pair of closely related invertebrate visual pigments. The effects were basically in accordance with Stiles' (1948) theory and similar to those observed in the visual pigments of a number of vertebrate species as well as the horseshoe crab *Limulus* (Srebro 1966; Lamb 1984; Koskelainen et al. 2000; Ala-Laurila et al. 2002, 2003). The photoactivation energy E_a could thus be estimated from the warming-induced rise in relative sensitivity at long wavelengths. The red-shift of λ_{max} in the visual pigment of the "Lake" population compared with the "Sea" population was indeed found to be associated with lower E_a. Thus, these pigments conform to the general correlation found among vertebrate visual pigments (Ala-Laurila et al. 2004b). In vertebrates, however, the average $E_a(\lambda_{max})$ relation was found to be somewhat shallower than predicted by the simple physical relation $E_a \propto 1/\lambda_{max}$, whereas in our present *Mysis* data, the E_a difference is even larger than given by this relation. Such deviations from the average are unsurprising, however, as vertebrate pigments exhibit considerable "residual" freedom for independent tuning of E_a and λ_{max} (Ala-Laurila et al. 2004b).

The third E_a value available for an invertebrate visual pigment comes from the *Limulus* 502-nm A1 pigment (ca. 50 kcal/mol: Srebro 1966), whose A2 pair would be spectrally similar to our "Sea" pigment, with expected $\lambda_{max} = 527$ nm (Dartnall and Lythgoe 1965). The relation of the *Mysis* (Sea) pigment and the *Limulus* pigment is rather accurately described by $E_a \propto 1/\lambda_{max}$ ($\lambda_{max2}/\lambda_{max1} = 502/529 = 0.949$ and $E_{a1}/E_{a2} = 47.8/50 = 0.956$). In vertebrate pigments, this relation has been found to hold quite precisely in cases where the spectral shift is achieved by a chromophore change in the same opsin, but only in those cases.

Evolutionary adaptation: for what, and how fast?

Both the *M. relicta* populations studied here are mainly active in very dim light, where "ultimate limits to visual sensitivity" are of immediate biological relevance. These limits are of two kinds. The most obvious factor is the number of photons captured by the eye. In the visual pigment, the main variable determining quantum catch is the "match" between the absorbance spectrum and the spectrum of available light. Although λ_{max} of the "Lake" pigment is red-shifted compared with the "Sea" pigment in qualitative correlation with the difference in the photic habitats, it still lies at much shorter wavelengths than

would be required to maximize quantum catch (true of the "Sea" pigment as well). Convolution of the illumination spectra of the two habitats with standard A2 visual-pigment templates indicates that quantum catch would be maximized for $\lambda_{max} = 590$ nm (Sea) and 650 nm (Lake); the observed values 529 nm and 554 nm would appear to be far from optimal (Jokela-Määttä et al. 2005). One possible explanation is that evolutionary adaptation is not yet complete (e.g. Lindström 2000). Since the populations have been separated for about 9,000 years, the time for differential adaptation corresponds to only 7,000–10,000 generations. This might be too short for optimal tuning to the downwelling light. It could also be, e.g., that Lake Pääjärvi has been less "red" for a significant part of its history.

Our results, however, support an alternative interpretation. A second main factor limiting visual sensitivity is the noise that interferes with detection (Barlow 1956). The functionally important noise of a visual pigment molecule consists of randomly occurring spontaneous thermal activations, which trigger false quantal responses in the photoreceptor cell, identical to those caused by a photon absorption (Baylor et al. 1980, 1984). Visual performance near absolute threshold in several vertebrate species including humans is consistent with the hypothesis that thermal activations of rhodopsin set an ultimate limit (Aho et al. 1988, 1993a, b; Donner 1992).

Barlow (1957) further suggested that the spectral absorbance and noise are interdependent properties of the visual pigment. E.g., red-sensitivity implies the capacity to be activated by photons of low energy, hence a low activation energy and a high probability for activation by thermal energy alone. Then shifting sensitivity towards longer wavelengths will be beneficial only as long as the increased photon catch outweighs the higher noise of the less stable pigment (Donner et al. 1990; Firsov and Govardovskii 1990).

We suggest that evolutionary adaptation works to maximize the SNR enabled by the visual pigment, not quantum catch per se. The picture then becomes very different, as indicated by the simple calculations illustrated in Fig. 3. SNR would be maximized by pigments with $\lambda_{max} \approx 510$ nm ("Sea" environment) and 590 nm ("Lake" environment), respectively. The actual "Sea" pigment ($\lambda_{max} = 529$ nm) now even falls slightly on the long-wavelength side of the optimum, and the "Lake" pigment ($\lambda_{max} = 554$ nm) is only moderately displaced from its calculated optimum. Moreover, the modelling in Fig. 3 is based on *average* relations in between λ_{max}, E_a and noise in vertebrate pigments. In the present work, we found that the shift from 529 nm to 554 nm in the *M. relicta* pigment was associated with an even stronger-than-average decrease in activation energy, and thus the mismatch could be even smaller. The spectral absorbance of both pigments would appear to be fairly well-tuned to maximize SNR in their respective light environments.

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References

Aho A-C, Donner K, Hydén C, Larsen LO, Reuter T (1988) Low retinal noise in animals with low body temperature allows high visual sensitivity. Nature 334:348–350

Aho A-C, Donner K, Helenius S, Larsen LO, Reuter T (1993a) Visual performance of the toad (*Bufo bufo*) at low light levels: retinal ganglion cell responss and prey-catching accuracy. J Comp Physiol A 172:671–682

Aho A-C, Donner K, Reuter T (1993b) Retinal origins of the temperature effect on absolute visual sensitivity in frogs. J Physiol 463:501–521

Ala-Laurila P, Saarinen P, Albert R, Koskelainen A, Donner K (2002) Temperature effects on spectral properties of red and green rods in toad retina. Vis Neurosci 19:781–792

Ala-Laurila P, Albert R-J, Saarinen P, Koskelainen A, Donner K (2003) The thermal contribution to photoactivation in A2 visual pigments studied by temperature effects on spectral properties. Vis Neurosci 20:411–419

Ala-Laurila P, Donner K, Koskelainen A (2004a) Thermal activation and photoactivation of visual pigments. Biophys J 86:3653–3662

Ala-Laurila P, Pahlberg J, Koskelainen A, Donner K (2004b) On the relation between the photoactivation energy and the absorbance spectrum of visual pigments. Vision Res 44:2153–2158

Audzijonytė A, Väinölä R (2005) Diversity and distributions of circumpolar fresh- and brackish-water *Mysis* (Crustacea: Mysida): descriptions of *M. relicta* Lovén, 1862, *M. salemaai* n. sp., *M. segerstralei* n. sp. and *M. diluviana* n. sp., based on molecular and morphological characters. Hydrobiologia (in press)

Audzijonytė A, Pahlberg J, Väinölä R, Lindström M (2005) Spectral sensitivity differences in two sibling *Mysis* species (Crustacea, Mysida): adaptation or phylogenetic constraints? J Exp Mar Biol Ecol (in press)

Barlow HB (1956) Retinal noise and absolute threshold. J Opt Soc Am 46:634–639

Barlow HB (1957) Purkinje shift and retinal noise. Nature 179:255–256

Baylor DA, Matthews G, Yau K-W (1980) Two components of electrical dark noise intoad retinal rod outer segments. J Physiol 309:591–621

Baylor DA, Nunn BJ, Schnapf JL (1984) The photocurrent, noise and spectral sensitivity of rods of the monkey *Macaca fascicularis*. J Physiol 357:575–607

Cronin TW, Caldwell RL (2002) Tuning of photoreceptor function in three mantis shrimp species that inhabit a range of depths. II. Filter pigments. J Comp Physiol A 188:187–197

Dartnall HJA, Lythgoe JN (1965) The spectral clustering of visual pigments. Vis Res 5:81–100

Donner K (1992) Noise and the absolute thresholds of cone and rod vision. Vis Res 32:853–866

Donner K, Firsov ML, Govardovskii VI (1990) The frequency of isomerization-like 'dark' events in rhodopsin and porphyropsin rods of the bull-frog retina. J Physiol 428:673–692

Dontsov AE, Fedorovich IB, Lindström M, Ostrovsky MA (1999) Comparative study of spectral and antioxidant properties of pigments from the eyes of two Mysis relicta (Crustacea, Mysidacea) populations, with different light damage resistance. J Comp Physiol B 169:157–164

Firsov ML, Govardovskii VI (1990) Dark noise of visual pigments with different absorption maxima (in Russian). Sensornye Sistemy 4:25–34

Goldsmith TH (1977) The effect of screening pigment on the spectral sensitivity of some crustacea with scotopic (superposition) eyes. Vis Res 18:475–482

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

Jokela-Määttä M, Pahlberg J, Lindström M, Porter M, Zak P, Ostrovsky M, Cronin T, Donner K (2005) Visual pigment absorbance and spectral sensitivity of the *Mysis relicta* species group (Crustacea, Mysida) in different light environments. J Comp Physiol A (in press)

Koskelainen A, Ala-Laurila P, Fyhrquist N, Donner K (2000) Measurement of thermal contribution to photoreceptor sensitivity. Nature 403:220–223

Lewis PR (1955) A theoretical interpretation of spectral sensitivity curves at long wavelengths. J Physiol 130:45–52

Lindström M (2000) Eye function of Mysidacea (Crustacea) in the northern Baltic Sea. J Exp Mar Biol Ecol 246:85–101

Lindström M, Nilsson HL (1983) Spectral and visual sensitivities of *Cirroleana borealis* Liljeborg, a deep-water isopod (Crustacea, Flabellifera). J Exp Mar Biol Ecol 69:243–256

Lindström M, Nilsson HL (1988) Eye function of *Mysis relicta* Lovén (Crustacea) from two photic environments. Spectral sensitivity and light tolerance. J Exp Mar Biol Ecol 120:23–37

Lindström M, Nilsson HL, Meyer-Rochow VB (1988) Recovery from light-induced sensitivity loss in the eye of the crustacean *Mysis relicta* in relation to temperature: a study of ERG-determined V/log I relationships and morphology at 4°C and 14°C. Zool Sci 5:743–757

Okada T, Ernst OP, Palczewski K, Hoffman KP (2001) Activation of rhodopsin: new insights from structural and biochemical studies. Trends Biochem Sci 26:318–324

Rieke F, Baylor DA (2000) Origin and functional impact of dark noise in retinal cones. Neuron 26:181–186

Ruuhijärvi R (1974) A general description of the oligotrophic Lake Pääjärvi, southern Finland, and the ecological studies on it. Ann Bot Fenn 11:95–104

Srebro R (1966) A thermal component of excitation in the lateral eye of *Limulus*. J Physiol (Lond) 35:495–517

Stiles WS (1948) The physical interpretation of the spectral sensitivity curve of the eye. In: Transactions of the optical convention of the worshipful company of spectacle makers. Spectacle Makers' Co., London, pp 97–107

Väinölä R (1986) Sibling species and phylogenetic relationships of *Mysis relicta* (Crustacea, Mysidacea). Ann Zool Fenn 23:207–221

Väinölä R, Riddoch BJ, Ward RD, Jones RI (1994) Genetic zoogeography of the *Mysis relicta* species group (Crustacea: Mysidacea) in northern Europe and North America. Can J Fish Aquat Sci 51:1490–1505

Yokoyama S, Yokoyama R (2000) Comparative molecular biology of visual pigments. In: Stavenga DG, DeGrip WJ, Pugh EN Jr (eds) Handbook of biological physics: molecular mechanisms of visual transduction 3. Elsevier, Amsterdam, pp 257–296