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# Visual pigments of Baltic Sea fishes of marine and limnic origin

MIRKA JOKELA-MÄÄTTÄ,[1*] TEEMU SMURA,[1*] ANNA AALTONEN,[1] PETRI ALA-LAURILA,[2] AND KRISTIAN DONNER[1]

[1]Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland
[2]Department of Physiology and Biophysics, Boston University School of Medicine, Boston Massachusetts

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**Abstract**

Absorbance spectra of rods and some cones were measured by microspectrophotometry in 22 fish species from the brackish-water of the Baltic Sea, and when applicable, in the same species from the Atlantic Ocean (3 spp.), the Mediterranean Sea (1 sp.), or Finnish fresh-water lakes (9 spp.). The main purpose was to study whether there were differences suggesting spectral adaptation of rod vision to different photic environments during the short history ($<10^4$ years) of postglacial isolation of the Baltic Sea and the Finnish lakes. Rod absorbance spectra of the Baltic subspecies/populations of herring (*Clupea harengus membras*), flounder (*Platichthys flesus*), and sand goby (*Pomatoschistus minutus*) were all long-wavelength-shifted (9.8, 1.9, and 5.3 nm, respectively, at the wavelength of maximum absorbance, $\lambda_{max}$) compared with their truly marine counterparts, consistent with adaptation for improved quantum catch, and improved signal-to-noise ratio of vision in the Baltic light environment. Judged by the shape of the spectra, the chromophore was pure A1 in all these cases; hence the differences indicate evolutionary tuning of the opsin. In no species of fresh-water origin did we find significant opsin-based spectral shifts specific to the Baltic populations, only spectral differences due to varying A1/A2 chromophore ratio in some. For most species, rod $\lambda_{max}$ fell within a wavelength range consistent with high signal-to-noise ratio of vision in the spectral conditions prevailing at depths where light becomes scarce in the respective waters. Exceptions were sandeels in the Baltic Sea, which are active only in bright light, and all species in a "brown" lake, where rod $\lambda_{max}$ lay far below the theoretically optimal range.

**Keywords:** Microspectrophotometry, Absorbance spectra, Quantum catch, Rod, Signal-to-noise ratio

## Introduction

The Baltic Sea is the largest brackish water body in the world, with a fish fauna that includes marine species (e.g., cod, flounder, and herring), anadromous (e.g., Atlantic salmon and Sea trout), and catadromous species (e.g., European eel), as well as fresh-water species (e.g., pike and perch). Its postglacial formation history is well-known, involving repeated making and breaking of connections with the ocean and concomitant salinity changes over a period from ca. 10000 to 4000 years before present as the ice shield receded and the land rose. The stepwise isolation of Finnish lakes after the ice age can also be dated quite accurately. The system offers a natural experiment for the study of short-term evolution in fish biology, including vision in changing light environments. In the Baltic Sea, light irradiance spectra are biased toward longer wavelengths compared with truly marine environments, and many lakes have still more long-wavelength-shifted spectra due to even higher concentrations of organic matter.

We have measured absorbance spectra of the visual pigments of the rods and some cones of 22 teleost species from the Baltic Sea, and as available to us, their marine (3 spp.) or limnic (8 spp.) conspecifics. The objectives are: (1) to look for shifts in rod absorbance spectra of Baltic fishes compared with populations of the same species from different photic environments, either lakes (Lake Vesijärvi, Lake Päijänne, and Lake Tuusulanjärvi), or salty seas (the east coast of Scotland, the west coast of Norway, and the Adriatic Sea); (2) to assess the adaptive value of given rod spectral sensitivities in terms of quantum catch and conceptual signal-to-noise ratios of dim-light vision in the photic environments studied.

## Materials and methods

### *Animals and preparation*

A total of 25 teleost fish species were studied (see Tables 1 and 2). We obtained samples of Baltic Sea populations from the coast of Finland (south-west coast: Tvärminne Zoological Station, Hanko and the Archipelago Sea near Nagu; south coast: Gulf of Finland at Kotka). Samples of freshwater populations were collected in

Address correspondence and reprint requests to: Mirka Jokela-Määttä, Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland, Division of Physiology, P.O. Box 65 (Viikinkaari 1), FI-00014 University of Helsinki, Finland. E-mail: mirka.jokela@helsinki.fi

*These authors contributed equally to the work.

three Finnish lakes (Lake Vesijärvi, four species, Lake Päijänne, one species, and Lake Tuusulanjärvi, six species). Marine specimens were obtained from the Eastern Atlantic (flatfish from Raunefjord south of Bergen, Norway and herrings originating from the east coast of Scotland received as a gift from the Sea Life Aquarium, Helsinki) and from the Adriatic Sea (sand gobies from near Venice, Italy).

After capture, fish were transferred in special water bags or tanks to the animal care facilities at the University of Helsinki. They were kept in aquaria with the salinity of their respective habitat (from 0.0% in the lakes via 0.6% in the Baltic to 3.5% in the Atlantic or Adriatic) at approximately 15°C and 12:12 h light/dark cycle, and supplied with appropriate food. The Baltic herrings were placed in dark tanks with ice after netting. Recordings on these were performed during the same or the following day. Some of the fish were frozen (−18°C) after netting and stored frozen in darkness until measurements. We found no significant difference in $\lambda_{max}$ of cells from retinas that had been frozen and then thawed compared with fresh retinas of fish of the same species from the same habitat.

Living fish were kept in darkness for at least 12 h before dissection. Frozen samples were thawed individually for approximately 30 min in darkness at room temperature. All subsequent manipulations were performed under dim red light (wavelength > 650 nm), which would cause negligible bleaching of visual pigments in rods and S- and M-cones, but could have affected L-cones significantly. Living fish were decapitated and pithed. Eyes were enucleated and dissected in teleost Ringer solution containing: 110 mmol/L NaCl; 2.5 mmol/L KCl; 1 mmol/L $CaCl_2$; 1 mmol/L $MgSO_4$; 10 mmol/L $NaHCO_3$, and 10 mmol/L glucose. The solution was buffered to pH 7.2–7.4 with 1 mmol/L HEPES. The lens was removed and pieces of retina separated from the pigment epithelium were transferred to a drop of Ringer on a cover slip and teased apart. Dextran (10–15%, $M_r$ = 70 kDa) was added to the Ringer to immobilize cells during recordings. The sample was covered with a second cover slip, sealed at the edges with Vaseline and placed on the MSP stage.

### *Microspectrophotometry (MSP)*

Absorbance spectra were recorded with a single-beam, computer-controlled, fast wavelength-scanning microspectrophotometer built at the University of Helsinki (Govardovskii et al., 2000; Ala-Laurila et al., 2002). Recordings were made on the outer segments (OSs) of isolated photoreceptor cells or of cells still attached to small pieces of retina. OS dimensions varied between species, rod OSs typically measuring approximately 2–3 $\mu$m × 20–40 $\mu$m. The size of cone OSs varied from 2 × 10 $\mu$m to 8 × 30 $\mu$m. The size of the measuring beam was adjusted to match the sample, typically to approximately three-quarters of the OS width and nearly the full OS length. The beam was linearly polarized in the plane of the discs. The wavelength calibration was checked regularly, at least at the beginning, and at the end of each experiment, against the spectrum of a "blue glass" standard, the spectrum of which had been accurately determined in a Hitachi spectrophotometer. The recordings were carried out at room temperature. For further technical details, see Govardovskii et al. (2000) and Ala-Laurila et al. (2002).

The data were stored on a computer hard disk for later analysis. The details of the analysis can be found in Govardovskii et al. (2000). Raw spectra from single cells were averaged and normalized within each individual, and the resulting within-individual average was corrected for zero offset. The position of the zero line was computed as a least-square linear fit to the long-wave tail where the absorbance of the visual pigment is close to zero. High-frequency noise components were removed by Fourier filtering, retaining 25–35 harmonics. Finally, the mean, zero-line-corrected and filtered spectrum from each individual was fitted with Govardovskii et al. (2000) templates. The fitting program applied the principle suggested by MacNichol (1986), finding the wavelength of 50% absorbance by fitting a least-square straight line to the long-wavelength limb between 70% and 30% of peak and calculating $\lambda_{max}$ from this. For further details, see Govardovskii et al. (2000). The width of the spectrum depends on the chromophore, and there is one (narrower) template for A1, and another (broader) template for A2 pigments. Conversely, the chromophore can be identified by the template that best fits the spectrum. When the pigment is a mixture of A1 and A2 molecules, the spectrum can be well fitted only with a sum of the templates, the relative weights revealing the ratio of the two chromophores. In these cases, the fitting procedure involved stepwise increasing the proportion of A2 template, starting from a pure A1 template. In the fitting program, the $\lambda_{max}$ of the A2 component is coupled to its A1 pair by the Hárosi (1994) relationship (see e.g., Firsov et al., 1994; Ala-Laurila et al., 2003). To summarize, both the parameter $\lambda_{max}$ and an estimate of the chromophore content were obtained by fitting of Govardovskii et al. (2000) templates to within-individual average spectra. The values given for each species are means ± SD calculated across individuals.

### *Light measurements*

In two of the lakes, the spectral distribution of downwelling light [quanta $m^{-2}$ $s^{-1}$ $nm^{-1}$], was recorded over the interval 400–750 nm using a QSM 2500 submersible quantum spectrometer (Techtum, Umeå, Sweden). Measurements were performed at 1, 5, and 10 m depth in Lake Vesijärvi and at 1.0, 2.7, and 3.7 m depth in Lake Tuusulanjärvi. The full depth of the lake at the measuring site was ca. 12 m in Lake Vesijärvi and ca. 9 m in Lake Tuusulanjärvi. The spectral distribution of the daylight during the measurements was also measured as control. All measurements were performed around noon on overcast days in October 2005. For the other habitats, published light spectra were used.

#### *Conceptual signal-to-noise ratios at absolute threshold*

We define two benchmarks for assessing the adaptedness of a rod pigment to a certain photic environment. The first is relative quantum catch ($QC_{rel}$). The second is a conceptual signal-to-noise ratio of vision near absolute threshold ($SNR_{dark}$), where the average thermal activation rates of rod pigments with given spectral properties are taken into account (Ala-Laurila et al., 2004*b*). Both ultimately measure the signal-to-noise ratio (SNR) of dim-light vision, but at slightly different light levels, the latter being relevant for absolute threshold, and the former in slightly brighter light. Model calculations are done for five representative spectra of downwelling light as shown in Fig. 1. They all represent the situation in a given water body at depths where the light has been strongly attenuated, resulting in a narrow spectrum that is strongly dependent on the transmission properties of the water.

The spectra are (from left to right): (1) JI = Jerlov's type I (open ocean) spectrum recalculated to 600 m depth; (2) JIII = Jerlov's type III (coastal ocean) spectrum recalculated to 90 m depth. The original values are from Jerlov (1968; Table XXVII);

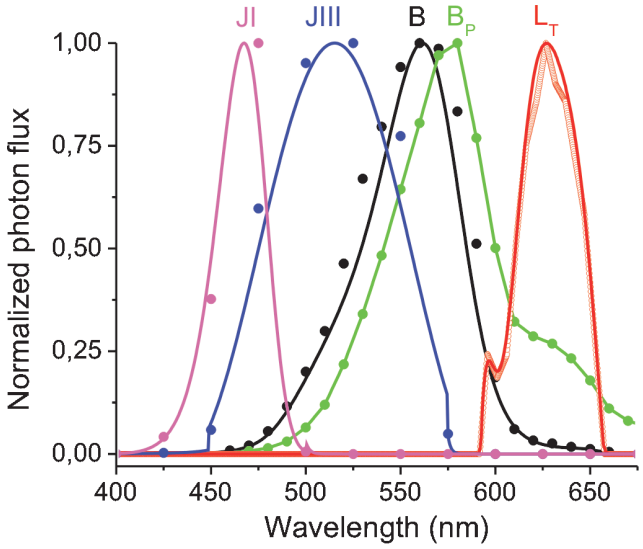


**Fig. 1.** The five representative aquatic illumination spectra considered, from left to right: JI represents the open ocean at 600 m depth, JIII coastal Atlantic water at 90 m depth, B the open Baltic Sea near the SW coast of Finland at 20 m depth, $B_P$ Pojoviken Bay of the Baltic Sea at 10 m depth, and $L_T$ Lake Tuusulanjärvi at 3.7 m depth. In this work, we studied specimens from all light environments except JI, which is included for comparison. See Text for details.

(3) B = open Baltic Sea outside Tvärminne Zoological Station at 20 m depth; (4) $B_P$ = Pojoviken Bay of the Baltic Sea at 10 m depth. Spectra (3) and (4) are based on direct measurements by Lindström (2000). (5) $L_T$ = Lake Tuusulanjärvi at 3.7 m depth, measured in the present study.

The calculations were done as follows (cf. Pahlberg et al., 2005). Downwelling irradiance spectra were linearly interpolated or fitted with a polynomial function (4–7$^{th}$ order) in the wavelength range where data points were available (see Fig. 1), and extrapolated at short and long wavelength borders to cover the whole wavelength range 100–1000 nm. (Note that extrapolation with power functions is equivalent to fitting straight lines on logarithmic ordinates.)

The product of irradiance spectra and Govardovskii et al. (2000) templates were calculated for pure A1 or A2 pigments with $\lambda_{max}$ between 430 and 700 nm (in some cases for $\lambda_{max}$ between 300 and 700 nm). The product of the visual pigment $\alpha$-peak and the irradiance spectrum was calculated in 0.5 nm steps between 100–1000 nm and the curves were integrated. This integral is proportional to the quantum catch *of the pigment* and was used as $QC_{rel}$. A number of other potential variables, e.g., outer segment lengths and volumes, rod densities, optical factors of the eye etc. will affect the actual quantum catch (spectrally as well as totally), and the amount of pigment-related noise, but in this paper, we restrict ourselves to pigment properties.

For calculating $SNR_{dark}$, assumed average rates of thermal "dark" events in rod pigments as function of $\lambda_{max}$ was taken from Ala-Laurila et al. (2004*b*). The intrinsic noise is proportional to the Poisson fluctuations of these photon-identical events. In a situation where the dark events are limiting, the SNR is proportional to $QC_{rel}$ divided by this intrinsic noise. Thus $SNR_{dark} = QC_{rel}/\sqrt{}$ (dark event rate). These SNR values all refer to a single temperature. Thermal noise will increase and conceptual SNR decrease with rising temperature, but the changes will, to a first approximation, be similar for different pigments (Baylor et al., 1980; Ala-Laurila et al., 2003).

Comparison of the performance of A1 and A2 pigments requires that the 1.42-fold lower photosensitivity of A2 pigments be taken into account (the difference is due to lower molar extinction, while the quantum efficiency of bleaching, ca. two-third, is the same for the two classes of pigments: Dartnall (1972)). $QC_{rel}$ of A2 pigments was therefore divided by the factor 1.42, as is evident from the lower peaks of the A2 curves in Fig. 3.

## Results

### *General*

Specimens from a total of 25 species belonging to 12 families were identified and studied. Absorbance spectra were recorded from 169 individuals originating from 38 different geographical locations. In each individual, recordings were made from 30 rods on average (range 15–82). Each within-individual average spectrum was fitted with a Govardovskii et al. (2000) template as described in the Materials and methods section to determine $\lambda_{max}$, as well as the chromophore of the pigment (A1 or A2 or, in the case of mixtures, the A1/A2 ratio). Knowing the chromophore is essential for both evolutionary and functional interpretation of shifts in $\lambda_{max}$. Chromophore substitution A1 $\rightarrow$ A2 will red-shift the absorbance spectrum of all opsins for which the A1 pigment has $\lambda_{max} >$ 430 nm (Dartnall & Lythgoe, 1965; Hárosi, 1994). As the chromophore content can be physiologically regulated in many species (e.g., Dartnall et al., 1961; Tsin & Beatty, 1980), such spectral shifts need not necessarily mean more than that the animals have been caught in different physiological states. For functional interpretations, it should also be noted that the A2 chromophore has lower photosensitivity (Dartnall, 1972) and a generally higher rate of thermal isomerization (Ala-Laurila et al., 2004*a*, 2004*b*), which from the viewpoint of SNR may outbalance apparent improvements in spectral match to the photic environment (see below).

Among the Baltic Sea fishes, we found examples of pure A1 or pure A2 pigments, as well as varying proportions of A1 and A2 (Table 1). All fishes of marine origin had only A1 chromophore whereas fishes of limnic origin used either A2 or a mixture of A1 and A2. The $\lambda_{max}$ values of rods in the species studied ranged from 485.6 nm to 515.7 nm for the A1 pigments and from 517.8 nm to 538.6 nm for the A2 pigments.

### *Lake species*

In the species where we compared Baltic and lake specimens, we found no indications of evolutionary divergence of the visual pigments. In the pure A2 group (bream, ruffe and perch), "Baltic" and "lake" rod absorbance spectra were indistinguishable to within measurement error. In the group with A1/A2 mixtures, more substantial variation of $\lambda_{max}$ within some species (notably pike and roach) could be satisfactorily explained by variation in A1/A2 chromophore ratios.

Our study included all three percid species living in Finland (perch, ruffe, and pikeperch), which all have pure A2 pigments. It is interesting to compare these closely related species. Rod $\lambda_{max}$ (mean ± SEM) was 538.6 ± 0.3 nm in perch, 532.2 ± 0.5 nm in ruffe, and 524.1 ± 0.5 nm in pike-perch. All three pairs differ statistically significantly (ANOVA, $P < 0.001$ followed by Scheffe's test, $P < 0.001$). Perch is a species generally active in somewhat brighter light, where the thermal noise generally asso-

**Table 1.** *The wavelength of peak absorbance ($\lambda_{max}$, mean ± SD) and chromophore identity in rods of 35 fish populations representing 25 species. N refers to the number of individuals studied. The locations of origin are: B = Open Baltic Sea outside Tvärminne, $B_P$ = Pojoviken Bay of the Baltic Sea, $B_K$ = Gulf of Finland near Kotka (Baltic Sea), $B_A$ = Achipelago Sea near Nagu (Baltic Sea), E = English Channel near Plymouth, A = Adriatic Sea near Venice, N = west coast of Norway, $L_V$ = Lake Vesijärvi, $L_P$ = Lake Päijänne, $L_T$ = Lake Tuusulanjärvi, $L_B$ = Lake (pond) in Bromarv (SW Finland).*

| Family | Species | Location | N | A1/A2 (%) | Rod λmax ± SD (nm) | λmax of A1–A2 (nm) |
|---|---|---|---|---|---|---|
| Ammodytidae | Greater Sandeel (*Hyperoplus lanceolatus*, Le Sauvage) | B | 1 | A1 (100) | 485.6 | |
| Sygnathidae | Straight-nosed Pipefish (*Nerophis ophidion*, L.) | $B_P$ | 1 | A1 (100) | 500.4 | |
| | Broadnosed Pipefish (*Syngnathus typhle*, L.) | $B_P$ | 4 | A1 (100) | 500.6 ± 0.4 | |
| Gobidae | Black Goby (*Gobius niger*, L.) | $B_P$ | 2 | A1 (100) | 504.8 ± 0.5 | |
| | Sand Goby (*Pomatoschistus minutus*, Pallas) | $B_P$ | 19 | A1 (100) | 508.3 ± 1.9 | |
| | | E | 10 | A1 (100) | 506.2 ± 1.0 | |
| | | A | 18 | A1 (100) | 503.0 ± 1.3 | |
| | Common Goby (*Pomatoschistus microps*, Krøyer) | $B_P$ | 11 | A1 (100) | 515.7 ± 1.3 | |
| Clupeidae | Baltic Herring (*Clupea harengus membras*, L.) | $B_K$ | 8 | A1 (100) | 512.3 ± 0.8 | |
| | Atlantic Herring (*Clupea harengus*, L.) | E | 5 | A1 (100) | 502.5 ± 0.2 | |
| Zoarcidae | Viviparous Blenny (*Zoarces viviparus*, L.) | B | 9 | A1 (100) | 512.8 ± 1.0 | |
| Pleuronectidae | Flounder (*Platichthys flesus*, Duncker) | B | 8 | A1 (100) | 512.2 ± 1.2 | |
| | | N | 4 | A1 (100) | 510.3 ± 1.3 | |
| Cottidae | Fourhorned Sculpin (*Myoxocephalus quadricornis*, L.) | $B_A$ | 6 | A1 (100) | 512.2 ± 1.0 | |
| Gasterosteidae | Three-spined Stickleback (*Gasterosteus aculeatus*, L.) | $B_P$ | 2 | A1/A2 (69/31) | 508.8 ± 0.6 | 504/531 |
| | Nine-spined Stickleback (*Pungitus pungitus*, L.) | $B_P$ | 4 | A1/A2 (4/96) | 526.8 ± 3.5 | 502/528 |
| Esocidae | Pike (*Esox lucius*, L.) | $B_P$ | 1 | A1/A2 (25/75) | 520.8 | 503/530 |
| | | $L_T$ | 1 | A1/A2 (10/90) | 529.8 | 506/534 |
| Osmeridae | Smelt (*Osmerus eperlanus*, L.) | $B_P$ | 2 | A1/A2 (95/5) | 517.8 ± 0.6 | 517/553 |
| Cyprinidae | Rudd (*Scardinius erythrophthalmus*, L.) | $B_P$ | 3 | A1/A2 (0/100) | 537.2 ± 1.4 | 507/537 |
| | | $L_T$ | 2 | A1/A2 (10/90) | 533.3 ± 9.4 | 508/538 |
| | Roach (*Rutilus rutilus*, L.) | $B_P$ | 6 | A1/A2 (35/65) | 523.8 ± 6.6 | 507/537 |
| | | $L_T$ | 2 | A1/A2 (11/89) | 534.2 ± 0.6 | 509/539 |
| | Bleak (*Alburnus alburnus*, L.) | $B_P$ | 4 | A1/A2 (100/0) | 514.6 ± 0.2 | 515/550 |
| | | $L_T$ | 2 | A1/A2 (89/11) | 514.6 ± 1.1 | 513/546 |
| | Crucian carp (*Carassius carassius*, L.) | $L_B$ | 2 | A2 (100) | 525.5 ± 0.6 | |
| | Bream (*Abramis brama*, L.) | $B_P$ | 3 | A2 (100) | 537.2 ± 0.9 | |
| | | $L_V$ | 1 | A2 (100) | 535.8 | |
| | Blue bream (*Abramis ballerus* L) | $B_P$ | 1 | A2 (100) | 517.8 | |
| | White bream (*Blicca bjoerkna*, L.) | $B_P$ | 1 | A2 (100) | 538.6 | |
| Percidae | Pike-perch (*Stizostedion lucioperca*, L.) | $L_T$, $L_V$ | 7 | A2 (100) | 524.1 ± 1.3 | |
| | Ruffe (*Acerina cernua*, L.) | $B_P$ | 3 | A2 (100) | 533.2 ± 1.1 | |
| | | $L_V$, $L_P$ | 5 | A2 (100) | 531.8 ± 1.8 | |
| | Perch (*Perca fluviatilis*, L.) | $B_P$ | 4 | A2 (100) | 538.7 ± 0.9 | |
| | | $L_T$, $L_V$ | 7 | A2 (100) | 538.6 ± 2.0 | |

N = Number of individuals
B = Baltic Sea
$B_P$ = Pojoviken Bay of the Baltic Sea
$B_K$ = Coast of Kotka in the Baltic Sea
$B_A$ = Archipelago Sea
E = Coast of England
A = Adriatic Sea
N = Coast of Norway
$L_V$ = Lake Vesijärvi
$L_P$ = Lake Päijänne
$L_T$ = Lake Tuusulanjärvi
$L_B$ = Lake Bromarv Pond

ciated with long-wavelength-sensitive pigments is not of crucial importance (see Discussion). On the other hand, its rod pigment affords the highest relative quantum catch among the three in Baltic or lake environments. Ruffe and pikeperch are species that are adapted to dim-light vision, as indicated e.g., by the presence of reflecting tapeta (Collette, 1977). Especially pikeperch stay in deep waters and its less long-wavelength-sensitive pigment might afford a higher signal-to-noise ratio of vision in very dim light, in spite of lower quantum catch (see Figs. 3C–3E and Discussion).

*Marine vs. Baltic populations*

All species of marine origin had pure A1 rhodopsins. For three of these (herring, flounder, and sand goby), we studied both Baltic and truly marine specimens. The pairs of representative individual

absorbance spectra for each species in Figs. 2A–2C illustrate that "Baltic" rods are consistently red-shifted compared with their marine counterparts. The between-population differences in $\lambda_{max}$ was statistically significant in all three cases (mean ± SEM): 512.3 ± 0.4 nm in Baltic herring (*Clupea harengus membras*) versus 502.5 ± 0.1 nm in Atlantic herring (*Clupea harengus*) (independent samples *t*-test, $P < 0.001$); 512.2 ± 0.4 nm in Baltic flounder (*Platichthys flesus*) versus 510.3 ± 0.6 nm in the same species from the west coast of Norway ($P < 0.05$), and 508.3 ± 0.5 nm in Baltic sand goby (*Pomatoschistus minutus*) versus 506.2 ± 0.3 nm and 503.0 ± 0.3 nm in the same species from English Channel and the Adriatic Sea, respectively ($P < 0.001$; cf. Jokela et al., 2003). Since all populations had the same chromophore (A1), the differences indicate differences in the protein part of the pigment.

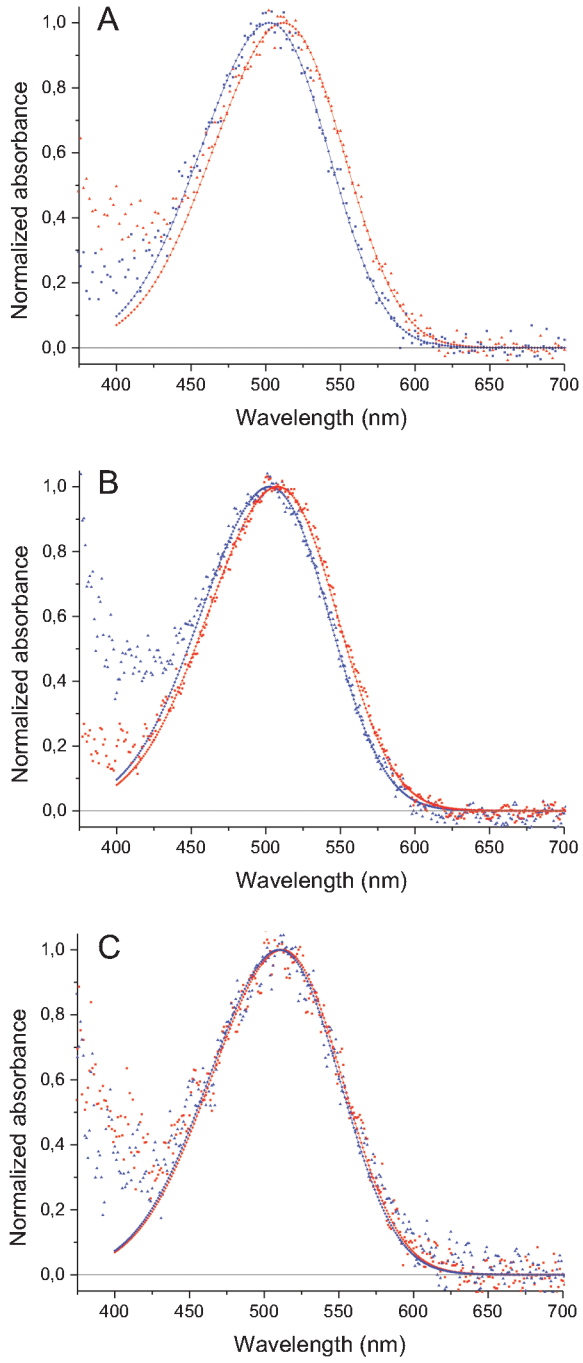


**Fig. 2.** Rod absorbance spectra from individual fish of the three species of which specimens were obtained both from the Baltic Sea (red spectra) and true marine environments (blue spectra). (**A**) herring, (**B**) sand goby, (**C**) flounder. The spectra have been zero-line-corrected, smoothed and normalized to unity peak absorbance as described in the Materials and methods. The curves are A1 templates of Govardovskii et al. (2000).

*Cones*

While our main purpose was to study rod pigments, we made somewhat unsystematical observations on cones that were encountered in the same preparations. The results from altogether 14 species are collected in Table 2. The table is presented only as an empirical data set without claims to completeness. A cone type may escape notice in MSP measurements for many reasons, e.g. rarity, bleaching, or morphological disintegration under the preparation procedures.

*Quantum catch and signal-to-noise ratio of rod vision*

In the following, we consider the functional meaning of tuning rod pigment absorbance to a certain spectral position, either by mutations in the protein part, or by chromophore exchange. In scotopic vision, functional considerations are fairly straightforward in principle, as rods are typically of a single kind and have the simple task to provide competitive absolute visual sensitivity, and achromatic contrast sensitivity in dim light. For this, rods must use what light there is and cannot afford, for example, to look at wavelength bands other than those at which the ambient illumination is maximal to enhance object contrast (cf. Lythgoe, 1979). Moreover, in deep-water conditions, where vision depends most strongly on rod performance, the spectral distribution of available light in aquatic environments is predominantly determined by the specific transmission properties of the water, often resulting in very different, and sometimes rather extreme requirements on spectral sensitivity in different water bodies. Most of the species studied here go into deep waters in some part of their annual lifecycle and are then exposed to such conditions. For example, the sand gobies stay at 20–30 m depth in the Baltic Sea after breeding near the shore in shallow waters between April and August, and sand gobies have been caught as deep as 300 m.

The most obvious criterion for how well rod pigment spectra match the spectral composition of the light is quantum catch. The sensitivity of vision, however, does not depend on quantum catch as such, but on the signal-to-noise ratio (SNR). At somewhat higher light levels, SNR will indeed increase monotonically with increasing quantum catch, but near the absolute threshold, it may be limited by the intrinsic noisiness of the visual pigment itself, i.e., the propensity of the pigment to be thermally activated and trigger the transduction cascade without absorbing any light. Such randomly occurring thermal activations will constitute an irreducible light-identical background noise. Sensitivity of the pigment to long-wavelength light implies that a low-energy quantum suffices for its activation. A relatively low activation energy is likely to imply a relatively high probability for activation by thermal energy alone, i.e., of signaling photon absorption even in the absence of photons. This is the simple logic behind the seminal hypothesis of Barlow (1957) that red-sensitivity is inevitably associated with a high rate of randomly occurring thermal "dark" events, i.e., noise. Barlow's prediction has been confirmed as a general empirical correlation, although not as a strict molecular-physical necessity

**Table 2.** *The wavelength of peak absorbance ($\lambda_{max}$, mean ± SD) and chromophore identity in cones encountered in the same preparations as the rods in Table 1. The estimates of A1/A2 ratio are less reliable than in rods due to generally noisier spectra.*

| Species (common name) | Location | N | A1/A2 (%) | Single cones | | | Double cones | |
|---|---|---|---|---|---|---|---|---|
| Sand goby | $B_P$ | 2 | A1 (100) | 447.2 ± 1.1 | 548.0 ± 2.5 | | 527.4 ± 1.0 | 527.4 ± 1.0 |
| Flounder | N | 1 | A1 (100) | 454.9 | 533.1 | | 536.9 | 536.9 |
| Flounder | B | 3 | A1 (100) | 450.6 ± 0.5 | 535.8 ± 0.5 | | 535.6 ± 2.9 | 535.6 ± 2.9 |
| V. blenny | B | 3 | A1 (100) | 473.6 ± 2.3 | 551.8 ± 1.0 | | 551.8 ± 1.0 | 551.8 ± 1.0 |
| F. sculpin | $B_A$ | 3 | A1 (100) | 450.0 ± 1.6 | | | 522.8 ± 0.7 | 556.0 ± 1.0 |
| Rudd | $B_P$ | 2 | A1/A2 (0/100) | 445.7 ± 2.4 | 531 ± 0.6 | 614.1 ± 0.7 | ND | ND |
| | $L_T$ | 1 | A1/A2 (10/90) | 448.2 | 527.6 | 611.4 | 530.0 | 614.8 |
| Roach | $B_P$ | 2 | A1/A2 (0/100) | 440.8 ± 2.0 | 532.5 ± 0.7 | 601.8 ± 0.6 | 532.9 ± 2.1 | 602.3 ± 1.0 |
| | $L_T$ | 1 | A1/A2 (36/64) | 453.0 | 514 | 578.0 | ND | ND |
| Bleak | $B_P$ | 2 | A1/A2 (100/0) | 409.6 ± 0.8 | 485.2 ± 4.5 | 570.3 ± 1.8 | | |
| | $L_T$ | 1 | A1/A2 (89/11) | ND | 496.4 | 564.4 | | |
| Crucian carp | $L_B$ | 1 | A2 (100) | 528.8 | 608.0 | 534.5 | 618.6 | |
| Bream | $B_P$ | 3 | A2 (100) | 451.0 ± 1.7 | 536.9 ± 1.2 | 619.7 ± 4.0 | 536.4 ± 1.6 | 619.5 ± 4.7 |
| | $L_V$ | 1 | A2 (100) | 452.7 | 532.0 | 617.8 | ND | ND |
| Blue bream | $B_P$ | 1 | A2 (100) | 440.1 | 523.7 | 571.3 | | |
| White bream | $B_P$ | 1 | A2 (100) | 443.7 | 533.1 | 602.5 | | |
| Pike-perch | $L_T$, $L_V$ | 4 | A2 (100) | | 529.9 ± 1.4 | | 597.6 ± 0.8 | 597.6 ± 0.8 |
| Ruffe | $B_P$ | 3 | A2 (100) | | 529.0 ± 1.2 | | 598.8 ± 1.2 | 598.8 ± 1.2 |
| | $L_V$ | 1 | | | 529.7 | | 600.0 | 600.0 |
| Perch | $B_P$ | 2 | | | 533.1 ± 1.3 | | 610.8 ± 1.7 | 610.8 ± 1.7 |
| | $L_T$, $L_V$ | 4 | A2(100) | | 534.3 ± 2.6 | | 610.9 ± 2.8 | 610.9 ± 2.8 |

(Ala-Laurila et al., 2004*a*, 2004*b*). Spectral tuning of pigments to maximize the SNR near the absolute threshold for seeing then appears as an optimization task, where possible increases in quantum catch must be weighed against possible increases in noise.

Thus we propose two measures for the performance of a rod visual pigment in a certain light environment. The first is relative quantum catch ($QC_{rel}$), which simply tells how well the pigment is able to utilize the available light. The second is the relative signal-to-noise ratio in extreme dim-light conditions, when spontaneous thermal activations of visual-pigment molecules may be the dominant limitation. We denote this $SNR_{dark}$. The calculation of $QC_{rel}$ and $SNR_{dark}$ as functions of pigment $\lambda_{max}$ under a given illumination is described in the Materials and methods section. In Fig. 3, panels A to E give results for each of the five irradiance spectra shown in Fig. 1. Four of these correspond to actual habitats of fish included in the present study (Baltic: B and $B_P$, coastal Atlantic: JIII, and Lake Tuusulanjärvi: $L_T$). Included for comparison are the calculations for a very short-wavelength-dominated environment (JI, curves in Fig. 3A), corresponding to great depths in the open ocean.

Each of the panels A–E includes four curves, two for A1 pigments (higher peaks) and two for A2 pigments (lower peaks). The two peaks at longer wavelengths (A1-black and A2-blue) give relative quantum catch, $QC_{rel}$. The two peaks at shorter wavelengths (A1-red and A2-green) give $SNR_{dark}$. The reason why the $\lambda_{max}$ for maximal $SNR_{dark}$ is lower than that for maximal $QC_{rel}$ is that moving $\lambda_{max}$ toward longer wavelengths will, on average, carry a cost in terms of increased thermal noise. In each light environment, the $SNR_{dark}$ peak defines a lower optimum and the $QC_{rel}$ peak an upper optimum position for pigment $\lambda_{max}$. These two positions represent the lower and upper bound of an "optimality interval" for $\lambda_{max}$ in that particular photic environment. If $\lambda_{max}$ lies outside this interval, the rod pigment cannot (in any simple sense) be regarded as optimal for those conditions.

Fig. 4 summarizes the $\lambda_{max}$-distribution of all pure A1 (blue histogram) and all pure A2 (red histogram) rod pigments in Table 1. This distribution can now be related to the optimality intervals for each of our five representative photic environments (JI, JIII, B, $B_P$, and $L_T$), which have been plotted as horizontal bars above the histograms (blue for A1 and red for A2 pigments). It is seen that no pigment is well-adapted to brown-water lakes like Lake Tuusulanjärvi. Otherwise the pigments of all but one population fall within the optimality intervals for the waters where the population lives. The single exception is the greater sand eel from the Baltic Sea, which has retained "marine" rod sensitivity seemingly ill-adapted to the present environment.

We would finally like to emphasize that the above considerations concern properties of the *visual pigment*. If we wanted to compare adaptations of *rod cells*, we would have to consider variations in outer-segment length, i.e., axial absorptance (rather than pigment absorbance), as well as outer-segment volume, on which the number of noise-producing pigment molecules depends. The variation in mean outer-segment length in our samples was generally moderate, most species falling in the range 25–40 $\mu$m. The flounder (18 $\mu$m) and the greater sand eel (21 $\mu$m) fell below and the pike (55.5 $\mu$m) and the perch (45 $\mu$m) above this range. The flounder also had the thinnest outer segments (1.5 $\mu$m dia) and the pike the thickest ones (5 $\mu$m), most of the other species falling in the range 2–3 $\mu$m. There is no immediately obvious interpretation for these differences.

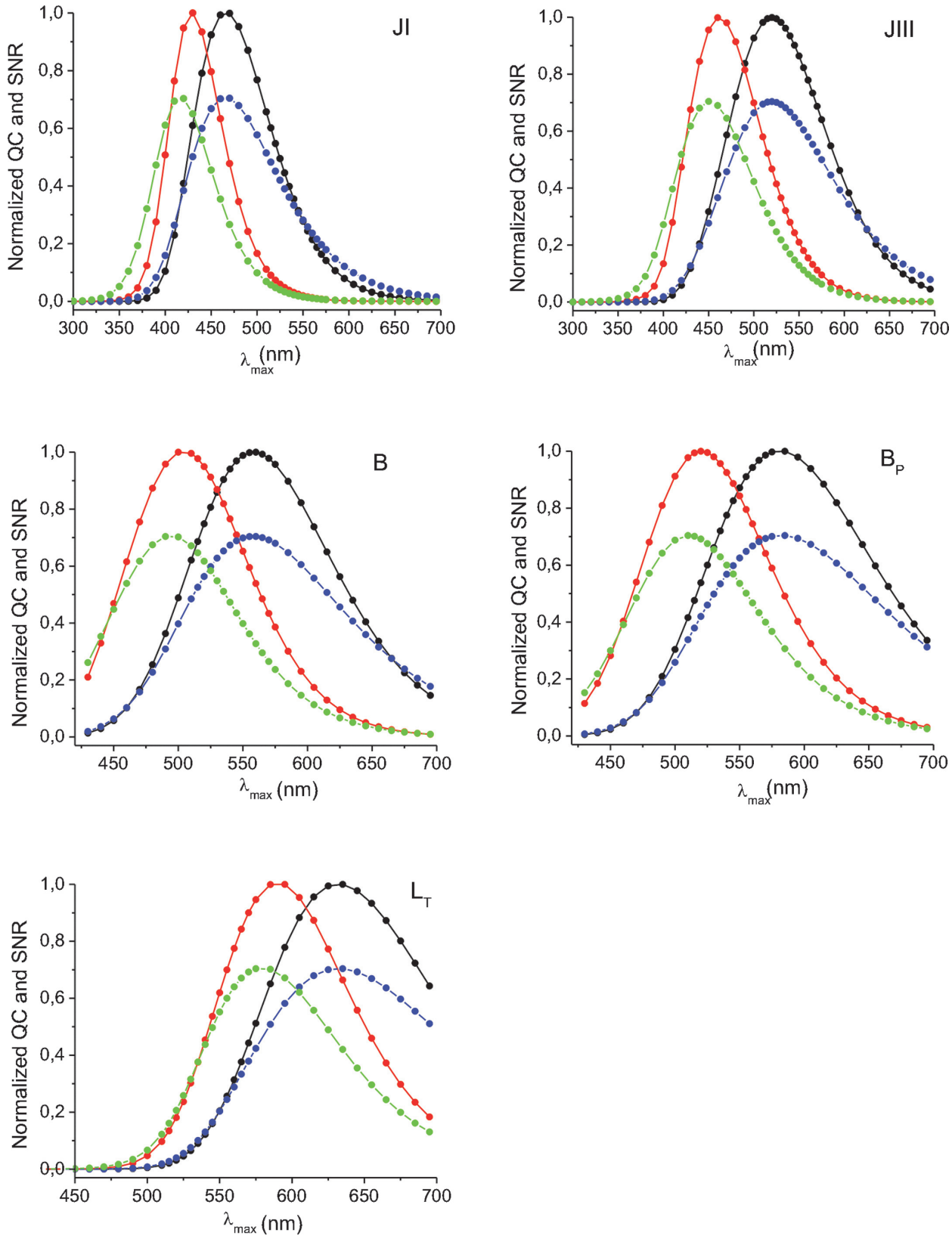


**Fig. 3.** Theoretical performance measures of visual pigments in the five representative spectral environments introduced in Fig. 1 (JI, JIII, B, $B_P$, and $L_T$), plotted as functions of the pigment's $\lambda_{max}$. Red and black curves refer to A1 pigments, green and blue curves to A2 pigments. In each panel, the two curves (red and green) that peak at shorter wavelengths give the signal-to-noise ratio near the absolute visual threshold, denoted $SNR_{dark}$ in the Text, and the two curves that peak at longer wavelengths (black and blue) give quantum catch, denoted $QC_{rel}$ in the Text. All values are given as fractions of the maximum values for A1 pigments, which have been normalized to 1.

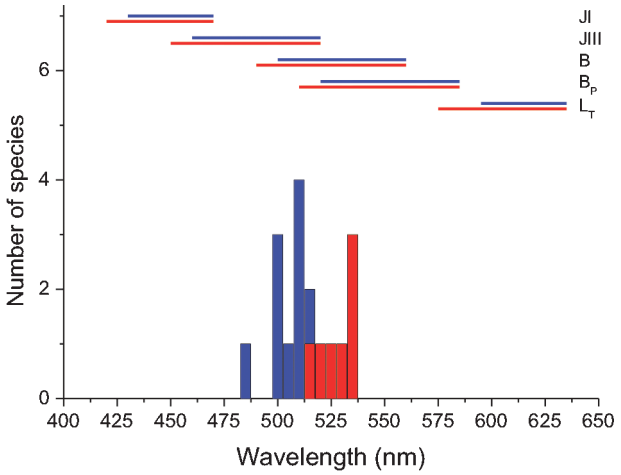


**Fig. 4.** Histogram showing the distribution of the $\lambda_{max}$ values of all pure A1 (blue) and all pure A2 (red) rod pigments in Table 1 (bin width 5 nm). The horizontal bars above the histogram show the optimality intervals (blue for A1 pigments, red for A2 pigments) in each of the five representative spectral environments, as indicated by the abbreviations on their right. The left edge of each optimality interval gives the $\lambda_{max}$ location estimated to maximize $SNR_{dark}$ in the environment in question, the right edge that which would maximize $QC_{rel}$. See Text for details.

## Discussion

### *What does it mean that a rod pigment is "adapted to the photic environment"?*

When discussing the "adaptedness" of rod pigments to different photic environments, it is useful to broadly distinguish three isotopic light levels that differ with respect to the optimization pressures they pose: (1) a level close to the absolute threshold of vision, (2) a level of slightly higher light intensities, where rods still operate predominantly in a photon-counting mode, (3) the high isotopic and mesopic range where the gain of transduction and transmission start to decrease and quantal fluctuations (whether from photons or from thermal events) no longer constitute the dominant source of noise. In the dim-light ranges (1–2), the SNR of rods, and thereby visual sensitivity, will strongly depend on the spectral and thermal properties of the visual pigment. In range (3), on the other hand, considerable variation in both spectral and thermal properties of rod pigments can be tolerated without much consequence for visual function.

In range (1), the SNR of rod vision is potentially limited not only by the pigment's capacity to catch photons (its spectral properties), but also by its propensity to undergo spontaneous thermal activations, which constitute an irreducible intrinsic noise (determining $SNR_{dark}$). In range (2), noise due to Poisson fluctuations in the numbers of photoactivations dominate over that from thermal activations. This photon-limited SNR grows in proportion to the square root of quantum catch and thus depends directly on the spectral properties of the pigment. The curves for $SNR_{dark}$ and $QC_{rel}$ in Fig. 3, delimit optimization ranges for the respective light milieus. A "well-adapted" rod pigment should have $\lambda_{max}$ between the wavelengths where the two curves reach their maxima, and the degree to which it is closer to one or the other might in principle indicate to what degree it is optimized for absolute sensitivity versus slightly brighter isotopic conditions. Discrepant properties may point to competing goals, lack of reproductive isolation, evolutionary constraints—or simply suggest that $SNR_{dark}$ and $QC_{rel}$ are not very critical for the visual "tasks" of the pigment, which may, e.g., be limited to brighter illumination. Cone pigments offer a striking example of the last-mentioned situation, where thermal isomerization rates some $10^4$-fold higher than in rod pigments can be tolerated (e.g., Kefalov et al., 2003; Ala-Laurila et al., 2004*b*).

### *Baltic and marine forms of the same species*

In all three species where we measured samples both from the Baltic and truly marine subspecies/populations of the same species, we found that the Baltic rod pigment was relatively long-wavelength-shifted. The same seems to apply to a fourth species (viviparous blenny), where we had the opportunity to study only Baltic specimens, but have to rely on literature data for marine populations. As all these pigments had pure A1 chromophore, the differences indicate polymorphism of the opsin. As the direction of all shifts is consistent with improved quantum catch in the Baltic light environment, it is difficult not to think that they reflect a real selection pressure.

*Herring*. The greatest $\lambda_{max}$ shift, 9.8 nm, was found between the Atlantic and the Baltic herring populations. Atlantic herring (*Clupea harengus*) is a plankton feeder living near the shore. Vision is used for foraging suitable prey. During the day the fish stay in deeper waters (up to 200 m) and come up near surface to eat at night (Whitehead *et al*., 1988). Baltic herring (*Clupea harengus membras*) is a small-sized subspecies of the Atlantic herring (Parmanne & Sjöblom, 1984). The shift from 502 to 512 nm gives Baltic herring a 35% gain in quantum catch with negligible change in $SNR_{dark}$. As seen in Fig. 3B, a 512 nm pigment would in fact catch more quanta (ca. 13%) even in the North Atlantic (JIII), but would decrease $SNR_{dark}$ by ca. 20% compared with the 502 nm actually used there. A similar value for marine herrings, $\lambda_{max}$ = 500 nm, has been obtained in an earlier study on rod pigment extract from Atlantic *Clupea harengus* (Blaxter, 1964) as well as an MSP study in young Pacific herrings, *Clupea pallasi* (Britt et al., 2001).

*Sand goby*. As mentioned before, for a considerable part of the year Baltic sand gobies stay at 20–30 meters depth in conditions described by spectrum B (possibly even $B_P$) in Fig. 1 and Fig. 3. The advantage then gained by shifting the A1 pigment from 503 nm to 508.3 nm is nearly 20%. By contrast, a similar red-shift in the Adriatic Sea would bring no advantage in quantum catch and a decrease in $SNR_{dark}$.

*Flounder*. Flounder is a migratory fish, which is found in estuaries most of the year. It is nocturnal and can burrow itself into the substrate (Cooper & Chapleau, 1998). During winter, adults retreat to deeper, warmer waters, where they spawn in spring. Juveniles live in shallow coastal waters and estuaries, which are also the summer feeding grounds for the adults. The 1.9 nm red-shift of the rod pigment in Baltic compared with Atlantic flounders would give the former a ca. 5% advantage in photon catch in the Baltic. Although the difference in mean $\lambda_{max}$ was statistically significant, we would be cautious in our conclusions. Most single amino acid substitutions within RH1 opsin genes lead to spectral shifts greater than about 5 nm, and our result may reflect a mean difference between two polymorphic populations. It is worth noting that even the Norwegian $\lambda_{max}$ is clearly red-shifted compared with typical ocean fish, increasing $QC_{rel}$ but decreasing $SNR_{dark}$ in its North Atlantic habitat (JIII). This may reflect

contradictory requirements on the estuarine and deep-water phases. Earlier measurements of rod pigment absorbance in "sea" flounders also suggest variability: Ali and Wagner (1975) give $\lambda_{max}$ = 507 nm, Loew and Lythgoe (1978) 512 nm.

*Viviparous blenny*. We can make a tentative comparison between Baltic and oceanic forms also in the viviparous blenny. We obtained $\lambda_{max}$ = 512.8 nm, whereas Ali and Wagner (1975) report ca. 493 nm. The difference is substantial and is unlikely to be an artifact due to differences in measurement techniques or analysis. In Baltic conditions (spectrum B in Figs. 1 and 3), this red-shift would bring an advantage of some 70% in $QC_{rel}$ as well as a small improvement in $SNR_{dark}$.

*Other marine species*. Not all marine species in the Baltic Sea appeared to be well-adapted to their present spectral environment. In three species of marine origin, rod $\lambda_{max}$ varied from 485.6 nm (greater sand eel) to about 500 nm (pipefish). For greater sand eel, the quantum catch in Pojoviken Bay ($B_P$) would be only 14% and in the open Baltic Sea (B) 27% of the theoretical maximum. Although performance appears less suboptimal when judged by the corresponding $SNR_{rel}$ values, which are ca. 70% ($B_P$) and 90% (B) of the maximal value, $\lambda_{max}$ lies on the short-wavelength-side of the $SNR_{dark}$ peak, i.e., outside the "optimality interval" (cf. Fig. 4). In this case, the apparent "maladaptation" probably reflects the fact that the animal is always active in such bright light that maximizing rod quantum catch becomes unimportant (light level (3) above). Winslade (1974) has shown that the swimming activity of the closely related lesser sand eel (*Ammodytes marinus*, Raitt) is limited by light level: they are active only in the daytime when there is a lot of light. At other times they protect themselves by burying quickly into sandbanks and gravel, and stay there. Even in the Baltic Sea, the 485.6 nm pigment is good enough for vision in bright light.

*Lake fish*

Of the two freshwater lakes included in this study, Lake Vesijärvi had peak spectral transmission around 570–585 nm (similar to Pojoviken Bay, $B_P$ in Fig. 1), while Lake Tuusulanjärvi is very brown with a transmission peak around 625–640 nm ($L_T$ in Fig. 1). All rods from Lake Tuusulanjärvi are poorly adapted at least to its deeper waters ($>$ 3 m), where even $SNR_{dark}$ peaks as high as 575 nm for A2 pigments (Fig. 3E). The general absence of evolutionary changes in the opsin between lake and Baltic populations is not surprising, as the current photic status of the lakes may be of considerably younger origin than their separation from the sea and in many lakes still does not differ much from parts of the Baltic. Moreover, more recent gene flow between the Baltic Sea and Finnish lakes cannot always be excluded.

The common use of A2 chromophore, either pure or mixed with A1, may be seen as a general adaptation to the longer-wavelength environments. The red-shift achieved by using A2 must be weighed against the lower photosensitivity (Dartnall, 1972) and higher rates of thermal activation of the A2 pigment (Donner et al., 1990; Ala-Laurila et al., 2004*a*, 2004*b*). The proportion of A1 and A2 can be regulated in response to environmental factors (temperature and photoperiod; Dartnall et al., 1961; Allen & McFarland, 1972). Spectral tuning by a plastic chromophore response can obviously be an advantage in the optically variable habitats of coastal, estuarine, and fresh waters. Different developmental stages of fish may recruit not only a chromophore switch, but also changes in opsin expression for (apparent) adaptation to shifts in habitat illumination (Shand et al., 2002). It is worth noting that the A1 $\rightarrow$ A2 substitution as such, would not increase $SNR_{dark}$ in *any* combination of visual pigment, and light environment considered here. Possibly, the use of A2 chromophore is always connected with conditions where quantum catch is the variable to be maximized. A full consideration of chromophore advantages, however, must necessarily take into account cone as well as rod vision (Liebman & Entine, 1968; Tables 1 and 2); although it is now evident that individual photoreceptor cells may be selective about their chromophore content and need not passively reflect proportions supplied by the pigment epithelium (Bowmaker et al., 1988; Parry et al., 2003).

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