A visual pigment of the sturgeon retina

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Summary. The visual pigments of hybrid sturgeon (a cross between *Acipenser ruthenus* (male) and *Huso huso* (female) have been studied both by the methods of incomplete partial bleaching and HPLC analysis. On the basis of the results obtained the relationship between the structure of opsins and the spectral characteristics of visual pigments is discussed.
Key words. Sturgeon; HPLC analysis; retina; visual pigment; porphyropsin.

The living Chondrostei belong to the subclass Actinopterygii of the class Osteichthyes and are today represented by Acipenseridae and a related family, the Polyodontidae. They include the twenty-odd species of sturgeons (and shovelnosed sturgeons) and two genera of spoonbills, *Polyodon* and *Psephurus*. Fundamentally, most sturgeons are anadromous although a few (and spoonbills) are confined to freshwater.

The existence of what has been referred to as the retina in the sturgeons is known through studies which were primarily concerned with the morphology of the eye [1-4], and produced no information on the visual pigment of the retina in this species, which is of considerable interest in view of the phylogenetic position of sturgeons.

Hybrids between *Acipenser ruthenus* and *Huso huso* can be produced by artificial fertilization; the hybrids are called "bester". The fertilized eggs were transported from USSR and subsequently hatched, grown and maintained (under natural photoperiod) in the outdoor culture ponds of the Mie Prefectural Freshwater Fisheries Experimental Station in Japan. For extraction of visual pigment, the routine procedure involved treatment with 4% potassium alum solution, which was removed by three rinses with distilled water, and extraction with 2% digitonin solution (Merck). 10% by volume of saturated sodium borate solution and the same amount of 0.2 M hydroxylamine solution were added before measuring the spectral absorbance. The absorption spectra were recorded with a Shimadzu UV-265 FS spectrophotometer.

The chromophore composition was determined by high-pressure liquid chromatography (HPLC) with a retinaloxime method, details of which have been reported by Suzuki and Makino-Tasaka [5]. HPLC analysis was performed on a Shimadzu LC-3A instrument equipped with a Zorbax SIL column (2.1 × 250 mm ID) and absorbance at 360 nm was monitored.

The absorbance spectrum showed only a small amount of pigment and is shown in figure 1, curve 1. Subsequently the pigment was bleached by exposure to orange light from a 500 W projection lamp with a color filter ($\lambda > 560$ nm; Toshiba V-R 56) in combination with a heat filter. The pigment was almost completely bleached. The spectral change in absorbance is shown in figure 2. The pigment band agrees with a 530-nm nomogram curve derived from Munz and Schwanzara's nomogram [6], and the product band is characteristic of 3-dehydroretinaloxime ($\lambda_{max} = 387$) confirming that the pigment is a porphyropsin.

HPLC separation (fig. 3) indicated the presence in the retina of 3-dehydroretinaloxime, principally as the 11-*cis*-isomer. No trace of 11-*cis*-retinaloxime was detected: the retina contained only porphyropsin. This finding supports the interpretation that the final photoproduct in figure 2 is 3-dehydroretinaloxime and is consistent with the best available evidence on the identity of the porphyropsin chromophore in this species. Total area of *syn*- and *anti*-peaks of the chromatogram was determined for each isomer by integrating the absorbance at 360 nm. The amount of each isomer was quantified from the peak area. The total chromophore amounts were estimated to be 2.3 nmol per retina of the 'bester'.

A succession of visual pigments can occur in lampreys [7-9], eels [10] and the Pacific salmon [11], in relation to the migrations

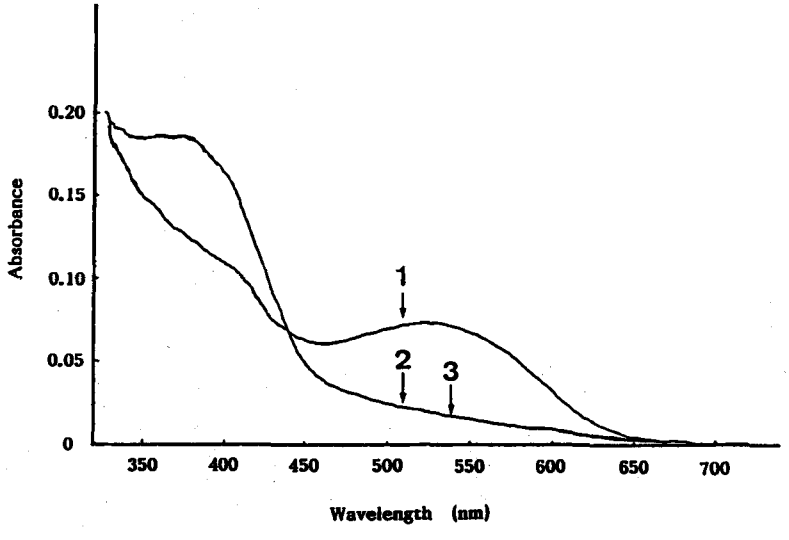

Figure 1. Series of absorption spectra obtained in a partial bleaching experiment. *1* before exposure; *2* after exposure to orange light, 30 s, $\lambda > 560$ nm; *3* after further exposure to orange light, 60 s.

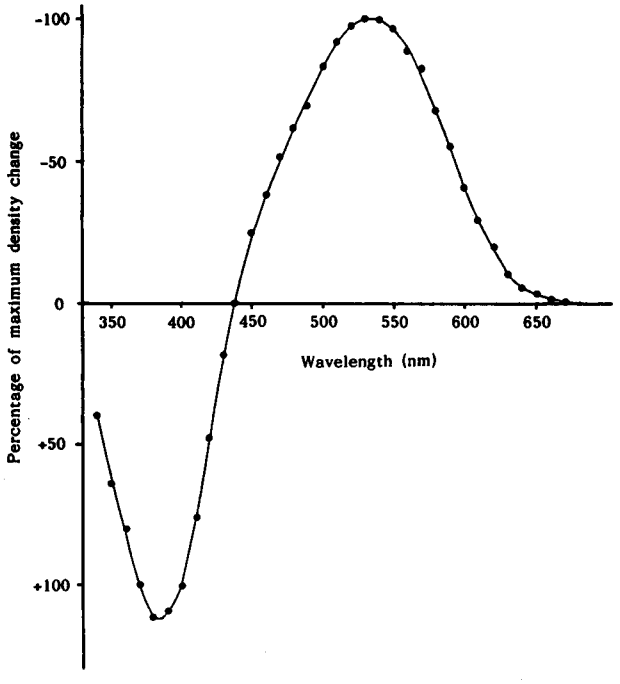

Figure 2. Difference spectrum of visual pigment of the 'bester'. Solid line represents the theoretical absorbance spectrum for VP 530_2 based on Munz and Schwanzara [13].

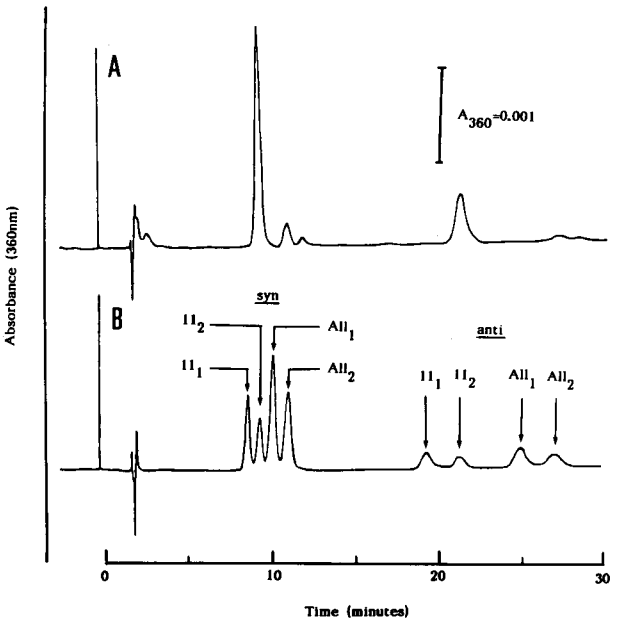

Figure 3. Chromatogram obtained from the retina of 'bester' (*A*), compared with standard oximes of 10 pmol each (*B*). 11_1, 11-*cis*-retinaloxime; 11_2, 11-*cis*-3-dehydroretinaloxime; All_1, all-*trans*-retinaloxime; All_2, all-*trans*-3-dehydroretinaloxime. The abscissa is a time axis; the ordinate is absorbance at 360 nm.

that characterize the life cycles of these forms. The 'bester' is a hybrid species; one of the parents is an anadromous and the other a landlocked form. The hybrid offspring is now confined permanently to fresh water. As reported by Munz and McFarland [12], and McFarland and Munz [13] in the case of the 'splake', the artificially fertilized hybrid between male brook char and female lake char, the specific differences in the opsins of each parental species are inherited with codominance, both being expressed in the heterozygotic hybrids. If visual pigments are always inherited with codominance, the absence of rhodopsin variants in this species suggests that it is homozygous for this condition. Thus, the most likely explanation is that the parental pigments of hybrid 'bester' are based on the same opsin, one feature being that the chromophore group is the same.

'Bester' porphyropsin has λ_{max} at 530 nm, in the same vicinity as that of primitive freshwater holosteans, which are closely related to the sturgeons. The visual pigments of two holosteans, *Amia calva* and *Lepisosteus platyrhincus* were studied by Bridges [14] who found two pigments, VP 525_2 and 523_2, respectively, in retinal extracts. This conforms to Wald's association of A_2-based pigment with the freshwater habitat [15]. Whether it should be regarded as a primitive and common feature of these fishes or an independent acquisition in each group is an open question. The possible biological significance of porphyropsin, with its absorbance at longer wavelengths, has been pondered in close association with the redder environment of freshwater lakes, ponds, streams and swamps by several authors [16, 17]. Perhaps coupled with this adaptation, most of the *Acipenseridae*, which are characterized by the anadromous habit, could evolve a permanent switch towards the pure porphyropsin condition, as occurs temporarily in some species during normal activity. However, it should be remembered that even fish belonging to purely freshwater species have not always discarded their rhodopsin, but maintain a mixture of the two pigments, often switching back and forth between them in response to a variety of influences [14, 18, 19].

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