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Visual Pigment of the Freshwater Stingray, *Paratrygon motoro*

In general the absorbance spectra of the visual pigments of deep-water and pelagic marine animals are blueshifted compared to those of coastal and estuarine animals, and the pigments of the latter are blueshifted compared with those of freshwater animals. This trend, which correlates with the transmittances of the waters, is best documented for the Osteichthyes (bony fishes)[1]. The marine members of this Class, except for some wrasses, have visual pigments based on vitamin A_1 (rhodopsins of λ_{max} 473 to 512 nm). In freshwater Osteichthyes, however, the need for longer-wave sensitivity is met by pigments based on vitamin A_2 (porphyropsins of λ_{max} 523 to 543 nm) or by mixtures of porphyropsin and rhodopsin or, in one case at least (the gwyniad[2]), by a rhodopsin of high λ_{max} (520 nm).

A similar trend of λ_{max} is apparent in the visual pigments of the Chondrichthyes (cartilaginous fishes). For example, Denton and Shaw[3] found that the rhodopsins extracted from three deep-sea sharks (λ_{max} 472 to 484 nm) are in the same range as those of deep-sea Osteichthyes (473 to 490 nm), while the extracted rhodopsins of surface-living sharks and rays have λ_{max}=497 to 499 nm (ref. 1). Until recently none of the very few cartilaginous fishes that inhabit freshwater has been examined in this respect. Consequently, the report[4] that the intact retina of the Amazonian stingray, *Paratrygon motoro*, absorbs maximally around 510 nm seems to provide a rare parallel to the visual pigments of the freshwater Osteichthyes, and to raise the question whether its pigment is a porphyropsin or a redshifted rhodopsin.

It should be noted, however, that other bottom-living fishes (Osteichthyes) from the same region were found in that investigation[4] to have retinal λ_{max} 20 to 30 nm longer than *Paratrygon*. Moreover measurements made on intact retinae may not be strictly comparable with those made on extracts[5]. Thus one cannot be sure that this apparent difference between the pigments of marine and freshwater rays is real. For this reason we examined a conventional extract.

It was made with three retinae from two specimens of *Paratrygon motoro* (Mueller and Henle) caught in the Rio Negro near Manaus, over 1,000 miles from the mouth of the Amazon. The retinae were dissected out, frozen and brought to Sussex for analysis. Facilities for keeping them frozen during transport were not good, and they thawed several times, which probably accounts for the small yield of pigment.

The photoreceptor outer segments were obtained as a minute pellet (by Saito's sugar flotation process[6]) which, after washing with neutral buffer, was extracted overnight with 0.5 ml 3% digitonin solution. The extract was made alkaline with sodium borate and 0.005 M in hydroxylamine. The absorbance spectrum showed only a small amount of pigment, insufficient for homogeneity tests. Consequently it was fully bleached by exposure to white light passed through a Wratten 15 filter. The spectral change in absorbance is shown in Fig. 1. The pigment band agrees with a 499-nm nomogram curve and the product band is characteristic of retinal oxime, confirming that the pigment

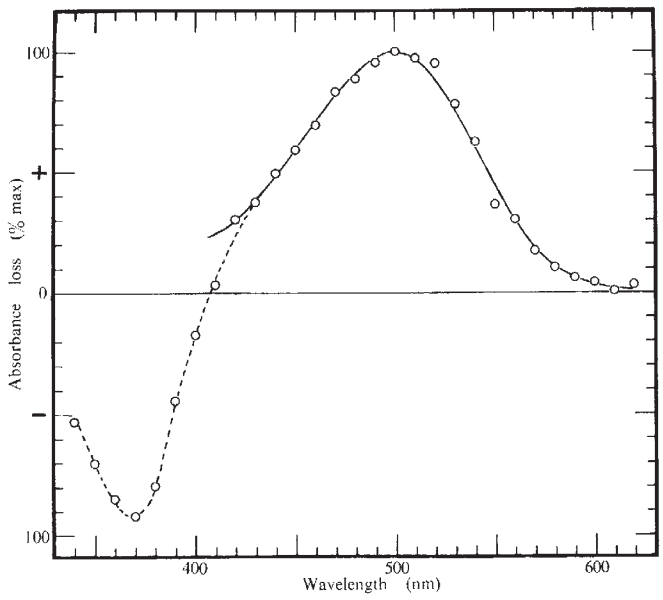

Fig. 1 Normalised difference spectrum (actual ΔA_{max}=0.0115) of the Stingray visual pigment (– – –) in the presence of 0.005 M hydroxylamine. The product band (λ_{max}~370 nm) is characteristic of the oxime of retinal. ——, A 499-nm 'nomogram' pigment. Optical path length 10 mm; volume of extract 0.55 ml, *p*H 8.45.

is a rhodopsin. Thus there is no significant difference between the rhodopsins of freshwater and marine rays.

Assuming that the 499-nm rhodopsin is the only pigment of the *Paratrygon* retina, it would seem that this animal is not well adapted to its freshwater environment. Its retina, however, is backed by a gold-coloured tapetum[4] and this will shift the effective sensitivity to longer wavelengths. Although it is possible, therefore, that the presence of a 'normal' rhodopsin means that neither a porphyropsin nor a redshifted rhodopsin could be evolved in this line, it could also mean that a golden tapetum was a simpler solution to the problem. The colour disappears on addition of glycerol[4], which suggests that, as in other Chondrichthyes, it is an interference phenomenon[7]. The reflectivity might, therefore, have moved to longer wavelengths, during evolution, by a change in the spacing of the tapetal plates.

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