Light-induced colour changes by the iridophores of the Neon tetra, *Paracheirodon innesi*

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Summary

The iridophores of the Neon tetra *Paracheirodon innesi* consist of alternating layers of guanine and cytoplasm. In the dark-adapted state the reflected light from constructive interference is in the ultraviolet or blue. When exposed to light the cytoplasm layers increase in thickness and as a result the reflections shift to longer wavelengths and the iridophores appear green. The iridophores are thought to contain a rhodopsin-like molecule and we suggest that the colour-change mechanism involves the light-induced opening of sodium channels in the plasma membrane, leading osmotically to an increase in thickness of the cytoplasm layers. Experimental support for this suggestion was obtained by the substitution of choline chloride for sodium chloride in the perfusing medium, which can be done without altering the osmotic strength of the perfusing medium. This procedure almost abolished the light response and makes it seem likely that sodium ions are necessary for the light response to take place.

Key words: *Paracheirodon*, iridophores, light.

Introduction

The first report that teleost iridophores could change colour in response to the direct action of light concerned the blue spots of the male Killiefish *Fundulus heteroclitus* (Foster, 1933). Since then similar colour changes have been described in the iridescent lateral stripe of the Cardinal tetra *Cheirodon axelrodi* (Rohrlich, 1974) and the Neon tetra *Paracheirodon innesi* (Lythgoe & Shand, 1981, 1982). In each case the colour of the iridescence in dim light is violet or blue, changing to longer wavelength green, orange or red when the light intensity increases. Iridescent colours are produced by the constructive interference of light from regular stacks of very thin alternating layers of transparent material of different refractive index. In fish iridocytes, the layers of low refractive index are cytoplasm and those of high refractive index are guanine crystals. Each pair of layers is of the order of half the wavelength of light and the colour of the light that is reflected depends largely upon the thickness of the layers (see Land, 1972, for a review of iridescent multilayers in biological systems). Most authors agree that it is changes in the thickness of the cytoplasm layers that separate the guanine plates that is responsible for the colour changes in fish iridophores (Foster, 1933, 1937; Rohrlich, 1974; Lythgoe & Shand, 1982; Oshima *et al.* 1985).

In all visual systems rhodopsin, or a closely related molecule, is the primary light receptor. This may also be true in some extra-retinal photoreceptors, for a rhodopsin-like molecule has been demonstrated in the avian pineal organ (Vigh *et al.* 1982) and the iridophores of the Neon tetra (Lythgoe *et al.* 1984). In the retina the activation of a rhodopsin molecule by a photon of light initiates a cascade of enzymic reactions that results in the closing of sodium channels in the plasma membrane of the rod outer segment (ROS) and the generation of a nervous impulse (for a recent account, see Stryer, 1987). In the light-activated iridophore we offer evidence that light causes sodium channels in the plasma membrane to open. The resulting change in the osmotic balance within the cell causes the thickness of the cytoplasm layers in the iridophore to increase and the colour of the light that it reflects to shift to longer wavelengths.

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Materials and methods

Neon tetras, *Paracheirodon innesi*, were purchased from a commercial supplier of aquarium fish in Bristol, who had imported them from commercial breeders in SE Asia. The fish were kept at a temperature of 23°C under a 12 h light/12 h dark regimen.

When alarmed, the iridescent lateral stripe of the Neon tetra becomes yellow-green in colour and does not show a shift to violet-blue iridescence in the dark (Lythgoe & Shand, 1982). We found, however, that with careful handling the light-induced colour changes were similar to those seen in undisturbed fish. Fish were killed by decapitation, and using a sharp blade a section of skin that included the dorsal part of the iridescent lateral stripe was removed and placed in the perfusion chamber. The chamber had a capacity of 450 ml with an inlet bore of 2 mm and an outlet bore of 4 mm. The chamber was lightly packed with non-absorbant lead-free glass fibre and the strip of skin was placed on it, outer surface upwards. The chamber was closed with a coverslip and sealed with high-vacuum silicone grease (Edwards High Vacuum).

All experiments were carried out at room temperature (21–23°C). The aerated solutions were supplied to the perfusion chamber through a two-way tap and a Watson Marlow peristaltic pump at a rate of 3 ml min^{-1}. Unless otherwise stated fish were perfused with Cortland Brown trout Ringer solution (Dawson *et al.* 1986) with an osmolarity of 273 mosmol kg^{-1} measured with a vapour pressure osmometer (Wescor). The solution had a pH of 7·1 at 25°C. The basic recipe for the Ringer solution is: 124·0 mM-NaCl, 5·0 mM-KCl, 1·56 mM-$CaCl_2 \cdot 2H_2O$, 0·9 mM-$MgSO_4 \cdot 7H_2O$, 2·9 mM-$NaH_2PO_4 \cdot H_2O$, 11·9 mM-$NaHCO_3$, 5·5 mM-glucose. To obtain hyposmotic solutions twice the normal amount of distilled water, and for hyperosmotic solutions half the quantity of water, was used. Equimolar quantities of choline chloride were used to replace NaCl when sodium-free solutions were required.

The perfusion chamber containing the specimen was mounted on the stage of the microscope and held in darkness for 30 min to permit the blue-violet colour typical of dark-adapted iridophores to become established, a reflectance reading at 570 nm was taken and the experimental procedures begun.

Spectral reflectance measurements were made using the method of Lythgoe & Shand (1982) and a microscope fitted with epi-illumination and a spectroradiometer coupled by means of a light guide to the exposure-meter port of a camera fitted onto the microscope. The spectroradiometer was a Macam SR 3010 A/B fitted with a holographic grating monochromater and a PM 1059 GaAs photomultiplier. The light source was a tungsten lamp fed by a regulated 6V, 4A supply and had a colour temperature of 2400K. The intensity of the source was adjusted by neutral density filters and the apparatus was calibrated against a sheet of polished aluminium, which has an almost flat spectral reflectance curve within the visible spectrum (Wyszecki & Stiles, 1967).

In order to calibrate the intensity of the experimental light a fibre-optic light guide was used to conduct the light from the level of the specimen directly to the spectroradiometer and the readings were integrated between 400 and 700 nm. The intensity of the light was adjusted by means of neutral density filters to $1{\cdot}46\times10^{18}$ photons $m^{-2}s^{-1}$, which is the light intensity to which the iridophores are maximally sensitive (Clothier, 1986). To ensure that all the incident light coming through the objective had been gathered by the light guide, a second light measurement was made with a Macam Quantum Radiometer/Photometer Q101 fitted with a fully cosine-corrected head to provide a 180° field of view. There was good agreement between the two sets of measurements. Spectral intensity measurements were adjusted to the percentage of the maximum reflectance, corrected so that polished aluminium showed a flat spectral reflection curve.

It is difficult to follow changes in spectral reflectance by measuring complete spectral reflectance curves because the light used in the measurements itself affects the course of the colour changes. However, the iridophores are relatively insensitive to light at 570 nm, yet spectral reflectance changes are easy to measure at that wavelength. A rise in reflectance at 570 nm marks a shift in spectal reflectance to longer wavelengths, and a reduction in reflectance at 570 nm marks a shift to shorter wavelengths (Lythgoe & Shand, 1982).

Results

The spectral reflectance curves for iridophores that had been dark-adapted for a total of 60 min in different experimental solutions are shown in Fig. 2. It is evident that in the dark various perfusion treatments have only a small effect on spectral reflectance, all tissues having the violet-blue colour that is typical of dark-adapted iridophores.

The changes in spectral reflectance at 570 nm following various light and osmotic treatments are shown in

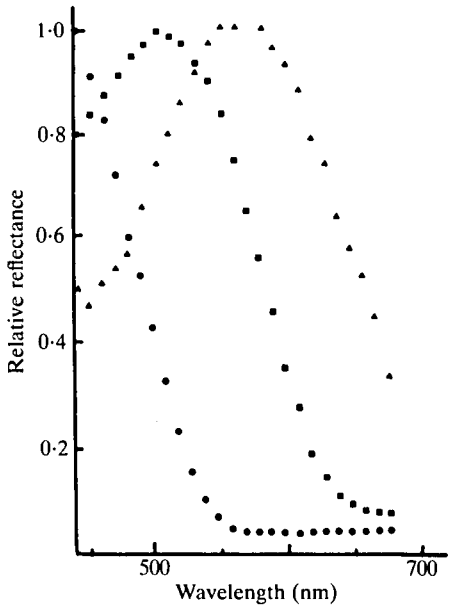

Fig. 1. Normalized spectral reflectance curves from the fully light-adapted iridescent lateral stripe of the Neon tetra perfused in Ringer solution of different strengths. (●) Double strength; (■) normal strength; (▲) half-strength Ringer solution.

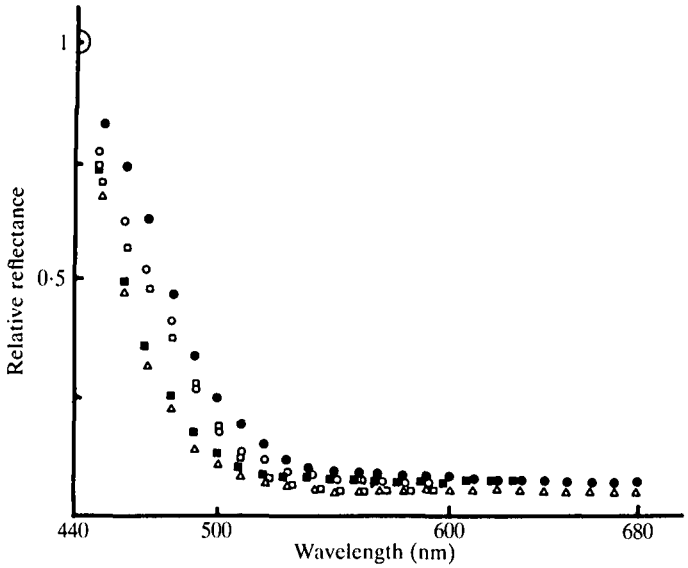

Fig. 2. Normalized spectral reflectance curves from strips of dark-adapted lateral stripe. Each curve is from a different fish and differences between the curves may not be significant. (■) Normal Ringer; (●) double-strength (hyperosmotic Ringer); (□) 200 mM-NaCl + normal Ringer; (○) 500 mM-NaCl; 500 mM-choline chloride.

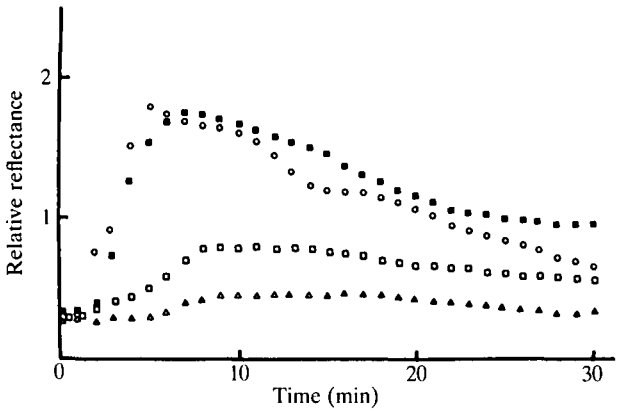

Fig. 3. Change in relative spectral reflectance at 570 nm of the iridescent lateral stripe perfused in various solutions and dark adapted for 60 min; continuous light began at 0 min. Symbols as in Fig. 2.

Fig. 3. The course of the colour change in normal Ringer solution following illumination is typical of that reported by Lythgoe & Shand (1982). There is a short initial period of about 60 s when there is no colour change and then a rapid rise to the maximum value, followed by a reduction in reflectance until a steady state is reached that is greater than the dark-adapted value. The substitution of 500 mM-NaCl for normal Ringer solution had little effect on the course of the light-induced reflectance change. However, the light response was significantly reduced if the perfusing fluid was made hypertonic by the addition of 200 mM-NaCl to the normal Ringer solution. Sodium-free Ringer solution made by substituting equimolar choline chloride for NaCl almost abolished the light response, which makes it seem likely that sodium ions are necessary for the light reaction to take place.

Discussion

It is helpful that the physical laws governing the constructive interference of light from multilayer reflectors are known and it is thus possible to understand how changes in the thickness of the reflecting layers affect the colour of the light that is reflected. In some situations the relationship between the refractive index and thickness of the reflecting layers is simple (see, e.g., eqn (1), below); but often the computations involved are so laborious that a computer is practically essential. Huxley (1968) has given a mathematical method for calculating the spectral reflectance of a regular stack of very thin transparent plates in biological tissues and it is his equations that have been incorporated into a computer program written for the BBC B computer (Clothier, 1986). The program can compute the spectral reflectance of a stack of plates of known thickness and refractive index, given the angle of incidence of the light, the plane of polarization of the incident light (required if the angle of incidence is other than normal), and the refractive index of the upper and lower boundary surfaces. In this study our chief concern was to investigate the effect of changing the thickness of the layers of low refractive index on the spectral reflectance of the whole plate. There is a simple formula for computing the wavelength of maximum spectral reflectance when the angle of incidence of the light is normal to the plane of the reflecting plates. This is:

$$\lambda = 2(n_a . d_a + n_b . d_b) , \qquad (1)$$

where n_a and n_b are the refractive index of the low- and high-refractive index plates, respectively, and d_a and d_b are the thicknesses of the low- and high-refractive index plates. For this, and other, useful formulae for calculating the reflectance of regular stacks of thin plates see Land (1972).

The exact thickness of the guanine plates has not been established. Land (1972) reported the thickness of the guanine plates in the blue-reflecting iridophores in Neon tetra iris to be 64 nm, on the basis of light-interference microscopy. Transmission electron microscopy (TEM) shows no plates of this thickness, although very thin plates estimated at being between 5 and 10 nm are visible (Lythgoe & Shand, 1982). Very thin plates have been noted in TEM pictures of the motile iridophore system of the blue Damselfish by Oshima *et al.* (1985). These authors considered the plates to be so thin that they treated each as a simple

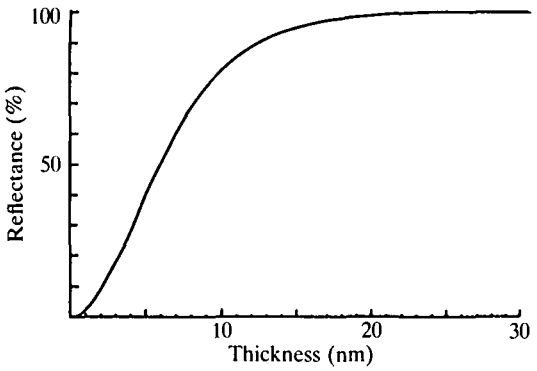

Fig. 4. Computed reflectance at 505 nm (the wavelength of maximum reflection) of a regular stack of 20 guanine and cytoplasm plate-pairs. The thickness of the guanine plates is indicated. The refractive index of the guanine and cytoplasm layers was 1·83 and 1·33, respectively. The thickness of the cytoplasm layers can be calculated from equation (1).

interface. Land (1972) used Huxley's model to show that the percentage reflectance of a multilayer system falls as the ratio of the thickness of the high- to the low-refractive index layers is reduced. The situation is illustrated for the Neon tetra in Fig. 4. It is evident that a system such as that envisaged by Oshima *et al.* (1985) would reflect no light, and a system of 20 guanine plates each 6 nm thick reflects 50 % of the light at the optimum wavelength for reflection. When the guanine plates are 10 nm thick the system reflects 80 % of the light.

If, as we think likely, the change in spectral reflectance of the iridophore is a result of the change in thickness of the cytoplasm layers of low refractive index, it is of interest to compute the change in thickness that would result in the observed shift in the wavelength of maximum reflectance. In Fig. 1 the normalized spectral reflectance curves of three light-adapted strips of skin perfused by hypotonic, normal and hypertonic Ringer are shown. Their spectral reflectance maxima are at about 440 nm, 505 nm and 560 nm. If the thickness of the guanine plates is considered to be 10 nm, the thickness of the cytoplasm plates and the percentage reflectance can be computed. The results are given in Table 1.

The measured spectral reflectance curves are broader than the curves computed for the situation that might pertain in the Neon tetra iridophore as set out in Table 1, for 505 nm (Fig. 5). The computed curve is for a situation where the dimensions of the layers are exactly regular and the angle of incidence of the light to the multilayer stack is precisely at normal incidence. The field of measurement includes approximately 50 iridophores, each of which has slightly different reflectance properties, and it is their average that was measured. Also, no account has been taken of the

Table 1. *Effect of altering the thickness of the cytoplasm layers on the reflectance of a guanine/cytoplasm non-ideal reflecting multilayer*

	Wavelength of maximum reflectance (nm)		
	440	505	560
Number of plates	20	20	20
Refractive index of guanine plates	1·83	1·83	1·83
Thickness of plates (nm)	10	10	10
Refractive index of cytoplasm	1·33	1·33	1·33
Thickness of cytoplasm layers (nm)	151·7	176·1	196·8
Light reflected (%)	86·8	80·6	75·1

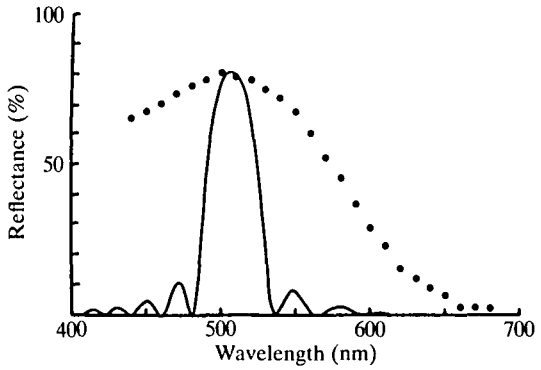

Fig. 5. Measured reflectance of light-adapted lateral stripe compared with the computed spectral reflectance of the guanine/cytoplasm multilayer stack that reflects maximally at 505 nm (see Table 1). The measured curve is the same as the curve for normal Ringer solution in Fig. 1, but has been scaled to give the same percentage reflectance at 505 nm as the computed curve. (●) Measured reflectance; (——) computed reflectance.

plasma membrane and any other layers that may differ slightly from the cytoplasm in refractive index. For these reasons it is to be expected that the measured spectral reflectance curve will be broader than the computed curve.

It is possible that the colour-change response in iridophores may involve changes in the sodium permeability of the plasma membrane similar to those that occur in rod outer segments (ROS) when they are activated by light. Korenbrot & Cone (1972) have shown that when frog ROS are exposed to osmotic shock by immersing them in NaCl of 3·5 times normal strength, the inter-disk spaces are reduced in volume and the ROS is reduced to 69 % of its original length. In the light the length remains at the 69 % level, but in darkness the length recovers to 83 % of its former level as the solute flows in and its concentration inside and outside the cell tends to equilibrate. The effect is specific to sodium and the explanation for the different behaviour in light and darkness is that in light sodium

channels in the plasma membrane are closed, whereas in darkness they are open.

Light causes the hyperpolarization of ROS (Tomita, 1970; Penn & Hagins, 1972), but depolarization in *Limulus* retinular cells, probably because light results in the opening rather than the closing of sodium channels in the plasma membrane (Hartline *et al.* 1952). The experiments reported here suggest that in the Neon tetra iridophores light permits the transport of sodium into the spaces between the guanine plates, and the water following it in causes an increase in volume of the inter-plate cytoplasm layers. This results in an increase in the wavelengths that are reflected and the iridophore changes from ultraviolet to green.

Under physiological conditions the dark-adapted iridophore has a maximum reflectance at 440 nm or less, and when light-adapted the reflectance shifts to approximately 520 nm (Lythgoe & Shand, 1982). A reasonable value for the thickness of the guanine plates is about 10 nm (Lythgoe & Shand, 1980), and the refractive index of the cytoplasm and guanine and cytoplasm plates is 1·33 and 1·83, respectively (Land, 1972). Provided there is no change in the refractive index of either the guanine or cytoplasm layers, and the thickness of the guanine plates does not change, it can be calculated from equation (1) that the thickness of the cytoplasm layer in the dark-adapted state is about 152 nm and in the light-adapted state about 182 nm. Supposing it is legitimate to allow for a 20·3 % volume change in the thickness of the cytoplasm plates, which is a similar amount to that found by Korenbrot & Cone (1972) for the inter-disk layers in ROS in hypertonic media in the dark compared to that in the light (from 69 % to 83 % of the original isosmotic level), one would expect a change in thickness of the cytoplasm plates from 144 nm when dark-adapted to 182 nm when light-adapted. This corresponds to a change in the wavelength of maximum reflectance from a dark-adapted 420 nm to a light-adapted 522 nm.

Rohrlich (1974) and Oshima *et al.* (1985) have noted the presence of microfilaments and microtubules in the cytoplasm layers and indicate that these may be involved in changes in their thickness. Oshima *et al.* (1985) quoted preliminary experiments that show that colchicine effectively inhibits the norepinephrine colour responses in Blue damselfish iridophores, and it is possible that they form part of the mechanism that controls the change in thickness of the cytoplasm layers or maintains the regularity of the multilayer stack whilst the cytoplasm layers are changing in thickness.

Colour change in the teleost iridophore may also be controlled by the sympathetic adrenergic system. The Blue damselfish *Chrysipter cyanea* is normally a beautiful cobalt blue colour, but when aroused it can darken to violet or fade to yellowish green (Oshima *et al.* 1985). Kasukawa *et al.* (1986) found that nervous stimuli and adrenergic agonists caused the iridiophores of the damselfish to change colour and the colouring response could be antagonized by alpha adrenolytic agents. The iridophores of the Killiefish and the Neon may also be under the control of the sympathetic nervous system as well as being directly activated by light. Over 50 years ago, Foster (1933, 1937) found that pituitrin caused a reddening of the iridophores in the Killiefish, and Clothier (1986) found that in the Neon tetra adrenergic agonists caused the iridophores to change from blue or violet to green or red. It is interesting to speculate that the receptors for both the light and the adrenergic mechanisms are located in the iridophore itself and indeed in similar rhodopsin-like molecules. It has recently been shown that the adrenergic receptor molecule is structurally homologous to rhodopsin (Dixon *et al.* 1986) and the effector mechanisms that follow the activation of the primary rhodopsin-like molecule are similar in both (Benovic *et al.* 1986).

References

Benovic, J. L., Mayor, F. Jr, Somers, R. L., Caron, M. G. & Lefkowitz, R. J. (1986). Light-dependent phosphorylation of rhodopsin by B-adrenergic receptor kinase. *Nature, Lond.* **322**, 869–872.

Clothier, J. R. (1986). Light-induced colour changes in a teleost iridophore. PhD. thesis, University of Bristol, UK.

Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1986). *Data for Biochemical Research.* Oxford: Clarendon Press.

Dixon, R. A. F., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohlman, H. G., Frielle, T., Bolanowski, M. A., Bennett, C. D., Rands, E., Diehl, R. E., Mumford, R. A., Slater, E. E., Sigal, I. S., Caron, M. G., Lefkowitz, R. J. & Strader, C. D. (1986). Searching of the gene and cDNA for mammalian adrenergic receptor and homology with rhodopsin. *Nature, Lond.* **321**, 75–79.

Foster, K. W. (1933). Color changes in *Fundulus* with special reference to the color changes of the iridosomes. *Proc. natn. Acad. Sci. U.S.A.* **19**, 535–540.

Foster, K. W. (1937). The blue phase in the color changes of fish with special reference to the role of guanin deposits in the skin of *Fundulus heteroclitus*. *J. exp. Biol.* **77**, 169–214.

Hartline, H. K., Wagner, H. G. & MacNichol, E. F. (1952). The peripheral origin of nervous activity in the visual system. *Cold Spring Harb. Symp. quant. Biol.* **17**, 125–141.

Huxley, A. F. (1968). A theoretical treatment of the reflexion of light by multilayer structures. *J. exp. Biol.* **48**, 227–245.

Kasukawa, H., Oshima, N. & Fujii, R. (1986). Control of chromatophore movements in dermal chromatic units of blue damselfish. II. The motile iridophore. *Comp. Biochem. Physiol.* **83C**, 1–7.

KORENBROT, J. A. & CONE, R. A. (1972). Dark ionic flux and the effects of light in isolated rod outer segments. *J. gen. Physiol.* **60**, 20–45.

LAND, M. F. (1972). The physics and biology of animal reflectors. In *Prog. Biophys. molec. Biol.* (ed. J. A. V. Butler & D. Noble), vol. 24, pp. 75–106. Oxford: Pergamon Press.

LYTHGOE, J. N. & SHAND, J. (1981). Diel colour changes in the neon tetra *Paracheirodon innesi*. *Env. Biol. Fish.* **8**, 249–254.

LYTHGOE, J. N. & SHAND, J. (1982). Changes in spectral reflexions from the iridophores of the neon tetra. *J. Physiol.* **325**, 23–34.

LYTHGOE, J. N., SHAND, J. & FOSTER, R. G. (1984). Visual pigment in fish iridocytes. *Nature, Lond.* **308**, 83–84.

OSHIMA, N., SATO, M., KUMAZAWA, T., OKEDA, N., KASUKAWA, H. & FUJII, R. (1985). Motile iridophores play the leading role in damselfish coloration. In *Pigment Cell 1985 – Biological, Molecular and Clinical Aspects of Pigmentation* (ed. J. Bagnara, S. N. Klaus, E. Paul & M. Scharti), pp. 241–246. Tokyo: University of Tokyo Press.

PENN, R. D. & HAGINS, W. A. (1972). Kinetics of the photocurrent of retinal rods. *Biophys. J.* **12**, 1073–1094.

ROHRLICH, S. T. (1974). Fine structure demonstration of ordered arrays of cytoplasmic filaments in vertebrate iridophores. *J. Cell Biol.* **62**, 295–304.

STRYER, L. (1987). The molecules of visual excitation. *Scient. Am.* **257**, 32–40.

TOMITA, T. (1970). Electrical activity of vertebrate photoreceptors. *Q. Rev. Biophys.* **3**, 179–222.

VIGH, B., VIGH-TEICHMANN, I., ROHLICH, P. & AROS, B. (1982). Immunoreactive opsin in the pineal organ of reptiles and birds. *Z. Zellforsch. mikrosk. Anat.* **96**, 113–129.

WYSZECKI, G. & STILES, W. S. (1967). *Colour Science*. New York: John Wiley.

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