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The Eyes of Deep-Sea Fish I: Lens Pigmentation, Tapeta and Visual Pigments

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Abstract—Deep-sea fish, defined as those living below 200 m, inhabit a most unusual photic environment, being exposed to two sources of visible radiation; very dim downwelling sunlight and bioluminescence, both of which are, in most cases, maximal at wavelengths around 450–500 nm. This paper summarises the reflective properties of the ocular tapeta often found in these animals, the pigmentation of their lenses and the absorption characteristics of their visual pigments. Deep-sea tapeta usually appear blue to the human observer, reflecting mainly shortwave radiation. However, reflection in other parts of the spectrum is not uncommon and uneven tapetal distribution across the retina is widespread. Perhaps surprisingly, given the fact that they live in a photon limited environment, the lenses of some deep-sea teleosts are bright yellow, absorbing much of the shortwave part of the spectrum. Such lenses contain a variety of biochemically distinct pigments which most likely serve to enhance the visibility of bioluminescent signals. Of the 195 different visual pigments characterised by either detergent extract or microspectrophotometry in the retinae of deep-sea fishes, *ca.* 87% have peak absorbances within the range 468–494 nm. Modelling shows that this is most likely an adaptation for the detection of bioluminescence. Around 13% of deep-sea fish have retinae containing more than one visual pigment. Of these, we highlight three genera of stomiid dragonfishes, which uniquely produce far red bioluminescence from suborbital photophores. Using a combination of longwave-shifted visual pigments and in one species (*Malacosteus niger*) a chlorophyll-related photosensitizer, these fish have evolved extreme red sensitivity enabling them to see their own bioluminescence and giving them a private spectral waveband invisible to other inhabitants of the deep-ocean. © 1998 Elsevier Science Ltd. All rights reserved

1. INTRODUCTION

Around 70% of the Earth's surface is covered by the sea. A small proportion of this (< 10%), covers the continental shelf, beyond which the ocean floor rapidly drops away before gradually levelling off into the abyssal plane at 4000–6000 m. The deep-sea can most easily be defined as that part of the ocean beyond the edge of the continental shelf, giving it an upper limit of around 200 m, which is also approximately the maximum depth to which sufficient light penetrates to allow photosynthesis. The deep-ocean is therefore by far the largest single environment on the planet, covering over 60% of the Earth's surface. This statistic becomes even more impressive when one remembers it is a three dimensional environment with an average depth in excess of 3000 m and a total volume of over 1.3×10^9 km^3.

Not surprisingly therefore, the deep-sea is home to the world's most abundant vertebrate genus; *Cyclothone*, and is also arguably the world's most diverse habitat in terms of the composition of its fauna (Angel, 1996). Despite this, it is still the least understood environment on Earth. In some ways it is also the most hostile, with pressures in excess of 1000 times atmospheric pressure at its deepest point and temperatures rarely above 4°C. Similarly, it is often said to be an environment of perpetual darkness. This, however, is quite clearly not the case as many of the 2500 species of fish inhabiting these depths have well developed and fully functional eyes. Furthermore, the presence of light in the deep-ocean is evident to anyone who has visited this environment in a submersible to witness the often dramatic bioluminescent displays produced by most of the animals inhabiting this region.

The deep-sea light environment, however, is very different to that experienced by most other animals. There are in fact two sources of illumination in the deep-sea; residual sunlight and bioluminescence. Downwelling sunlight is rapidly attenuated with depth until, even in ideal conditions, at around 1000 m insufficient light penetrates to allow vision in even the most sensitive fish (Denton, 1990). Usually, however, sunlight becomes visually irrelevant at shallower depths; the precise limit depending on the body of water, latitude and time of day (see Section 4.2.2.1.1). Not only is the intensity of this downwelling light reduced as one descends the water column, its spectral composition also becomes increasingly restricted. Due to spectral filtering by the water, at depth light primarily consists of a narrow band of radiation between 470 and 480 nm (Kampa, 1970; Jerlov, 1976; Kirk, 1983), although enough ultraviolet (UV) penetrates to allow some crustaceans living at depths of up to 600 m to be sensitive to these wavelengths (Frank and Widder, 1996; Section 5.1).

The second source of light in the deep-sea is the bioluminescence produced by the animals themselves, whose peak emission is usually, but not always (see Section 4.2.2.1.2) close to the same wavelengths as the remaining sunlight (Herring, 1983; Widder *et al.*, 1983; Latz *et al.*, 1988; Mensinger and Case, 1990, 1997). Although such bioluminescence is rare in terrestrial and fresh water organisms (Herring, 1996), in the deep-sea it is the norm, with over 80% of deep-oceanic species having the ability to produce their own light. These emissions serve a variety of functions including; intra and inter-specific signalling, counterillumination camouflage (see Section 2.3), a means of startling predators, an attractant to prey, and a way of simply illuminating their darkened world (Herring, 1996). In the upper reaches of the water column both low intensity residual sunlight and bioluminescence will be available for vision. Deeper, where no sunlight penetrates, the light produced by the animals themselves is the only source of illumination.

The eyes of the fish living in this unique visual environment have been the subject of much research. The purpose of this, and the following paper (Wagner *et al.*, 1998) is to summarise developments in the field of deep-sea fish ocular anatomy, biochemistry and physiology since the last major review of this topic (Locket, 1977). Here we will concentrate on the spectral properties of the visual system. The most appropriate way of specifying the spectral response of any animal is to perform either electrophysiological recordings from various cells within its visual pathway, or preferably to undertake some form of behavioural analysis of spectral sensitivity and/or colour discrimination. Unfortunately, catching deep-sea fish, using either mid-water (Roe and Shale, 1979) or bottom (Merrett and Marshall, 1981) trawls, often from considerable depths, takes many hours. The combination of physical abrasion and the changes in pressure and temperature experienced during capture, usually results in animals arriving on board ship either dead, or at best moribund. Even the rare individuals that are caught in relatively good condition are difficult to maintain alive due to problems in replicating the animal's normal environment. Not surprisingly therefore, electrophysiological recordings (ERGs) have only been successfully performed on one species of deep-sea fish (O'Day and Fernandez, 1976) and no psychophysical observations have so far been possible. In the absence of such data one can only assess the spectral response properties of these animals indirectly.

The spectral information available to an animal's visual system depends both on the wavelengths of the photons reaching its photoreceptors and the visual pigments contained within them. The former is governed by the chromatic stimuli present in the environment and by the degree to which light from these sources is modified through intraocular filters before being absorbed by the visual pigments. We therefore begin by outlining how environmental visual stimuli incident on the cornea can be altered by photostable pigments contained within the lenses of many species and by the spectrally selective properties of reflective tapeta lying behind the photoreceptors. We then summarise current knowledge of the visual pigments of deep-sea fish, highlighting members of the family stomiidae, whose visual systems have become modified to perceive their own far red bioluminescence.

2. LENS PIGMENTATION

The lens is primarily thought of as a transparent refractive device that, along with the cornea, focuses an image on the retina. However, in many animals it also contains a variety of shortwave absorbing pigments and therefore performs a secondary function, serving as a wavelength-selective filter. Such filters are common in a number of structures in the eyes of both vertebrates and invertebrates (Douglas and Marshall, in press for review). In vertebrates they are usually associated with animals living in comparatively high light levels and are thought, among other things, to be involved in protecting the retina by both absorbing those wavelengths most likely to cause damage and by acting as free radical scavengers. Such filters might also serve to increase image quality by decreasing both chromatic aberration and certain forms of scatter (Douglas and Marshall, in press).

While shortwave absorbing filters are relatively common in the eyes of both shallow living fish (Heinermann, 1984; Douglas and McGuigan, 1989; Thorpe *et al.*, 1993 for reviews) and terrestrial animals (Douglas and Marshall, in press for review), it is surprising to also find them in the eyes of some deep-sea teleosts (Denton, 1956; Somiya and Tamura, 1971; Muntz, 1976, 1983; Somiya, 1976, 1979, 1982; McFall-Ngai *et al.*, 1986, 1988; Yu *et al.*, 1991; Douglas and Thorpe, 1992; Thorpe *et al.*, 1992; Douglas *et al.*, 1995) and cephalopods (Denton and Warren, 1968; Muntz, 1976; Douglas and Marshall, in press), since they inevitably decrease both the spectral bandwidth and the intensity of the already restricted retinal illumination, removing up to 80% of all downwelling light (Douglas and Thorpe, 1992). Such filters must therefore confer a significant adaptive advantage, since they decrease overall sensitivity in species for whom maximising photon capture would seem to be at a premium.

2.1. Distribution of Shortwave Absorbing Lens Pigments and their Effect on Lens Transmission

The lenses of the majority of the over 150 species of deep-sea fish examined to date contain no detectable amounts of shortwave absorbing pigment and hence transmit short wavelengths well (Douglas and Thorpe, 1992; Douglas *et al.*, 1995 for reviews). However, even in the absence of such filters, no ocular structure will transmit significant amounts of radiation below about 310 nm due to absorption by its nucleic acids and various structural protein components, particularly aromatic amino acids. In such unpigmented lenses the decline in transmission in the UV is smooth and the "cut-off" wavelength (defined here as the wavelength of 50% transmission) is generally between 310–350 nm (Fig. 1, curve a). Variation between individuals with such unpigmented lenses is due largely to differences in lens diameter; larger lenses absorbing a greater proportion of the shortwave radiation, and to a lesser extent on the species; probably reflecting differences in the structural lens proteins (O'Rourke, 1974).

However, 27 species of mesopelagic fish (Douglas and Thorpe, 1992; Douglas *et al.*, 1995 for reviews) and several midwater cephalopods (Douglas and Marshall, in press for review), have a variety of shortwave absorbing pigments within their lenses, resulting in a range of absorption characteristics in the UV/blue part of the spectrum. Such lenses either have transmission spectra which, in contrast to the unpigmented lenses described above, do not have a smooth cut-off in the UV (Fig. 1, curve b), or have a wavelength of 50% transmission above 400 nm (Fig. 1, curve c).

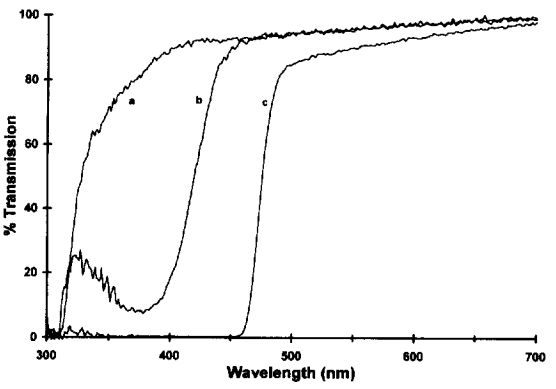

Fig. 1. Transmission spectra of intact lenses (a) *Opisthoproctus soleatus* (diameter 7.9 mm), (b) *Aristostomias tittmanni* (2.6 mm), (c) *Scopelarchus analis* (4.8 mm). All data are from Douglas (unpubl).

The latter group of lenses appear visibly yellow (Fig. 6b). While such pigmented lenses are relatively common in mesopelagic animals living in the upper 1000 m of the water column (Douglas and Thorpe, 1992), they are not found in demersal species living below this depth (Douglas *et al.*, 1995).

Age-related changes in lens transmission occur in most vertebrates (Douglas and Marshall, in press for review), including shallow water fish (Douglas, 1989; Thorpe and Douglas, 1993). Some age-related change is inevitable in all species as the vertebrate lens grows continually throughout life. Thus, the pathlength the light has to traverse will increase with age, leading to a reduction in shortwave transmission in older animals. In addition to this, in pigmented lenses age-related changes in the rate and type of pigment production will lead to further changes in the transmission characteristics of the lens. Not surprisingly therefore, age-related transmission changes have been reported in the pigmented lenses of several mesopelagic teleosts (Muntz, 1983; McFall-Ngai *et al.*, 1986, 1988; Yu *et al.*, 1991; Douglas and Thorpe, 1992) with larger lenses removing significantly more shortwave radiation than younger smaller ones (e.g. Figure 2). While exposure to shortwave radiation, and in particular UV, has been implicated in causing, for example, at least part of the age-related "yellowing" of the human lens (e.g. Andley and Clark, 1989; Lerman, 1980; Lerman *et al.*, 1985; Zigman, 1983, 1985), and the formation of some shallow water fish lens pigments (Zigman, 1971; Villermet and Weale, 1972; Zigman and Gilbert, 1978), this is unlikely to be the case in mesopelagic species. Although physiologically significant amounts of UV do penetrate up to 600 mm in clear tropical waters (Frank and Widder, 1996), this is unlikely to be sufficient to affect the lens.

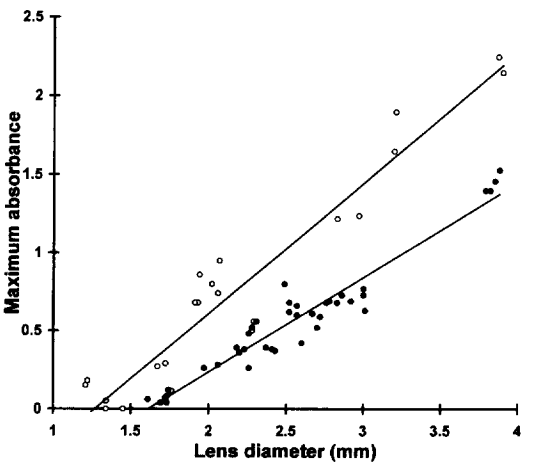

Fig. 2. Peak absorbance of lens carotenoid pigments as a function of lens diameter in *Argyropelecus sladeni* (solid circles, maximum absorbance 454–459 nm) and *A. affinis* (open circles, maximal absorbance at 429–438 nm) (from Douglas and Thorpe, 1992).

Such ageing, as in *Stylephorus chordatus* (Douglas and Thorpe, 1992; Thorpe *et al.*, 1992), often appears to be the result of simple pigment accumulation. However in other species there might also be qualitative changes in pigment type. The absorption spectra of intact *Argyropelecus affinis* lenses, for instance, suggest they contain at least two different carotenoids whose relative concentrations change with age (Douglas and Thorpe, 1992). *Malacosteus niger*, a species whose unusual tapetum (see Sections 3.3 and 4.3.2.3) and visual pigments (see Section 4.3.2.3) are discussed elsewhere in this review, may show a similar age-related change in pigment composition. The absorption profile of this species' intact lens is most unusual (Fig. 3a) consisting of two maxima at 429 and 460 nm (Somiya, 1982; Muntz, 1983; Douglas and Thorpe, 1992). The relative height of these two maxima shows a degree of variability amongst individuals (Muntz, 1983), with larger lenses generally showing a relatively higher optical density at 429 nm compared to smaller and hence younger lenses (Fig. 3a,b). This suggests the *M. niger* lens contains at least two, as yet unidentified, pigments the relative concentrations of which can vary. Interestingly, visible pigmentation in the *M. niger* lens is restricted to the inner core of the lens (Douglas and Thorpe, 1992), which represents the oldest fibres, while in *Argyropelecus affinis*, for instance, pigment is restricted to the outer and hence younger layers of the lens (McFall-Ngai *et al.*, 1986; Yu *et al.*, 1991; Douglas and Thorpe, 1992). In yet other mesopelagic species the pigments are freely diffusible and therefore found throughout the lens (McFall-Ngai *et al.*, 1988; Douglas and Thorpe, 1992).

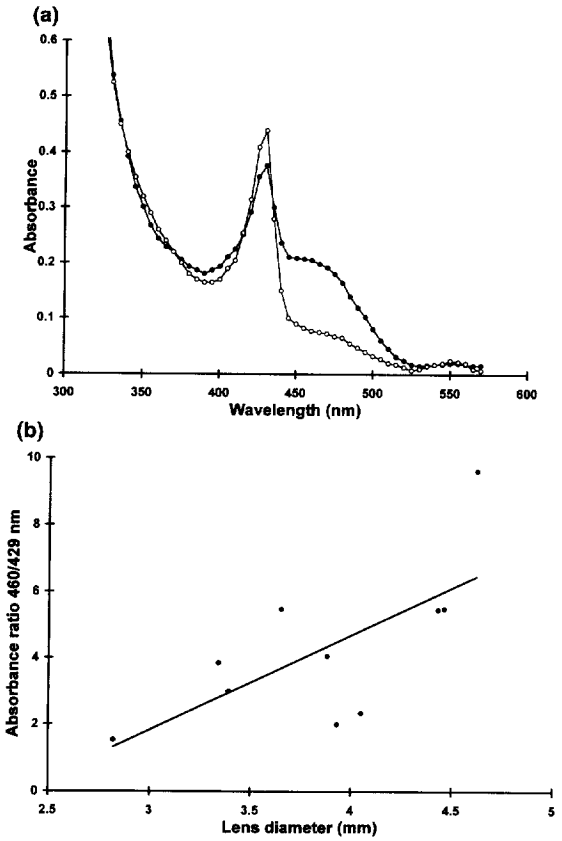

Fig. 3. (a) Absorbance spectra of two *Malacosteus niger* lenses (closed circles: diameter 3.95 mm; open circles: 4.46 mm). (b) Intact lens absorbance ratio at 429 nm and 460 nm as a function of lens diameter, demonstrating an age-related change in the relative concentrations of two lens pigments.

2.2. Identity of Lens Pigments

In shallow water fish lens pigments are usually a variety of dietary-derived mycosporine-like amino acids; palythine, which has a wavelength of maximum absorbance (λ_{max}) at 320 nm, asterina (λ_{max} 330 nm), palythinol (λ_{max} 332 nm), and palythene (λ_{max} 360 nm) (Dunlap *et al.*, 1989; Thorpe *et al.*, 1993) or, as in several mammals (Douglas and Marshall, in press for review), a number of tryptophan-derived kynurenine pigments with an absorbance maximum in the near UV around 365–375 nm (Truscott *et al.*, 1992; Thorpe *et al.*, 1993). Both of these pigment groups are also found in the lenses of deep-sea fish (Douglas and Thorpe, 1992; Thorpe *et al.*, 1992), along with carotenoid pigments (McFall-Ngai *et al.*, 1986; Yu *et al.*, 1991; Douglas and Thorpe, 1992) end a number of, as yet, unidentified pigments (λ_{max} values 325–412 nm; Douglas and Thorpe, 1992 for review) (Fig. 4). While the mycosporine-like amino acids and carotenoids almost certainly have a dietary origin, kynurenine is most probably derived within the lens from the catabolism of tryptophan in a manner analogous to the human lens (van Heyningen, 1973). In all, a total of at least 11 different shortwave absorbing lens pigments have been identified in mesopelagic fish.

All of these pigments, although chemically quite distinct, result in lenses absorbing short wavelengths and thus removing significant amounts of the UV/blue part of the spectrum impinging on the eye and are therefore probably functionally equivalent. The great diversity of lens pigmentation in the deep-sea suggests this trait has evolved separately on a number of occasions and is a clear testament to its functional importance.

2.3. Function of Shortwave Absorbing Lens Pigments

Given the low level of illumination in the deep-sea and the high degree of convergence in the retinae of most deep-sea fish (Wagner *et al.*, 1998), it is unlikely that shortwave absorbing pigments in the lenses of these species serve either to protect the retina from excessive shortwave radiation or to enhance the quality of the image, the functions they most likely perform in shallow water and terrestrial species (Douglas and Marshall, in press). Although a number of other uses have been proposed for mesopelagic lens pigments (McFall-Ngai *et al.*, 1986, 1988; Douglas and Thorpe, 1992; Douglas *et al.*, 1995), their most likely function is to enhance the visibility of bioluminescent signals.

In the mesopelagic zone (200—1000 m) both bioluminescence and downwelling sunlight may be present (see Section 1). Although the wavelength of peak emission of most bioluminescent photophores lies between 450–500 nm, matching the wavelengths that most readily penetrate the water column, there are several exceptions to this and peak emissions at longer wavelengths are not

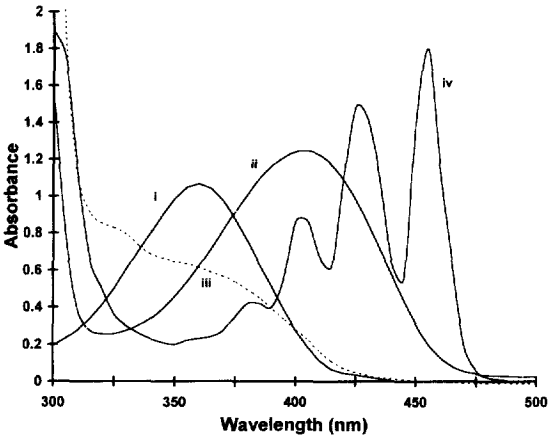

Fig. 4. Absorbance spectra of pigments extracted from the lenses of: (i) *Stylephorus chordatus* (λ_{max} 365 nm, identified as kynurenine, Thorpe *et al.*, 1992), (ii) *Scopelarchus analis* (λ_{max} 403.5 nm, unidentified, Douglas and Marshall, in press), (iii) *Aristostomias tittmanni* (λ_{max} values *ca.* 320 nm and 360 nm, probably the mycosporine-like amino acids palythine and palythene, dotted line, Douglas unpubl), (iv) Intact lens of *Argyropelecus sladeni* displaying a profile typical of a carotenoid (Douglas and Thorpe, 1992).

uncommon (Herring 1983; Widder *et al.*, 1983, 1984). Furthermore, the emission spectra of the bioluminescence often contain more longwave radiation than the surrounding spacelight. Shortwave absorbing filters will therefore decrease the intensity of downwelling sunlight more than that of the relatively longwave rich bioluminescence, thereby enhancing the contrast of the bioluminescence against the residual sunlight and making it more visible (Somiya, 1976; Muntz, 1976, 1983; Douglas and Thorpe, 1992). The absence of significant amounts of intraocular pigmentation in the lenses of fish living deeper than 1000 m, where insufficient sunlight penetrates to provide a visible background, is consistent with such a hypothesis (Douglas *et al.*, 1995). If lens pigments had other functions, such as stabilising lens crystallins (McFall-Ngai *et al.*, 1986, 1988; Douglas and Thorpe, 1992), which are unrelated to detecting bioluminescence, one would expect to find them in species inhabiting all depths.

Bioluminescence in the deep-ocean has a variety of potential uses (Herring, 1996; Section 1). Of all the proposed functions, however, perhaps the most interesting is the counterillumination camouflage employed by many animals. A dark animal observed from below by a potential predator will inevitably cast a silhouette against the residual sunlight. Consequently, many animals have photophores on their ventral surface matching the intensity of the downwelling illumination, thereby obliterating this shadow. If there is a difference between the photophore emission spectra and the irradiance spectrum of the downwelling light, yellow lenses would effectively break this counterillumination camouflage by removing much of the background light, thereby enhancing the visibility of the bioluminescence and making the animal an easy and well illuminated target (Muntz, 1976).

3. TAPETA

3.1. Introduction and General Functional Considerations

Eyeshine is a common phenomenon in nocturnal, crepuscular or low-light habitat animals (Nicol, 1989) and is a prominent feature of the eyes of many but not all species of deepsea fish (Somiya, 1980; Nicol, 1989). It usually originates from the tapetum lucidum, a layer of reflective tissue situated beneath the photoreceptors (Walls, 1963; Best and Nicol, 1980; Nicol, 1981), positioned in such a way as to reflect light back through the photoreceptors (Denton and Nicol, 1964; Nicol, 1989). This function, coupled with its presence in animals inhabiting light limited environments, has led to the assumption that tapeta increase the sensitivity of eyes by doubling the photoreceptor path length. In a study of chondrichthian tapeta however, Denton and Nicol (1964) noted that the photoreceptors of sharks such as *Deania calceus* and *Centrophorus squamosus* and the chimera *Hydrolagus affinis* which all possess tapeta, have around half the visual pigment density of species lacking such structures. This would result in similar quantal catch in tapetal and non-tapetal animals. High visual pigment density increases the thermal noise within a photoreceptor and this is disadvantageous for eyes operating at the low-light limit of vision as signal and spontaneous noise approach each

other (Barlow, 1956; Land, 1981; see also Section 4.2.2.2.1). It may therefore be that tapeta enhance sensitivity by increasing the signal to noise ratio rather than by simply increasing the rate of quantum capture (Denton and Nicol, 1964; Muntz, 1990).

Many of the shallow water relatives of deep sharks, such as *Squalus* or *Scyliorhinus*, have occlusible tapeta in which a pigmentary layer covers more or less of the tapetum during the diurnal light cycle thereby regulating light flux within the eye (Denton and Nicol, 1964; Nicol, 1989). In the deep-sea, where light is more constant, this function may be redundant, however in-depth studies of tapeta in chondrichthians are sparse (Franz, 1913, 1934; Denton and Nicol, 1964; Best and Nicol, 1980).

Due to light scatter from the reflective surface, tapeta can drastically reduce resolution (Nicol, 1989). The extent of image degradation depends on the tapetal type (see below), the distance between photoreceptors and tapetum and the structure of the tapetum relative to the photoreceptors (Nicol, 1989). The photoreceptors of some deep-sea genera, such as *Omosudis* (Frederiksen, 1976; Locket, 1977) and *Notolepis* (Locket, 1977), have their own individual tapetal reflectors (see Section 3.4), presumably in an attempt to boost the signal and yet retain resolution. However, most animals with tapeta show a high degree of neural summation with many photoreceptors converging on fewer retinal ganglion cells (Nicol, 1989). Photoreceptors in such retinae need not remain optically isolated. Other optical mechanisms for sensitivity increase such as, optical photoreceptor coupling (Locket, 1977), large eye size and wide pupil apertures (Marshall, 1979; Land, 1981) also usually accompany the possession of tapeta.

The tapetum lucidum can be categorised according to a number of parameters such as exact anatomical position, chemical composition, fine structure and whether it reflects in a specular or diffuse manner (Walls, 1963; Nicol, 1981, 1989). Two broad anatomical tapetal types are known; those located in the choroid and those found in the retinal pigment epithelium (RPE), and examples of both types are known in deep-sea fish (Denton and Nicol, 1964; Somiya, 1980; Nicol, 1989). Walls (1963) further divided choroidal tapeta into a tapetum cellulosum, tapetum fibrosum or tapeta containing guanine, depending on the nature of the cellular material producing the reflection. The outer layer of the choroid, the argenteum, is silvery in colour in most fish and it is known to have tapetal function in a variety of shallow water fish (Best and Nicol, 1980). Choroidal tapeta in fish almost invariably employ guanine crystals arranged in parallel plates to produce specular (that is shiny) or occasionally diffuse reflection (Nicol *et al.*, 1973; Nicol, 1989 for reviews and some exceptions). Specular reflectance from choroidal tapeta is the result of constructive interference of light reflected from each crystalline layer and is often silvery or gold in colour (see Land, 1972 for a useful mechanistic review of multilayered structures).

Choroidal tapeta are more common in chondrichthyes, the only teleost examples so far known being priacanthids (Somiya, 1980), *Epigonus atherinoides* an apogonid (Somiya, 1980), *Rurettus pretiosus* a gempylid (Nicol, 1989), sturgeons (Nicol, 1989), birchirs (Nicol, 1989) and the coelacanth *Latimeria* (Locket, 1974). Of these, the marine fish generally live in deeper water (although the priacanthids are also found commonly in shallow waters) and all of the teleosts are perciforms. The crystal layers in both chondrichthian and teleost choroids are usually arranged at right angles to the incoming light, thus changing orientation from the fundus to the periphery in order to reflect light back along the path of the photoreceptor rather than into other regions of the eye (e.g. Denton and Nicol, 1964).

Teleosts more commonly possess diffuse reflecting RPE tapeta (Nicol *et al.*, 1973) in which light is scattered back over a broad angle by small particles constructed from a variety of substances such as lipid, uric acid, pteridine, melanoids and in *Malacosteus*, a deep-sea species discussed below (see also Section 4.3.2.3), carotenoid. RPE tapeta containing specular material (again usually guanine) are known for a limited variety of teleosts such as the deep-sea aulopiforms like *Scopelarchus* and in many of the myctophids (deep-sea lantern fish, Locket, 1977). In all myc-

tophids examined guanine is employed, arranged in parallel plates within the cells of the RPE (Locket, 1977). Somiya (1980) and Nicol (1989), however describe choroidal tapeta in some myctophid species, indicating some confusion worthy of further study in this family.

As well as silvery or white tapeta which reflect light over most of the spectrum, tapeta may be coloured as a result of physical phenomena, pigment or a combination of both. They may be: violet (e.g. some myctophids—Nicol, 1989), blue (e.g. *Latimeria*—Locket, 1974; Nicol, 1989), green (e.g. some myctophids) yellow/brown (e.g. catfish—Nicol *et al.*, 1973), orange (e.g. priacanthids—Wang *et al.*, 1980) or red (e.g. some parrotfish and *Malacosteus*—O'Day and Fernandez, 1974; Nicol *et al.*, 1973). Colours may be generated by interference mechanisms in tapeta containing multi-layer structures such as guanine (Land, 1972; Nicol, 1989). RPE tapeta containing uric acid, lipid or pteridine particles are often white, the particles scattering light of all wavelengths (Nicol, 1989). Such tapeta are common in benthic deep-sea fish like the gadoid *Mora moro* (Locket, 1977). The RPE tapetum of the cusk eel *Ophidion welshi*, which may be found in moderately deep water and has a variety of deep-sea relatives, contains lipid spheres of a size and refractivity which selectively reflect blue light by Mie scattering (Nicol *et al.*, 1975; Born and Wolf, 1965).

The colour of tapeta can often be related to the environment inhabited. Yellow, orange or red tapeta are frequently present in the eyes of fish living in turbid fresh water, a habitat where long wavelengths predominate due to plant matter in the water (Lythgoe, 1979). Coloured tapeta in deep-sea fish reflect at short wavelengths from UV through to green and most often with blue peak reflectance (Fig. 5). These are the principle wavelengths which penetrate deep, clear oceanic water and which are emitted as bioluminescence from deep-sea inhabitants (Sections 1 and 4.2.2.1.2) There are also many deep-sea fish possessing white, silver or golden tapeta all of which reflect efficiently over much of the spectrum. Advantages of coloured and broad band tapeta are reviewed below (Section 3.3).

3.2. Methods of Measuring Tapetal Reflectance

The colour of eyeshine in intact eyes is dependent on both the tapetal reflection and the spectral characteristics of the ocular media, such as the cornea, lens, vitreous and retina, lying between the tapetum and the entry point of light into the eye. This refluxed light is sometimes taken as a measure of tapetal reflectance. However, two main problems exist in using this approach: Firstly, light emitted from an eye has passed through each optical element twice and without knowledge of their spectral absorption, the reflection characteristics of the tapetum alone cannot be determined. Light may also be reflected directly from any of these structures before reaching the tapetum (Nicol, 1989). Individual measurements of the reflection and absorption of the optical elements of an eye are therefore desirable. This is illustrated, for instance, by the bigeye, *Priacanthus hamrur*, which has a tapetum that reflects effectively from 400–700 nm with considerable loss in efficiency below 400 nm (Fig. 5a) and would by itself therefore appear silvery. Eyeshine of the intact eye in this species is, however, golden orange (Fig. 6a, Wang *et al.*, 1980; Somiya, 1980). This coloration is clearly influenced by optical elements other than the tapetum (Fig. 5a). Similarly, the pearleye, *Scopelarchus analis*, possesses a bright yellow lens (Figs 1 and 6b; Douglas and Thorpe, 1992; Douglas and Marshall, in press) which totally dictates the colour of light emitted from the eye (Fig. 5b). The tapetum of *Rinoctes nasutus*, a deep-sea alepocephalid, on the other hand, has broad-band reflectance which extends to 300 nm (Fig. 5b). Since the cornea, lens, vitreous and retina of this species absorb only slightly below 550 nm, the reflection measured from the intact eye is little different to that of the isolated tapetum.

Clearly measuring tapetal reflectance independently of other optical components is desirable. However, for practical reasons this may not always be possible or indeed give the most accurate results, as the removal of the interfering pretapetal media may itself alter the reflection characteristics of the isolated tapetum. This is illustrated by the behaviour of the tapetum of

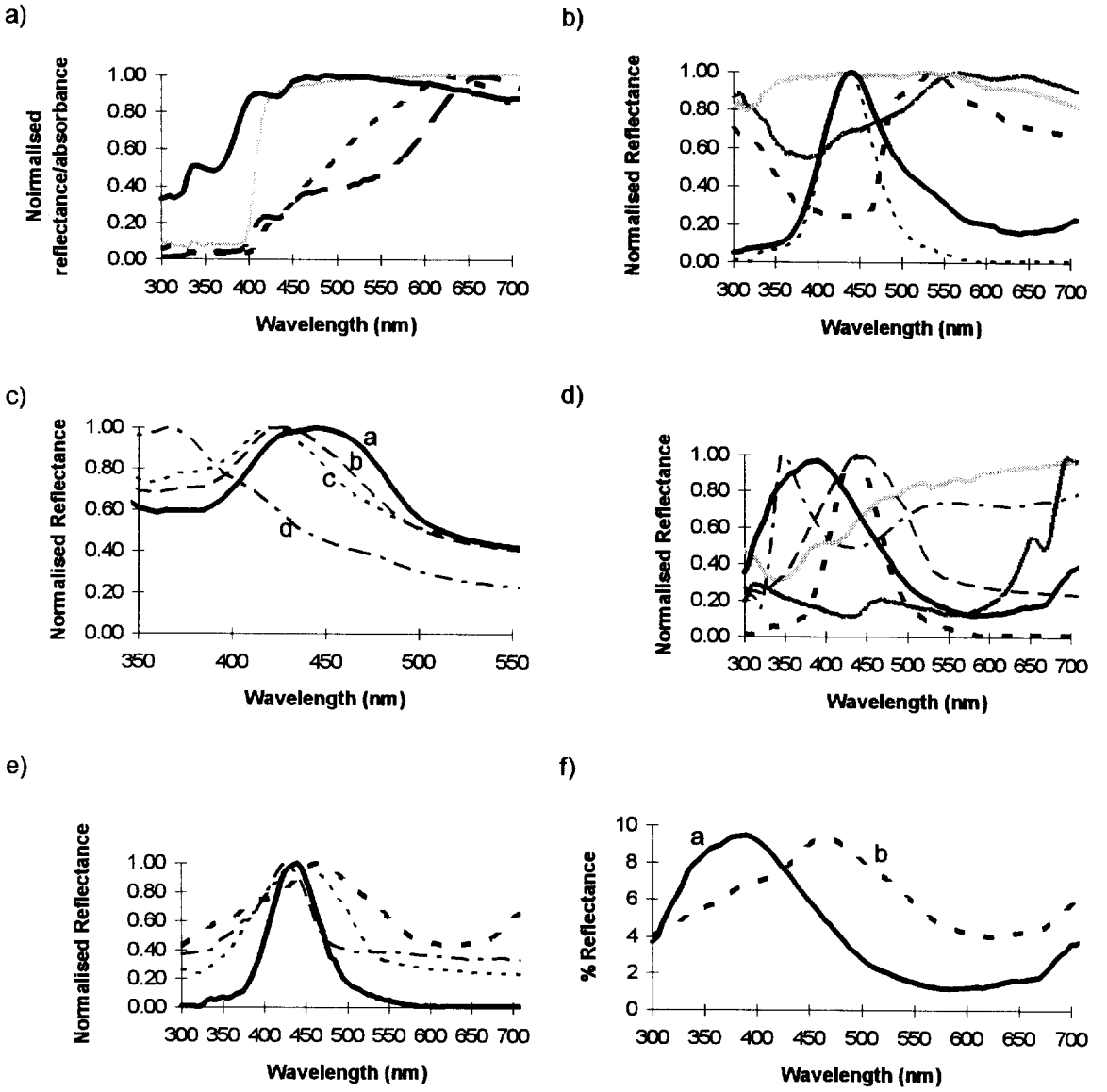

Fig. 5. (a) Normalised tapetal reflection through a variety of optical components and lens absorbance plotted on the same scale in the big-eye, *Priacanthus hamrur*. The lens (thin grey line) absorbs most light below 400 nm. Tapetum alone: solid line, light emitted from intact eye; dotted line, reflection measured with lens and cornea removed but with vitreous and retina still in place—dashed line. When lens and cornea are removed, degradation in optical quality means that reflectance is significantly reduced between 450 nm and 650 nm while viewing through vitreous and retina. (b) Thick dotted line: intact eye emission from the tube eye of *Scopelarchus analis*. The spectrum of this reflection is largely influenced by the yellow lens (Fig. 6b). Thin dotted and solid black lines: *Benthosema glaciale* "tapetal reflection" measured through the aphakic gap and with tapetum only respectively. Dark grey and light grey lines: emission through all optical elements and tapetum only of *Rinoctes nasutus* respectively. (c) Change in *Benthosema glaciale* tapetal reflectance over time (a: T = 0 min, b: T = 5 min, c: T= 10 min, d: T = 15 min) after removal of lens and cornea. Note these spectra are more diffuse than those for the same species in (b) due to disruption of the retina. (d) Tapetal reflections in a variety of deep-sea teleosts measured with eye whole or dissected as noted for each curve (and see text for details). Solid line: Dorsal tapetum in *Notoscopelus resplendens* (tapetum only). Thick dotted line: *Benthosema glaciale* (through aphakic gap). Dark grey line: *Malacosteus niger* (through aphakic gap). Light grey line: *Bathysaurus mollis* (tapetum only). Dashed line: *Diaphus rafnesquei* (intact eye through lens). Dot/dash line: *Argyropelicus aculeatus* (tapetum only). (e) Blue tapeta in a variety of myctophids: *Benthosema glaciale*—solid line, *Diaphus rafinesque*—thin dotted line, *Notoscopelus resplendens*—thick dotted line, *Diaphus dumerilii*—dot/dash line. (f) The (a) dorsal and (b) ventral tapetal regions of *Notoscopelus resplendens*. Measurements for tapetum only.

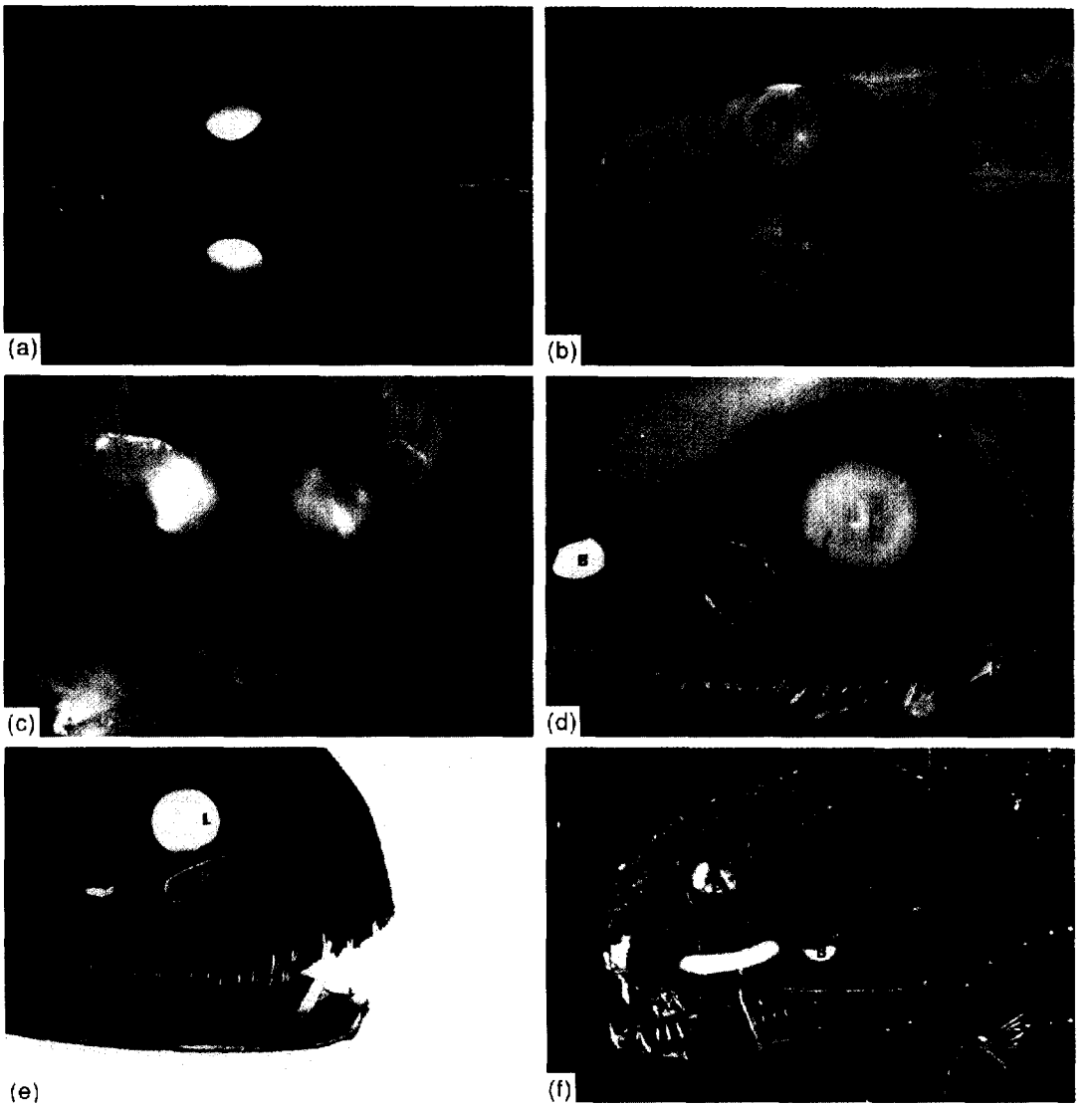

Fig. 6. (a) Orange, gold eyeshine in *Priacanthus hamrur*. (b) Dorsal view of the upward looking tube eyes of *Scopelarchus analis*. Light reaching the retina and tapetum in this species is greatly influenced by the yellow lenses (Douglas and Marshall, in press). (c) The reduced eyes of *Nybelinella*. Only retina and tapetum remains in this fish, visible through the transparent skin of the head. Each eye is shaped like a parabola, an ideal light capturer. (d) The eye and photophores of *Malacosteus niger*. The red tapetum can clearly be seen and measured through the aphakic gap in this species. L: lens, R: longwave emitting photophore, B: shortwave emitting photophore. (e) The eye and photophores of *Aristostomias grimaldii*. Labels as for (d) (courtesy Dr P.J. Herring). (f) The eye and photophores of *Pachystomias microdon*. Labels as for (d) (courtesy Dr P.J. Herring).

Benthosema glaciale, a deep-sea myctophid, following the removal of the pre-tapetal elements (Fig. 5b). The tapetum in myctophids is situated in the RPE (although see Somiya, 1980 and Nicol, 1989). It is generally an intense blue colour, and is observable and measurable from the outside of the eye both through the lens and through an aphakic aperture. This colour is disrupted by removing the other optical elements (Fig. 5b) and also changes rapidly over time to reflect shorter wavelengths post dissection (Fig. 5c). Such colour changes have been noted previously in other fish and it is suggested may be the result of a reduction in intraocular pressure

or osmotic effects (Nicol *et al.*, 1975). Myctophid tapetal colours are generated by the interference of light reflected from sequential layers of guanine crystals. This precise arrangement, coupled with the position of the crystals in the relatively easily deformed RPE (compared to choroidal specular tapeta), may make such tapeta particularly susceptible to damage. For *B. glaciale* at least, where an aphakic aperture is available to measure through and whose other optical components are relatively transparent, better results are obtained from intact eyes.

3.3. Tapetal Spectral Reflectance

The deep-sea is dominated by blue light due either to the wavelengths of light penetrating from the surface or the bioluminescent emissions from creatures living there (Section 1). At first glance it is therefore not surprising to find deep-sea fish with tapeta having maximum reflection at short wavelengths. Denton and Nicol (1964) demonstrated an 85% maximum reflective efficiency around 475 nm for two deep-sea sharks and in myctophids, for example, reflective peaks are situated between 430 nm and 480 nm (e.g. Figure 5a). As well as approximately matching the ambient light, these wavelengths coincide fairly well with the absorption maximum of the rod visual pigments of most deep-sea fish (Fig. 7; Section 4.2). Superficially therefore shortwave tapetal reflection appears well tuned to the deep-sea environment. However, such an interpretation does not account for the variability in tapetal reflection seen both within myctophids (Fig. 5; Nicol, 1989; Somiya, 1980; Locket, 1977) and other families of deep-sea fish (e.g. Figure 5a). Furthermore, a tapetum with a high reflection over a broad range of wavelengths, such as those of *R. nasutus* (Fig. 5b) or *Bathysaurus mollis* (Fig. 5d) and other benthic deep-sea fish, will always be more efficient for light capture than one reflecting only a limited part of the spectrum. Another explanation is therefore needed for the function of blue tapeta.

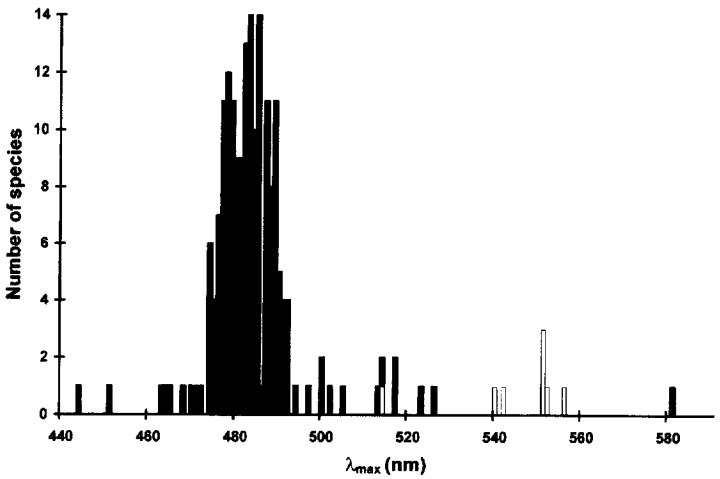

Fig. 7. Wavelength of maximum absorption (λ_{max}) for 195 visual pigments isolated from 175 species of deep-sea fish. Only those data obtained by extract spectrophotometry and microspectrophotometry have been include as these two methods give insignificantly different results (Douglas and Partridge, 1997). If pigments have been characterised by both methods the extract data have been used. Data derived from wholemount spectroscopy have not been included as this method shifts the λ_{max} towards longer wavelengths compared to the other methods (Douglas and Partridge, 1997; Douglas *et al.*, 1995), resulting in the exclusion of 8 species from the graph. If more than one author have studied a particular species we have, where possible, used our own data. The solid bars represent A_2-based visual pigments and open bars those using an A_2-based chromophore. Data were derived from the following sources; Beatty (1969), Bowmaker *et al.* (1988), Crescitelli (1989, 1991a), Crescitelli *et al.* (1985), Denton and Locket (1989), Denton and Nicol (1964), Denton and Shaw (1963), Denton and Warren (1956, 1957), Douglas and Partridge (1997, unpubl), Douglas *et al.* (1995), Fernandez (1978), Knowles and Dartnall (1977), Munz (1957, 1958), O'Day and Fernandez (1974, 1976), Partridge and Douglas (1995), Partridge *et al.* (1988, 1992), Wald *et al.* (1957).

Notoscopelus resplendens has taken the match of intraocular filter and environment one stage further. The tapetum in this myctophid is divided into a blue ventral region (470 nm peak) and a violet dorsal region (390 nm peak, Fig. 5f). It is striking that this colour distribution matches the spectral distribution of light in mid-water (Jerlov, 1976) where *N. resplendens* lives. Light from above, which would fall on the ventral tapetum, is dominated by the blue of remaining sunlight, while light scattered up from the depths, which would strike mainly the dorsal tapetum, is relatively rich in UV and short wavelengths (Shelton *et al.*, 1992). Similar matching to the environment is shown by the visual pigments of a shrimp, *Systellaspis debilis*, which inhabits similar depths in the ocean. The dorsal upward-looking photoreceptors in the compound eye of this species are dominated by blue (498 nm) photoreceptors, while the ventral downward pointing photoreceptors peak in the violet (410 nm, Gaten *et al.*, 1992, Cronin and Frank, 1996). The tapeta of other myctophids are a more uniform blue, however due to methodological problems outlined above or because specimens are often brought up damaged from the depths, subtle gradients in spectral reflectance could easily be missed.

In common with other fish, many myctophids possess a ventral, upward looking darker area where the tapetum is reduced (Nicol, 1989; and see next section) and this may also be correlated with the light distribution in the ocean. At mesopelagic depths, 200 times more light comes from above than is scattered up from below (Land, 1990; Denton, 1990) reducing the need for a tapetum in the ventral, upward looking part of the retina. Deepsea shrimps also possess tapeta morphologically matched to the light field in a similar way (Shelton *et al.*, 1992). A further function of this dark region in both shrimps and fish may be to obliterate eyeshine in an upwards direction and thus prevent detection (Denton and Nicol, 1964; Nicol, 1989; Shelton *et al.*, 1992). A blue rather than a highly silvered tapetum will also enable mesopelagic fish to remain unseen in their blue dominated environment. Myctophids show a complex array of camouflage mechanisms including ventral blue bioluminescence (Herring, 1977, 1983), highly oriented guanine reflectors in their scales (Denton, 1970) and thin or transparent bodies, all for concealment. It may be that the reflectance characteristics of tapeta are an addition to this complement of camouflage mechanisms.

Another intriguing tapetal adaptation to life in the deep-sea is the red tapetum constructed from small particles of the carotenoid astaxanthin found in the eye of *Malacosteus niger* (Denton and Herring, 1971; Somiya, 1982). This is clearly visible external to the eye through an aphakic aperture (Fig. 6d) and reflects well beyond 600 nm but most efficiently at 700 nm (Fig. 5d). This is one of several adaptations that enable this species to see its own far red bioluminescence and is discussed further (Section 4.3.2.3).

3.4. Tapeta in "Unusual" Eyes

Not all fish tapeta are uniformly distributed over the retina, and as argued above, dark regions in the ventral retina can generally be related to the environmental light field and may act as a means of camouflage (Denton and Nicol, 1964; Nicol *et al.*, 1973; Nicol, 1989). Perhaps the most interesting examples of regionalised tapeta are those that are found in fish also possessing regionally specialised retinae. The scopelarchids, for example, have upward looking tube eyes within which there are a variety of retinal subregions (Munk, 1966; Collin and Partridge, 1997). Locket (1971, 1977) describes this in some detail for *Scopelarchus*. The rostral portion of the main retina, which is situated at the bottom of the tube eye, contains single photoreceptors backed by a diffuse RPE tapetum made from randomly arranged crystals which are probably guanine (Locket, 1977). In the caudal part the rods are grouped in bunches of around 23 receptors positioned in an RPE tapetal cup made of highly aligned crystals, again most likely guanine. The tapetal cups are therefore probably specular (Locket, 1977) in the close relative *Benthalbella* where the bottom of the cup is flat and can be observed as shinny. Light entering the tapetal cup of *Scopelarchus* will be trapped and undergo multiple reflections through the outer segments within the cup. Locket (1971) suggests based on this and

other neural interconnection observations, that each group of rods functions as a highly sensitive if coarse grain macroreceptor.

An extreme example of receptor pooling is to be found in the eye of *Nybelinella*, a benthic aphyonid. In this fish the distal optical components of the eye, cornea and lens, have been discarded. All that remains of the eye is a parabolic shaped retina and highly reflective tapetum, clearly observable from the mud of a benthic trawl through the transparent skin of the head (Fig. 6c; and Peter Shelton pers. com). The parabolic eye cup shape is ideal for trapping light like the macroreceptor cups in *Scopelarchus*. However, as the retina is spread evenly over this surface, the whole eye effectively acts as a single macroreceptor. The only directionality available is left/front and right/front from the angle of view of the parabolic shape of each eye (Fig. 6c).

Notolepis rissoi, which, like *Scopelarchus*, is aulopiform, also has grouped rod photoreceptors situated in eyes which have a "normal" shape (Locket, 1977). Most of the retina is grouped into macroreceptors, each with a tapetal cup containing crystals which act as a specular reflection system. The area centralis of this species however contains single photoreceptors, which show characteristics of both rods and cones (Locket, 1977), and each is sheathed in its own tapetal cup. Although these photoreceptors are larger than those in the grouped retinal region (Locket, 1977), they are smaller than the macroreceptors and perhaps retain a relatively high acuity while also increasing sensitivity through possession of a tapetum?

Omosudis lowei, another deep-sea aulopiform, is unusual in having an almost pure cone retina. There is an RPE tapetum over much of the retina in which single cones are optically isolated in tapetal cups, much like the single rod/cones of *Notolepis* (Frederiksen, 1976; Nicol, 1989). The cells at the bottom of each cup contain flat crystals while the RPE cells, which construct the walls of the container, contain rod-shaped, more randomly oriented crystals (Nicol, 1989). The ventrotemporal retina in *Omosudis* lacks a tapetum and contains mainly double cones perhaps sensitive enough to respond to downwelling light without a tapetum (Frederiksen, 1976).

The tapetum of *Howella brodiei* is of the diffuse type, the RPE cells containing tightly packed lipid spheres which scatter light. Interestingly this tapetum is situated in the tempro-ventral region and sights through a rostral aphakic gap (itself an adaptation to increase sensitivity) (Nicol, 1989) so, with the fish upright, the photoreceptors over the tapetal area would look forward and up (Best and Nicol, 1978). *Howella* undergoes extensive vertical migrations from 30 m at night to 300–1800 m during the day. It is not known what position the fish swims in to perform this extensive journey. However one could imagine if tilted down for the daytime dive, the tapetal region would boost sensitivity for looking into the dim depths and at night the same would be true for examining the dark waters above.

Best and Nicol (1980) note a bright green reflecting region in the tempro-dorsal region of the retina in the stomiatoid *Photonectes*. Unusually this reflective surface lies on the surface of the retina and would therefore reflect light before it reached the photoreceptors. Where this reflected light ends up is unknown and though producing eyeshine, this structure may best be viewed as a filter rather than tapetum.

4. VISUAL PIGMENTS

4.1. General Structure and Physiology

The basic properties of deep-sea fish visual pigments do not differ in any significant way to those of other vertebrates. Light, after passing through the ocular media, and following reflection from a tapetum (if present), impinges on the visual pigments lying within the disc membranes of the photoreceptor outer segments. All vertebrate visual pigments consist of two components; the chromophore, an aldehyde of vitamin A, which absorbs the light, and a protein, opsin, which determines the spectral absorption characteristics of the chromophore.

The chromophore in most vertebrates is retinal, a derivative of vitamin A_1. However, some fish (including a few living in the deep-sea; Section 4.3), reptiles and amphibia possess an additional chromophore, 3,4-dehydroretinal, derived from

vitamin A_2. All visual pigments with retinal as their chromophore are known as rhodopsins, while vitamin A_2-based pigments are referred to as porphyropsins. A visual pigment consisting of a given opsin and using retinal as the chromophore, will have a narrower absorption spectrum peaking at shorter wavelengths, than a pigment composed of the same opsin bound to the A_2-derived 3,4-dehydroretinal (Bowmaker, 1991, 1995 for reviews). Due to the golden colour of extracted deep-sea fish visual pigments, Denton and Warren (1956) called them chrysopsins, a practice that has in some cases continued until recently (e.g. Crescitelli, 1991a). However, as already pointed out by Wald *et al.* (1957) (see also Munz, 1958), such naming is not very helpful and deep-sea fish visual pigments are best referred to as either rhodopsins or porphyropsin, depending on the nature of their chromophore.

All opsins have a similar structure, consisting of a chain of around 350 amino acids that cross the outer segment disc membrane seven times in the form of α-helices. Isolated retinal and 3,4-dehydroretinal absorb at *ca.* 380 and 400 nm respectively. When bound to the opsin, always via a Schiff's base linkage at lysine 296 on the seventh transmembrane helix, the amino acids "tune" the chromophore to absorb at longer wavelengths (see Section 4.2.1). Thus, the absorption spectrum of a visual pigment depends on both the identity of the chromophore and on the amino acid composition of the opsin surrounding that chromophore (Bowmaker, 1991, 1995 for reviews).

The spectral characteristics of visual pigments are usually studied either in detergent extracts of homogenised retinal tissue (Knowles and Dartnall, 1977) or by microspectrophotometry (MSP) of individual intact outer segments (Liebman, 1972; Partridge and DeGrip, 1991; Partridge *et al.*, 1988, 1989), although we have recently successfully employed a retinal wholemount technique (Douglas *et al.*, 1995; Partridge and Douglas, 1995). While MSP produces data representing visual pigment absorption directly, this is not the case for either detergent extracts or retinal wholemounts since they represent a mixture of all the accessible visual pigments within a retina as well as any other pigments and/or impurities. In order to isolate the constituent visual pigments within such mixtures, they must be subjected to partial bleaching (Knowles and Dartnall, 1977; Douglas *et al.*, 1995; e.g. Figure 13a). Such methods reveal that visual pigments have bell-shaped absorption spectra (e.g. Figure 13b) that are most easily characterised by their wavelength of maximum absorption (λ_{max}) at which the pigment is most sensitive. Of all classes of vertebrate, fish exhibit the greatest range of visual pigments with λ_{max} values from around 350 nm in the UV to 635 nm in the far red (Bowmaker, 1990, 1991, 1995 for reviews).

When dark adapted, the chromophore of a visual pigment is in the "bent" 11-cis configuration and bound to the opsin. On the absorption of a photon the chromophore "straightens out" into the all-trans conformation and separates from the opsin. Following the chromophore isomerisation, the visual pigment "bleaches" via a series of intermediate stages and triggers an enzyme cascade that ultimately results in the closure of ion channels, causing a graded receptor hyperpolarisation and changes in the release of neurotransmitter at the photoreceptor synapse (Yau, 1994 for review). This electrophysiological signal is then processed by the other neurones of the retina and visual pathway ultimately resulting in visual perception.

Most vertebrates, including shallow water fish, have two types of photoreceptor within their retinae; cones subserving high light level (photopic) vision, which allow the perception of detail and colour, and rods, which become active in lower (scotopic) levels of illumination and are adapted for optimising sensitivity. Although some deep-sea fish do possess cones (e.g. Munk, 1965, 1981, 1984; Fröhlich *et al.*, 1995), the majority have retinae containing just a single class of rod. To date, the visual pigments of 183 species of deep-sea fish have been characterised. Not surprisingly, the vast majority of these (88.5%) contain only a single visual pigment within their retinae.

4.2. Distribution of Deep-Sea λ_{max} Values

Of the 195 different pigments characterised by either detergent extract or by MSP in the retinae of deep-sea fishes, 86.7% have their λ_{max} within

the range 468–494 nm (Fig. 7). This contrasts with the majority of other vertebrates that have been examined, which have rod visual pigments with λ_{max} values close to 500 nm (Lythgoe, 1972b). This hypsochromatic shift in deep-sea visual pigments can be explained at two levels (Goldsmith, 1990, 1991); the proximate explanation (concerning the way in which shortwave sensitive rod pigments are constructed) and the ultimate explanation (outlining the adaptive significance of such visual pigments). Both will be addressed below.

4.2.1. *Clustering of λ_{max} values, amino acid sequences and tuning*

Dartnall and Lythgoe (1965) and Bridges (1965) reported the spectral clustering of vertebrate rhodopsin λ_{max} values around certain points of the spectrum. This, at the time perhaps unexpected observation, can now be interpreted in the light of what we know about how the visual pigment chromophore is "tuned" by amino acids within the opsin. A single change in an appropriately positioned amino acid can lead to a relatively large discrete shift in λ_{max}. That λ_{max} values occur at only certain points in the spectrum related to different amino acids substitutions therefore appears reasonable (Bowmaker, 1995 for review).

Since deep-sea fish visual pigments are similarly tuned by the amino acids within the opsin (see below), it is not surprising that Partridge *et al.* (1989, 1992) also noted clustering among the visual pigments of 52 such species. The present summary of the available data from all mesopelagic and demersal species studied to date (Fig. 7), also shows that visual pigments are not normally distributed and suggests λ_{max} grouping at particular points of the spectrum. However, this clustering is less well defined than that observed in previous studies. This might either be a true reflection of visual pigment distribution or it could be attributable to the large number of different studies that provided data for Fig. 7. Partridge *et al.* (1989, 1992) used only data from their own laboratory, derived using a single method (MSP). Figure 7, on the other hand, uses values taken from a large number of studies, most of which used difference spectra derived from the partial bleaching of detergent extracts to determine pigment λ_{max} although some MSP data are included. In general, the spectral characteristics of deep-sea fish visual pigments can be determined with a great deal of accuracy, using both extract spectrophotometry and MSP, due to the high total amount of visual pigment and the large size of the outer segments respectively (Partridge *et al.*, 1989; Douglas and Partridge, 1997). It is nonetheless possible that even slight variations induced by methodological differences between studies might blur the underlying clustering in Fig. 7. It is, however, equally possible that the spread in the λ_{max} data, resulting in λ_{max} values between cluster points is not artefactual and in fact reflects the effects of amino acids additional to the major tuning residues causing the stepwise shifts in λ_{max} which underlie the main cluster points. Such additional amino acid substitutions may have a lesser effect on λ_{max} tuning due, for instance, to being more distantly located from the chromophore.

As outlined in Section 4.1, the λ_{max} and spectral absorption of a visual pigment are determined both by the nature of the chromophore employed and the amino acid sequence of the opsin. All deep-sea fish with rod visual pigments having λ_{max} values below 510 nm appear to utilise retinal as their chromophore (Lythgoe, 1972b; Partridge *et al.*, 1989), and thus the hypsochromatic shift in λ_{max} compared with most vertebrate rod rhodopsins must be due to the amino acid sequence of the opsin protein. To date, only one study has specifically addressed this subject (Hope *et al.*, 1997). Partial sequences (908 nucleotide base pairs) of the rod opsin encoding genes of four species of demersal and mesopelagic deep-sea fish (*Hoplostethus mediterraneus*, *Cataetyx laticeps*, *Gonostoma elongatum*, *Histiobranchus bathybius*), which included the coding regions for all 7 transmembrane α-helices of the opsin proteins, were compared to those of shallow living teleosts and the European eel (*Anguilla anguilla*). The latter expresses two rod opsins which form visual pigments with λ_{max} values at 502 and 482 nm (Wood and Partridge, 1993; Archer *et al.*, 1995). This comparison suggests that two amino

acid sites have a critical role in the spectral tuning of deep-sea fish visual pigments. The hypsochromatically shifted deep-sea visual pigments, with λ_{max} values close to 480 nm, are based on opsins with an amide-bearing, uncharged residue (asparagine) at position 83 and a hydroxyl-bearing, polar residue (serine) at position 292. In contrast, shallow living teleosts, with visual pigments close to 500 nm, have an acidic, negatively charged residue (aspartate) at position 83 and a non-polar residue (alanine) at position 292. The same two substitutions have been proposed by Hunt *et al.* (1995) to account for the λ_{max} values, ranging from around 500 nm to about 480 nm, in the rods of different species of cottoid fish living in Lake Baikal. Amino acid substitutions at sites 83 and 292 have previously also been shown, by site-directed mutagenesis, to result in spectral shifts in artificially modified visual pigments (Nathans, 1990; Nakayama and Khorana, 1991; DeCaluwé *et al.*, 1995).

Although only two sites have been identified as being responsible for the shortwave shift in deep-sea fish λ_{max}, it is unlikely that the shortwave sensitive opsins of all deep-sea fish will be found to have these same two substitutions. At present, however, there are simply insufficient data to know whether given λ_{max} values can only be achieved in one particular way, (i.e. by employing specific amino acids at certain sites), or if a particular λ_{max} (i.e. from a certain cluster point) might be achieved in a variety of ways.

4.2.2. *Adaptive significance of λ_{max} in the majority of deep-sea fish*

Although the proximate explanation for λ_{max} tuning of deep-sea fish visual pigments has now been at least partly answered (see Section 4.2.1), its adaptive significance is less well understood, despite frequent consideration (Bayliss *et al.*, 1936; Clarke, 1936; Denton and Warren, 1957; Munz, 1958; Denton and Shaw, 1963; Dartnall and Lythgoe, 1965; Lythgoe, 1972a; Dartnall, 1975; Levine and MacNichol, 1982; Crescitelli *et al.*, 1985; Denton, 1990; Crescitelli, 1991a,b,c; Partridge *et al.*, 1988, 1989, 1992; Douglas *et al.*, 1995).

It has in fact been argued that the precise location of the λ_{max} of deep-sea fish visual pigments is of little consequence due to their relatively broad absorption spectrum resulting from their unusually high concentration within the outer segments (Munz, 1965; Crescitelli, 1991a; Bowmaker, 1995). However, in a photon limited environment such as the deep-sea, it is likely that there will be a significant adaptive advantage in any gain in visual sensitivity, and there is thus likely to be selection for visual pigments that confer the highest possible sensitivity.

In the following sections we shall first examine whether visual pigments are adapted for the detection of downwelling sunlight or bioluminescence and then examine any other factors not directly related to light, that might influence their spectral characteristics.

4.2.2.1. *Adaptation to photic environment*

The spectral composition of shallow bodies of water is highly variable due largely to differences in the quantity and identity of the substances dissolved or suspended within them (Partridge, 1990; Douglas, 1991). One thus finds, for instance, red/brown freshwater that transmits mainly longwave radiation, yellow/green coastal waters in which intermediate wavelengths dominate, and clear blue shortwave transmitting oceanic waters. Not only does the spectral content of the underwater light environment vary, so does its intensity, decreasing both with depth and in turbid waters. Consequently, fish live in almost every conceivable optical environment. For this reason, in attempts to relate the spectral properties of the pigment to the photic environment, the visual pigments of shallow living fish have been the subject of much research (Bowmaker, 1990, 1991, 1995 for reviews). In such animals the position of the visual pigment λ_{max} values can be interpreted in two ways; they can either closely match the predominant wavelengths in the background to maximise sensitivity (sensitivity hypothesis), or they might be offset from the spacelight to enhance the contrast of relatively bright objects with a different spectral radiance to the background (Lythgoe, 1966, 1972a, 1980, 1984; McFarland and Munz, 1975; Munz and McFarland, 1977).

When considering the adaptive significance of the spectral location of visual pigments within the deep-sea two sources of illumination need to be considered; downwelling residual sunlight and bioluminescence. Visual pigments might be adapted to either one or both of these types of stimuli.

4.2.2.1.1. *Maximisation of sensitivity to downwelling light*

In nearly all habitats, light from the sun is by far the most significant source of photons for vision. In the open oceans, as in many natural waters with little suspended sediment, this light is attenuated by absorption by water, chlorophyll and dissolved organic matter (DOM). As noted above, different waters, including those of the open oceans, vary in their concentrations of DOM and chlorophyll, causing wide variations in the spectral transmission characteristics. This property was utilised by Jerlov (1968, 1976) who proposed a classification of sea-water based on spectral transmission properties; open ocean water being divided into five divisible subtypes, I, IA, IB, II and III, from clearest to least transparent.

The wavelengths of maximum transmission (λ_{Tmax}) of these different waters have on occasion been aligned with the λ_{max} values of deep-sea fish visual pigments, since it was predicted over 50 years ago (Clarke, 1936; Bayliss *et al.*, 1936) that as the residual downwelling sunlight in the deep-sea is largely restricted to the shortwave part of the spectrum, fish living in this environment should also have visual pigments absorbing maximally at these wavelengths. As pointed out by Govardovskii (1976), however, the simple alignment of λ_{max} and λ_{Tmax} does not take sufficient account of the asymmetry in the absorptance spectra of visual pigments in photoreceptors, nor the spectral distribution of downwelling light. Instead, the calculation of the most sensitive visual pigment in an environment with a given spectral irradiance demands the numerical integration of these two functions with respect to wavelength. Such calculations demonstrate that the optimal λ_{max} is often significantly displaced from λ_{Tmax}, the amount of displacement depending on both the spectral irradiance distribution of the light and the spectral absorptance of the visual pigment (itself determined by the rhodopsin λ_{max}, the specific absorbance (per μm) of the visual pigment in the rod outer segment, and the length of the outer segment itself; see Partridge, 1990; Partridge and Cummings, in press).

One of the main problems with such calculations is that there are very few measurements of spectral distributions of light at mesopelagic depths. For this reason the spectral irradiance distribution has often been calculated from tabulated values of the spectral diffuse attenuation coefficients of open ocean waters (e.g. Sverdrup *et al.*, 1942; Jerlov, 1976). Such calculations demonstrate that as depth increases, so more shortwave sensitive visual pigments are required and, with judicious consideration of depth and water type, a convincing match between the calculated most sensitive visual pigment and those actually found in deep-sea fish can be demonstrated (e.g. Lythgoe, 1972a; Dartnall, 1975; Partridge, 1990; Goldsmith, 1990, 1991). Indeed, this match has become one of the most cited examples of an adaptation of a sensory system to the environment and led Bowmaker (1995) to write about such data: "one of the most striking correlations in vision ecology is the apparent "perfect" fit to the photic environment of the visual sensitivity of deep-sea (mesopelagic) fish living at depths down to approximately 1000 m".

However, as more data have accumulated, such a simple conclusion is in need of revision. For instance, although in comparison to shallow water species the spectral range covered by the visual pigments of deep-sea species is quite restricted, it is nonetheless greater than would be predicted by a simple matching to the background spacelight (Fig. 7). Animals caught within a single trawl and inhabiting apparently similar photic environments often have visual pigments whose sensitivity maxima differ by more than 20 nm. Furthermore, the sensitivity hypothesis would predict a gradual blue shift of visual pigments with depth, at least within the upper 1000 m. This is quite clearly not the case (see Section 4.2.2.2.2). Furthermore, a simple sensitivity hypothesis cannot explain the presence of more than one visual pigment within the retinae of several species (see Section 4.3).

There are also several problems inherent in the calculations used to confirm the sensitivity hypothesis in explaining the position of deep-sea fish λ_{max} values. Often, for instance, these calculations obtain a good match with observed deep-sea fish λ_{max} data by employing the diffuse spectral attenuation coefficients of some "intermediate" type of open ocean water (usually Jerlov Type II). These coefficients are not adjusted for depth, and absolute photon fluxes are rarely estimated. In fact, examination of the map of the distribution of ocean water types given by Jerlov (1976) reveals that Jerlov Type II open ocean water is relatively scarce in global terms, and most open ocean water is significantly more transparent than Type II water, a fact recently confirmed by satellite imagery. Moreover, Jerlov's diffuse attenuation coefficients were derived from measurements made in surface water which contains chlorophyll, which rapidly diminishes at depths below the euphotic zone (phytoplankton falling out of this zone soon being consumed and degraded). Finally, the calculations usually continue to depths at which photon flux would be so low as to be irrelevant for vision. Even in the clearest ocean water (Jerlov Type I) the depth at which downwelling light becomes too dim for a fish to see it has been calculated to be *ca.* 1000 m, at midday in the tropics (Dartnall, 1975; Denton, 1990). In all other water types, and at all other times of day and at higher latitudes, this depth is considerably less.

Partridge (in prep) has repeated the computation of downwelling light in different Jerlov water types, adjusting the diffuse attenuation coefficients for the lack of chlorophyll below the euphotic zone, and calculating the most sensitive rhodopsin visual pigment at different depths. The preliminary results of these calculations are shown in Fig. 8. At shallow depths in all water types rhodopsins with' relatively longwave λ_{max} values give greatest sensitivity, but as depth increases more shortwave rhodopsins are required, until asymptotic values are reached. In fact, the values of these asymptotes are largely irrelevant in Jerlov Type II and III waters because, even on the brightest tropical day, the downwelling light becomes too dim to be seen by fish before the asymptotes are reached. Indeed, these depths are so shallow as to be hardly "deep-sea" and thus, in regions of the globe where such water types are found, downwelling light will never be relevant to deep sea fish. Only in clearer ocean waters (e.g. pure water, Jerlov Types I, IA, or IB) will sunlight reach truly deep-sea depths to levels sufficient for detection by fish. Examination of the terminal values of λ_{max} in these different water types, shown in parentheses in Fig. 8,

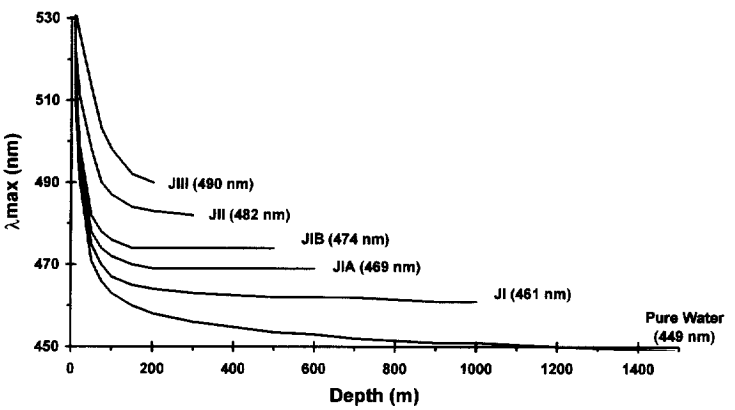

Fig. 8. The calculated λ_{max} values of visual pigments most sensitive to downwelling sunlight at different depths in different open ocean waters. The calculations assume Jerlov's (1976) water types (I, IA, IB, II and III) but Jerlov's tabulated spectral diffuse attenuation coefficients were adjusted at depths below the euphotic zone to compensate for the lack of chlorophyll in deeper water. Diffuse attenuation coefficients for pure water were obtained from Smith and Baker (1981) and Baker and Smith (1982). The lines end at the depths at which downwelling light becomes too dim for deep-sea fish to detect and at this depth the λ_{max} of the most sensitive visual pigment is given in parentheses. For details see Partridge (in prep).

shows that deep-sea fish should, in most of the world's oceans (i.e. in Jerlov Type IB, IA and I waters, or even in pure sea water), have visual pigments with rhodopsins shorter than 474 nm. In fact, of the 154 deep-sea fish with only one rhodopsin pigment in their retina that have been examined (see Fig. 11) only four have visual pigments with λ_{max} values less than 474 nm Thus deep-sea fish seem to be poorly adapted for maximising sensitivity to downwelling light.

4.2.2.1.2. *Maximisation of sensitivity to bioluminescence*

In most deep-sea habitats the primary source of light for vision is not the sun but bioluminescence, as the majority of deep-sea animals, including deep-sea fish and their prey, produce their own light (Herring, 1987; Mensinger and Case, 1990, 1997). It is possible that the visual pigments of deep-sea fishes may be under significant selective pressure for the detection of bioluminescence, a fact that has been recognised previously (e.g. Bayliss *et al.*, 1936) but has not been fully considered by calculation of optimal visual pigments.

Most deep-sea bioluminescence appears blue to the human observer and, to a first approximation, the wavelength of maximum emission (λ_{Emax}) is close to the wavelength of maximum transmission (λ_{Tmax}) of deep-ocean waters, and thus close to the λ_{max} of deepsea fish visual pigments (e.g. Herring, 1983; Widder *et al.*, 1983). In order to calculate the λ_{max} value of the visual pigment most sensitive to bioluminescence, however, information is needed not just about λ_{Emax} but also about the emission spectrum of the bioluminescence. Such data are scarce (Herring, 1983; Widder *et al.*, 1983) but, when combined with data about the absolute brightness of bioluminescent events (e.g. see Mensinger and Case, 1990, 1997), it is possible to estimate the spectral radiance of bioluminescence at different visualisation distances from a photophore. Such estimates have been made by Partridge (in prep), who modelled the spectral radiance of a typical fish photophore by assuming that the light emitted was decreased with distance both by an inverse square law and by the spectral beam attenuation coefficients of open ocean water (Jerlov, 1976), and calculated the most sensitive visual pigment at different visualisation distances. The results of such calculations are shown in Fig. 9, which indicates the λ_{max} of the visual pigment most sensitive to "typical" fish bioluminescence at different ranges in pure sea water and in Jerlov Type IB water (with spectral beam attenuation coefficients adjusted for the absence of chlorophyll at depth). These water types bound most open ocean waters and the two photoreceptor sizes considered (10 μm and 100 μm) span the limits of rod outer segment lengths seen in most deep-sea fish retinas. Clearly, a rela-

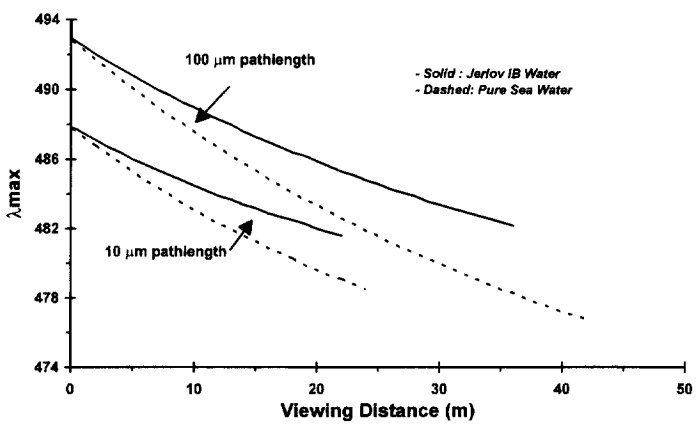

Fig. 9. The calculated λ_{max} values of the visual pigment most sensitive for the visualisation of bioluminescence operating at different visualisation ranges. The results of the calculations are shown for two water types, Jerlov Type IB (but without chlorophyll) and pure sea water, and for two photoreceptor sizes, these conditions bounding those found in most ocean waters and in most deep-sea fish retinas. As viewing distance increases, so the λ_{max} of the most sensitive visual pigment shifts to shorter wavelengths.

tively longwave sensitive visual pigment is required when bioluminescence is visualised close up, but at greater ranges a pigment with a shorter wave λ_{max} is more sensitive. The exact λ_{max} of the most sensitive visual pigment depends not only on range but also on the water type and the length of the photoreceptor, but in all cases a maximum range is ultimately reached at which point the bioluminescence becomes too dim to see (for details see Partridge, in prep). This maximum range naturally depends on the absolute intensity of the photophore emission but is also dependent on the pupil area of the observing fish, this (rather than eye f-number) determining the brightness of a retinal image of a point source of light (Land, 1981). For this reason small fish, with small eyes, cannot see bioluminescence at such large ranges as larger fish with larger eyes.

Considering the case of a bioluminescent event being visualised at the maximum detection range, maximum sensitivity will be conferred by a visual pigment with a particular λ_{max}, the value of this depending on eye size, as well as photoreceptor outer segment length and the spectral beam attenuation coefficients of the particular sea water being considered. Figure 10 shows typical results of such calculations (for details see Partridge, in prep). Small eyes, operating at close ranges because their small pupil area and consequent low sensitivity to point sources precludes visualisation of distant bioluminescence, have relatively longwave λ_{max} values, whereas larger eyes need relatively shorter wave sensitive visual pigments if they are to have maximum sensitivity at their furthest working range. The most sensitive visual pigment is, however, determined by a combination of eye size, visualisation range and photoreceptor path length, as well as water type. A large range of λ_{max} values, extending between *ca.* 468 nm and 490 nm is suggested by this model, depending on such variables. The predictions of which visual pigments will confer maximum sensitivity to bioluminescence are thus somewhat different from those predicted to confer maximum sensitivity to downwelling light. Figure 11 shows how the two predictions compare with λ_{max} data from deep-sea fish visual pigments. Clearly, the results of these calculations suggest that the detection of bioluminescence exerts the primary selective pressure on deep-sea fish visual pigment λ_{max} values. Although such a conclusion is attractive, it is equally possible that deep-sea visual pigment λ_{max} values are, in fact, constrained by factors unrelated to the photic environment (see Section 4.2.2.2). If this is the case, then it follows that the emission spectra of bioluminescence are likely to be under selective pressure to be "tuned" to the observers' visual systems, rather than *vice versa*.

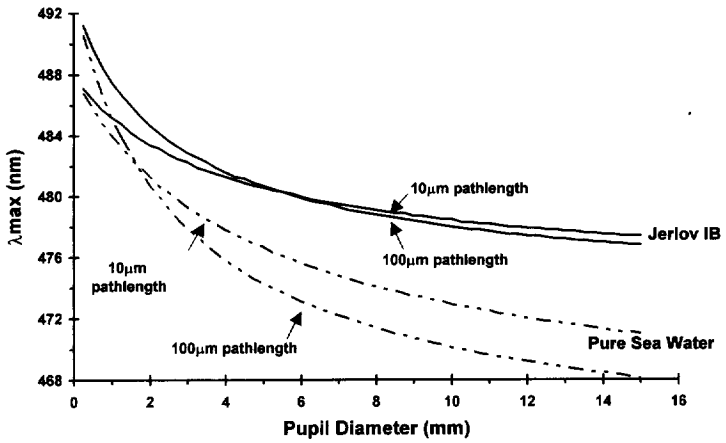

Fig. 10. The calculated λ_{max} of the visual pigment most sensitive to bioluminescence for eyes of different sizes visualising the bioluminescent event at maximum visualisation range. Data are shown for two water types, Jerlov Type IB (but without chlorophyll) and pure sea water, and for two photoreceptor outer segment lengths. Small eyes, operating at short ranges because they lack sensitivity to point sources of light, need relatively longwave sensitive visual pigments, but larger eyes need visual pigments with shorter λ_{max} values for maximum sensitivity.

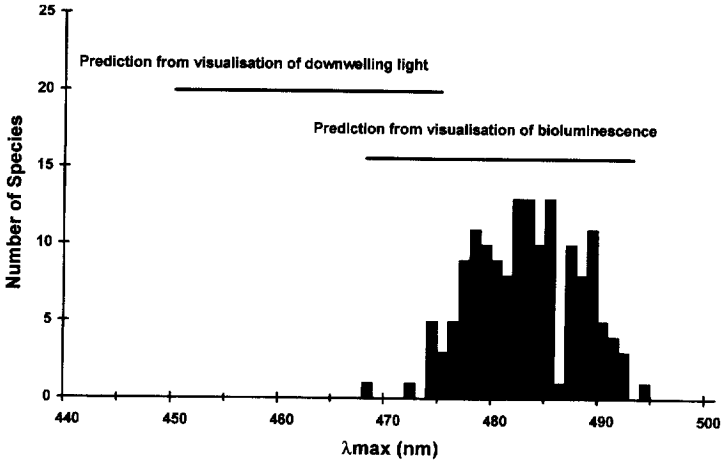

Fig. 11. Histogram showing all λ_{max} values measured from those deep-sea fish having single rhodopsin visual pigments (data from sources listed in the legend to Fig. 7). Above the histogram are the ranges of λ_{max} values predicted to confer maximum sensitivity to downwelling light from the sun, or for the visualisation of fish bioluminescence (for details see text and Partridge, in prep.).

4.2.2.2. *Factors unrelated to photic environment*

In a photon limited environment such as the deep-sea it is tempting to assume that retinal photon capture rate is maximised by the spectral tuning of visual pigment λ_{max} values through evolution to the light source being detected. However, it is possible that the precise location of a pigment's λ_{max} is determined by factors other than the photic environment.

4.2.2.2.1. *Maximisation of signal to noise ratio and thermal stability*

The ability of an animal to detect light at low levels is determined by the signal to noise ratio (S/N) of the signal rather than by just the signal itself (Land, 1981). Although the capture of light by a photoreceptor can be modelled adequately as a Poisson process, and hence the S/N will rise with increasing S (Land, 1981), such calculations assume that all noise in the photoreceptor output is determined by the stochastic nature of photon capture. In practice this is not the case and photon noise ("channel noise") is combined with noise in the visual system itself ("receptor noise"; Dusenbury, 1992). If receptor noise in a visual system is dependent on visual pigment λ_{max} then it is very likely that there will be selective pressure on deep-sea fish for the evolution of visual pigments that have inherently low N, as well as on those that confer high S, the ultimate λ_{max} being determined by some compromise that maximises S/N.

Electrophysiological measurements from vertebrate rods have indeed demonstrated spontaneous "dark" noise, in the form of discrete bumps in the electrical potential of the receptor that are indistinguishable from the effects of single photon absorption (Baylor *et al.*, 1980; Donner *et al.*, 1990; Barlow *et al.*, 1993). It is not understood exactly how such thermal activation of ion channel closure operates and it is unlikely to involve the spontaneous isomerisation of 11-cis retinal to the all-trans isomer (Goldsmith, 1991; Barlow *et al.*, 1993) but Aho *et al.* (1988) have shown that this dark noise can limit visual thresholds, as hypothesised by Barlow (1956). Nevertheless, there are thermodynamic reasons to expect that visual pigments with longwave sensitivity will be more prone to thermal noise (Barlow, 1957), and there is empirical evidence that dark noise is less in photoreceptors containing visual pigments with shortwave λ_{max} values (Firsov and Govardovskii, 1990). Thus, the shortwave sensitivity of deep-sea fish visual pigments could, at least in part, be an adaptation for noise minimisation as much as for signal maximisation. Such a conclusion is supported, at least circumstantially, by two related facts. Firstly, it is well known that shallow living fish (and, indeed, terrestrial animals) have rod visual pigments too

shortwave sensitive to confer maximum sensitivity at night (Lythgoe, 1972a; Dartnall, 1975). This may be an adaptive compromise between the need for a longwave sensitive visual pigment that would have a greater photon capture (high S) and the need for a pigment with low noise (N). Secondly, the deep-living cottoid fishes of Lake Baikal also generally have shortwave shifted visual pigments, with rod λ_{max} values close to 482 nm, and yet downwelling light in this freshwater lake is unlikely to be so shortwave biased as in the open oceans, and there is no reported bioluminescence (Bowmaker, 1995). Thus, the visual pigments of deep-sea fishes may not have λ_{max} values spectrally located to maximise photon catch (whether from downwelling light or bioluminescence) but, in fact, may be ideal for the maximisation of S/N ratios.

4.2.2.2.2. *Ambient pressure*

The amino acids which tune the visual pigment chromophore are contained within 7 helical loops than span the outer segment disc membrane (Sections 4.1 and 4.2.1) and are therefore part of a complex tertiary protein structure. It is possible that the pressures experienced at great depth (1 atmosphere for every 10 m below the surface) may influence this tertiary structure and hence affect visual pigment absorption characteristics. This has two possible consequences:

Firstly, at depth it might be impossible to manufacture opsins with λ_{max} values other than those observed in deep-sea fish. The relatively restricted range of visual pigments observed (Fig. 7) might therefore reflect an effect of pressure rather than adaptation to the photic environment. However, it is unlikely that the few amino acids that are involved in visual pigment spectral tuning (Section 4.2.1) could present an insurmountable problem to the evolution of λ_{max} values other than those found in deep-sea fish.

Secondly, some proteins in very deep-living organisms are known to be specifically "designed" for operation at great pressure, with correct protein structure only being achieved under pressure (Somero *et al.*, 1983). It is therefore conceivable that opsin structure might also be affected by pressure. Consequently, the λ_{max} values of deep-sea fish visual pigments shown in Fig. 7, which are all recorded at atmospheric pressure, might change when the pigment is subjected to its normal environmental pressure.

If there were an effect of pressure on visual pigment absorption characteristics one might expect a direct relationship between a species' λ_{max} and the depth it normally inhabits. Although it has long been recognised that deep-sea fish generally have shorter wave absorbing visual pigments than surface dwelling species, it would be of interest to establish a more precise relationship between λ_{max} and depth. However, this is not possible for mesopleagic animals, which comprise the bulk of the species for whom visual pigment data are available, as too little is known of their distribution and many undertake extensive diurnal vertical migrations, making it difficult to establish their habitat depth with any precision. Nevertheless, when mesopelagic species have been grouped into general depth-related categories (Partridge *et al.*, 1989) it appears that there is no clear relationship between an animal's depth range and its visual pigment, the same range of visual pigments occurring at all depths. For demersal species, which habitually live on or very near the bottom and generally do not undertake diurnal migrations, the depth of capture gives a reasonable estimate of the animal's true habitat. However, in such animals there is also no obvious shift in λ_{max} with depth, although the range of pigments expressed by species living in the upper 1000 m is greater than found in the more "conservative" deeper dwelling species (Fig. 12; Douglas *et al.*, 1995). It thus appears unlikely that pressure has a great effect on the spectral characteristics of the visual pigments.

4.2.2.2.3. *Phylogenetic constraints*

There appears to be a degree of phylogenetic conservatism of λ_{max} within certain families and genera of deep-sea fish, indicating the visual pigment absorption characteristics of a species may be partly determined by phylogenetic constraints as well as by ecological pressures (Partridge *et al.*, 1989, 1992; Douglas and Partridge, 1997), although, to date, such a conclusion has not been formally analysed by appropriate statistical treatment (Harvey and Pagel, 1991).

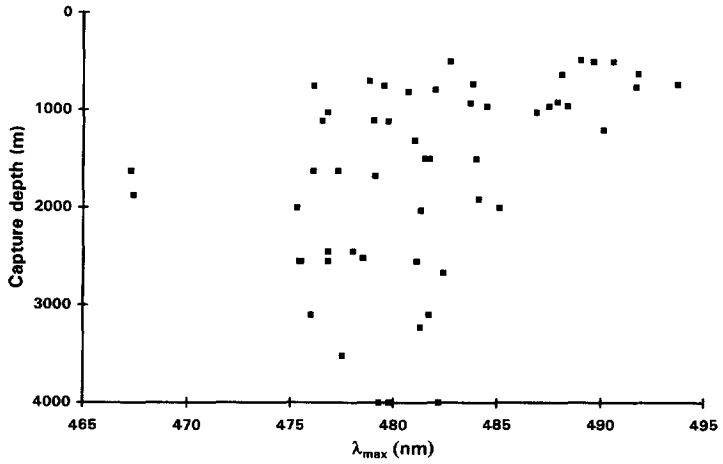

Fig. 12. Visual pigment λ_{max} values as a function of capture depth for 52 species of demersal deep-sea fish (from Douglas *et al.*, 1995)

One of the most widespread and numerous families of deep-sea fish, for instance, are the Myctophidae. Although most myctophids are mesopelagic species with broadly similar habitats, inhabiting deeper water during the day and migrating towards the surface at night, different species occur at significantly different depths (e.g., Gartner *et al.*, 1987; Hulley, 1984, 1992; Clark, 1973). Furthermore, some species do not undergo diel migrations (Gartner *et al.*, 1987). Despite such differences, which will result in different species being exposed to quite different photic environments, all myctophids have similar visual pigments, with λ_{max} values generally between 485–490 nm (Denton and Warren, 1957; Munz, 1958; O'Day and Fernandez, 1976; Fernandez, 1978; Partridge *et al.*, 1989, 1992; Crescitelli, 1991a; Douglas and Partridge, 1997), suggesting a phylogenetic constraint may help determine the spectral properties of these pigments.

4.2.2.3. *Summary*

The hypsochromatic shift observed in the visual pigments of most species of deep-sea fish (Fig. 7) in comparison to shallower living species and terrestrial vertebrates is most likely an adaptation for the detection of bioluminescent light, but may also be influenced by the need for a visual pigment inherently unlikely to generate noise in the receptor, the ambient pressure and the animal's phylogeny. The exact "best" visual pigment for a given species will thus depend on details of the species' morphology, its visual tasks and its general ecology (Partridge and Cummings, in press).

4.3. Deep-sea Fish With Multiple Visual Pigments

As discussed in Section 4.2, most deep-sea fish have single visual pigments with λ_{max} values between 468 and 494 nm. However, *ca.* 11% of deep-sea fish visual pigments recorded to date are maximally sensitive at longer wavelengths, while *ca* 3% have their λ_{max} below 468 nm. All of these "unusual" pigments are contained within retinae possessing more than one spectral class of photoreceptor. At present, data are restricted to species with only rods; no cone absorbance spectra having been measured from those few deep-sea fish that also have retinal cones. Broadly speaking there are three categories of deep sea fishes with multiple rod visual pigments: those that have two visual pigments with λ_{max} values within the "normal" range of 468 to 494 nm; those that have multiple pigments including at least one pigment outside this range; and those that have very long-wave displaced λ_{max} values. The last group includes only the stomiid dragon fishes which are discussed in Section 4.3.2.

4.3.1. *Multipigment species without far-red sensitivity*

Excluding the stomiids, thirteen species of deep-sea fishes are known to have more than one rod visual pigment (Table 1). Of these, five species have two visual pigments with λ_{max} values within the range 468 to 494 nm that is typical of the vast majority of deep-sea fishes. The remaining species have at least one visual pigment displaced either to shorter than usual wavelengths, as in the perciform *Howella sherborni*, or, more commonly, having one pigment displaced to longer than normal wavelengths. Only one species, the pearleye *Scopelarchus analis*, has been shown to have three rod visual pigments, although it is likely that the closely related *Bethalbella infans* will also be found to have a similar set of visual pigments, despite the fact that, to date, only one pigment, with a λ_{max} at 451 nm, has been described (Partridge *et al.*, 1989).

Almost all deep-sea fish visual pigments are rhodopsins, utilising retinal as their sole chromophore. The only exceptions are the far red sensitive stomiidae (Section 4.3.2) and *Bonapartia pedaliota* (Table 1), which have retinae containing both rhodopsin and porphyropsin visual pigments. In the stomiidae these form "pigment pairs"; that is a single opsin that in some photoreceptors is bound to the A_1-based chromophore, and in others to 3,4-dehydroretinal (see Section 4.1). Surprisingly, this is not the case in *Bonapartia pedaliota*, where the two visual pigments appear to be composed of two different opsins bound to the different chromophores (Douglas and Partridge, 1997).

Theoretically the possession of more than one visual pigment can have two advantages in comparison to retinae containing just a single pigment; increased spectral range and colour vision. Some form of hue discrimination would appear advantageous to many deep-sea fish as it would, for instance, allow the detection and identification of other animals on the basis of the colour of their bioluminescence (Denton and Locket, 1989). The function of multiple visual pigments depends critically on the location of these pigments within the retina and the neural interactions between the photoreceptors containing them; colour vision usually requiring some form of neural opponency between receptors housing distinct visual pigments. MSP measurements from 8 of the 13 species tasted in Table 1 have shown that, in all cases bar one, the two pigments are located in separate types of rod photoreceptor, fulfilling one of the minimal requirements for colour vision (but see below). The exception to this rule is *Scopelarchus analis* in which the 444 nm λ_{max} visual pigment was found in the vitreal part of

Table 1. Summary of visual pigment λ_{max} values (nm) from deep-sea fishes with more than one rod pigment but lacking longwave photophores

Species	λ_{max}	Method	References
Alepocephalus bairdii	467, 481	wholemount	Douglas *et al.* (1995)
Antimora rostrata	475, 483	extract	Douglas and Partridge (1997); Douglas *et al.* (1995); Dartnall and Lythgoe (1965)
Bathylagus berricoides	464, 500	extract	Douglas and Partridge (1997); Dartnall and Lythgoe (1965); Partridge *et al.* (1988)
Bathylagus euryops	465, 497	extract	Douglas and Partridge (1997)
Bathylagus longirostris	474, 502	extract	Douglas and Partridge (1997); Partridge *et al.* (1989)
Bathylagus wesethi	478, 500	extract	Munz (1957, 1958)
Bonapartia pedaliota	471, 514	extract	Douglas and Partridge (1997)
Diretmus argenteus	484, 500	wholemount	Denton and Locket (1989)
Howella sherborni	463, 492	MSP	Partridge *et al.* (1989)
Lepidion eques	476, 484	extract	Douglas and Partridge (1997); Douglas *et al.* (1995)
Malacocephalus laevis	477, 485	MSP	Douglas *et al.* (1995); Partridge *et al.* (1988)
Scopelarchus analis	444, 479, 505	MSP	Partridge *et al.* (1992); Muntz (1976)
Stylephorus chordatus	470, 481	MSP	Partridge *et al.* (1992)

All visual pigments are rhodopsins except for the 514 λ_{max} pigment of *Bonapartia pedaliota* which is a porphyropsin. Where data were available from more than one source we chose, where possible, our own.

the rod outer segments that also contained the 505 nm λ_{max} visual pigment more sclerally (Partridge *et al.*, 1992), a finding interpreted as being the manifestation of a switch between the expression of one opsin to the expression of another (as occurs in the European eel *Anguilla anguilla*; see Partridge and Cummings, in press).

Many deep-sea fishes have multiple banks of photoreceptors (Locket, 1977; Denton and Locket, 1989; Fröhlich *et al.*, 1995) and in these species the effective spectral sensitivities of the photoreceptors differ from layer to layer because of the spectral screening by more vitreal banks. As a result, colour vision is theoretically possible, even if only one visual pigment is present in the retina (Denton and Locket, 1989). However, where two or more classes of photoreceptor with different visual pigments exist in a retina, some form of colour vision is more likely, albeit mediated, in most deep-sea fishes, by inhibitory interactions between rod rather than cone photoreceptors. Unfortunately, due to a lack of physiologically viable material, little is known about the neural interactions that occur in deep-sea fish retinae, and far less about their behaviour in response to colour stimuli.

Horizontal cells form an early link in the processing of chromatic information. Thus, animals such as shallow water fish, which are believed to have well developed cone-based colour vision, show extensive and specific connectivity in their outer plexiform layers involving more than one type of horizontal cell. However, horizontal cells are relatively scarce in deep-sea fish with pure rod retinae, and those that are present appear to be of a single morphological type (Wagner *et al.*, 1998). One might therefore argue that such deep-sea fish are unlikely to have colour vision (Wagner *et al.*, 1998). However, such a conclusion may be unwarranted since rod-based hue discrimination in deep-sea fish quite probably involves quite different neural pathways to the colour vision of shallow water species which relies on classical cone–cone interactions. However, if the outputs of the spectrally distinct photoreceptors of multipigment deep-sea fish do simply interact in an additive manner, although the animals will not have colour vision, they will have an increased spectral range in comparison to animals with a single visual pigment of intermediate λ_{max}. Douglas and Partridge (1997) modelled the theoretical spectral sensitivity of *Bathylagus euryops* and concluded that the dual visual pigments found in this species would confer significantly greater sensitivity to wavelengths beyond 521 nm than a single pigment of intermediate λ_{max}, but that there would be little difference in shortwave sensitivity.

Table 2. Summary of stomiid visual pigment λ_{max} values (nm)

	Method of analysis			
	extract	MSP	wholemount	References
Aristostomias grimaldii	517, 552x			Bowmaker *et al.* (1988)
Aristostomias scintillans	524, 552x			Crescitelli (1991a)
Aristostomias scintillans	526, 551x			O'Day and Fernandez (1974)
Aristostomias xenostoma	514, 551x			Knowles and Dartnall (1977)
Aristostomias tittmanni	523, 551x	518, 550x, 581	*531, 548x, 588	Partridge and Douglas (1995)
Pachystomias sp.			575	Denton *et al.* (1970, 1985)
Pachystomias microdon	(524), 539x	515, 543x		Bowmaker *et al.* (1988)
Pachystomias microdon	513, 539x	513, 539x		Partridge *et al.* (1989)
Pachystomias microdon	513, 540x		520, (563x), 595	unpubl.
Malacosteus danae	514, 556x			Crescitelli (1989, 1991a)
Malacosteus niger	517, 545x	522, 548x		Bowmaker *et al.* (1988)
Malacosteus niger		521, 538x		Partridge *et al.* (1989)
Malacosteus niger	510			Somiya (1982)
Malacosteus niger	*517, 542x		*522, 534x	Douglas *et al.* (in press)

All pigments are vitamin A_1-based rhodopsins except those marked by (x) which are A_2-based porphyropsins.

The λ_{max} values quoted here for species indicated by an asterisk (*) represent averages and therefore differ from the individual data shown in Figs 14, 20 and 21.

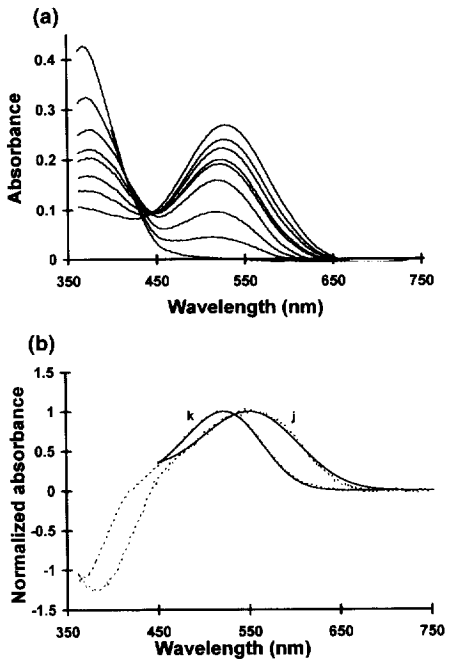

Fig. 13. (a) Partial bleach of a retinal pigment extract from *Aristostomias tittmanni* (for method see Douglas *et al.*, 1995). The curves represent the absorption spectra of the extract following various bleaches; in order of decreasing longwave absorbance (a) initial measurement of the unexposed extract 10 min after the addition of 50 µl 1M hydroxylamine to *ca.* 0.5 ml of extract, (b) after 30 min exposure to monochromatic light of 709 nm, (c) another 30 min 709 nm, (d) 30 min 699 nm, (e) another 30 min 699 nm, (f) 15 min 666 nm, (g) 3 min 624 nm, (h) 1 min 609 nm, (i) 5 min 521 nm. (b) Difference spectra (dotted lines) constructed using the curves shown in (A); (j) a–b, (k) h–i. These are best fit (Partridge and DeGrip, 1991) to the templates of Stavenga *et al.* (1993) revealing a porphyrops in with λ_{max} 551 nm and a rhodopsin with λ_{max} 523 nm respectively (solid lines) (Douglas and Partridge, unpubl.).

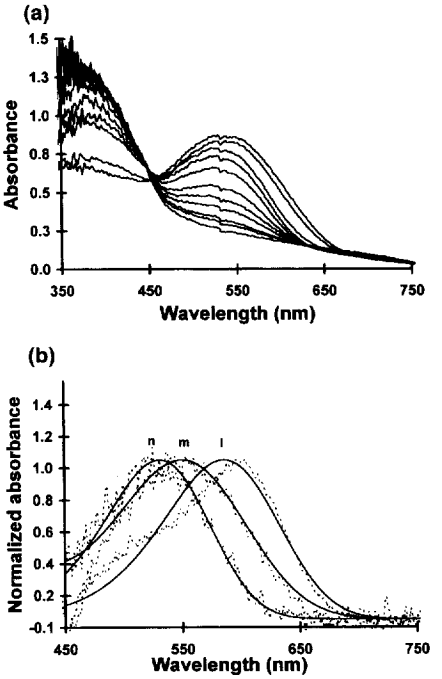

Fig. 14. (a) Absorbance spectra of a fresh retinal whole-mount preparation from *Aristostomias tittmanni* following various bleaches; in order of decreasing longwave absorbance (a) initial measurement of the unexposed extract 10 min after the addition of 50 mM hydroxylamine, (b) after 10 min exposure to monochromatic light of 699 nm, (c) another 60 min 699 nm, (d) 10 min 666 nm, (e) another 30 min 666 nm, (f) another 60 min 666 nm, (g) another 60 min 666 nm, (h) another 60min 666 nm, (i) 77 sec 609 nm, (j) another 77 sec 609, (k) 6 min 521 nm. (b) Normalized difference spectra constructed using the curves shown in (b); (l) a–c, (m) c–e, (n) h–j. These are best fit by a rhodopsin template with λ_{max} 586, a porphyropsin with λ_{max} 550 nm and a rhodopsin with λ_{max} 531 nm respectively (smooth lines) (from Partridge and Douglas, 1995).

An attractive hypothesis is that the dual visual pigments found in at least some species have λ_{max} values spectrally located in one case for maximum sensitivity to downwelling light and in the other for maximum sensitivity to bioluminescence (see Section 4.2.2.1). If colour vision exists in these species it will be well suited to the differentiation of bioluminescence and downwelling light on the basis of hue, and thus to the breaking of bioluminescence-based ventral camouflage (see Section 2.3). Alternatively, if there are no opponent processes operating, it might be expected that the different visual pigments of multipigment species would be located in different regions of the retina, subserving different regions of visual space. Such regionalization has been reported in the beryciform *Diretmus argenteus*, in which the ventral retina contains the longwave sensitive visual pigment (λ_{max} 500 nm) and the dorsal retina the more shortwave sensitive pigment (λ_{max} 486 nm; Denton and Locket, 1989) although modelling predicts (see Section 4.2.2.1) that the ventral retina should contain the more shortwave sensitive visual pigment. Similarly, in the pearl eye *Scopelarchus analis*, the main retina (which subserves the dorsal visual field) contains two visual pigments (λ_{max} 445 and 507 nm) whereas the accessory retina, which subserves the lateral

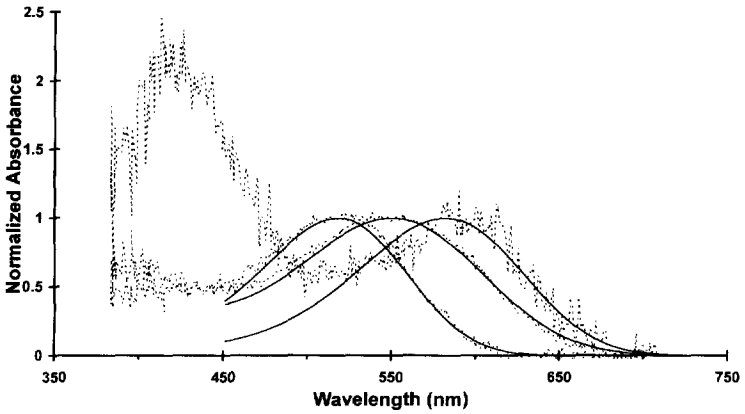

Fig. 15. Normalized average absorbance spectra of the 3 types of visual pigment recorded by microspectrophotometry from the eye of *Aristostomias tittmanni*. The smooth curves represent best-fitting visual pigment templates for rhodopsins with λ_{max} 518 and 581 and a porphyropsin with λ_{max} 550 nm. The absorbances at the λ_{max} and the number of scans included in each average are 0.027 (n = 18), 0.022 (n = 42) and 0.014 (n = 2), respectively (from Partridge and Douglas, 1995).

visual field, contains an additional pigment (λ_{max} 479 nm). Complex regional differences in visual pigment are also evident in the deep-sea squid *Watasenia scintillans* (Matsui *et al.*, 1988; Kito *et al.*, 1988; Seidou *et al.*, 1990; Michinomae *et al.*, 1994). Nevertheless, Douglas and Partridge (1997) failed to find evidence for regional distributions of different visual pigments in five species of deep-sea fish (*Bathylagus berricoides*, *B. longirostris*, *Antimora rostrata*, *Lepidion eques* and *Howella sherborni*) and regional variation in visual pigments in the retinae of deep-sea species may prove to be the exception rather than the rule, reflecting the relatively uniform spectral distribution of light at most depths in the deep-ocean.

4.3.2. *Longwave sensitive dragon fishes*

The most clear cut example of visual pigment adaptation to specific bioluminescence is provided by three genera of deep-sea stomiid dragon fish (*Malacosteus*, *Aristostomias* and *Pachystomias*), which have two light producing organs around their eyes (Fig. 6d–f); a postorbital photophore producing blue–green bioluminescence similar to that produced by most other deep-sea organisms, and a second, suborbital, photophore producing far-red bioluminescence with spectral emissions peaking sharply at wavelengths beyond 700 nm (Denton *et al.*, 1970, 1985; Widder *et al.*, 1984). The vast majority of deep-sea organisms have visual pigrnents maximally sensitive below 500 nm (Fig. 7; Section 4.2). They will therefore be unable to see the far red light produced by the suborbital photophores of these dragon fish as their visual pigments simply will not absorb significantly in this part of the spectrum. The stomiids, however, have evolved a number of adaptations to enable them to perceive their own far red bioluminescence.

4.3.2.1. *Aristostomias*

It has been known for over 20 years, based on data derived from partial bleaching of detergent extracts (Section 4.1), that the *Aristostomias* retina contains two visual pigments that are long-wave shifted compared to those of other deep-sea animals (λ_{max} values *ca.* 520 nm and 551 nm; Table 2; Fig. 13a,b). These two pigments form a rhodopsin/porphyropsin "pigment pair" (see Sections 4.1 and 4.3.1). Similar pigment pairs are found in *Pachystomias* and *Malacosteus* (see below). In shallow water species with such pigment pairs both visual pigments occur as a mixture in the same photoreceptor (Bowmaker, 1995). In all three stomiid genera, however, the rhodopsin and porphyropsin analogues are located, more or less exclusively, in separate types of rod (Bowmaker *et al.*, 1988; Partridge *et al.*, 1989). It is not known how the retinal pigment

epithelium, which supplies re-isomerised (11-cis) chromophore to the photoreceptors, effects this topographical separation of chromophore supply, which results in adjacent rods having very different rhodopsin:porphyropsin ratios.

Although such pigments will certainly increase sensitivity to longwave radiation compared to the other inhabitants of the deep-ocean, the correspondence to bioluminescent emissions at over 700 nm is still far from perfect (Fig. 17). However, using retinal wholemounts of fresh material, we have recently been able to demonstrate the existence of a third, longer wave absorbing, pigment (λ_{max} *ca.* 588 nm; Partridge and Douglas, 1995; Fig. 14a,b) in the retina of *Aristostomias tittmanni* which will further enhance longwave sensitivity. Template fitting suggests that this third pigment is a rhodopsin. Three distinct types of rods containing these same three pigments have also been identified by MSP (Table 2; Fig. 15). Interestingly, wholemounts of previously frozen material show only the same two visual pigments previously revealed by extraction (Fig. 16). The inability to isolate this additional longwave pigment by extraction and its disappearance following freezing, suggests that it is very labile.

Thus, the *Aristostomias* retina contains at least three visual pigments; a shortwave opsin bound to both retinal and 3,4-dehydroretinal forming a pigment pair (λ_{max} values *ca.* 520 nm and 551 nm) and a separate longwave opsin combined with retinal, forming a longwave sensitive rhodopsin (λ_{max} *ca* 588 nm). This latter pigment is by far the most longwave sensitive rod pigment ever described. Nonetheless, even this pigment, is still not a very good fit to the animal's longwave bioluminescence (Fig. 17). However, it would not be unreasonable to expect an even more red-sensitive visual pigment in the *Aristostomias* retina made up of the longwave opsin combined with 3,4-dehydroretinal. Using the formula of Whitmore and Bowmaker (1989) to predict the λ_{max} of a porphyropsin from a known rhodopsin utilising the same opsin, *Aristostomias* might have an A_2-based pigment with λ_{max} 669 nm. This would produce an excellent fit to their longwave bioluminescence (Fig. 17). However, this is far more red-sensitive than any photoreceptor pigment isolated to date and it is quite likely that the relationship between rhodopsin and porphyropsin λ_{max} values developed by Whitmore and Bowmaker (1989), and others, does not hold for such extreme pigments. However, even an A_2-based pigment such as that found, for instance, in rudd cones (λ_{max} 635 nm; Loew and Dartnall, 1976) would substantially increase the sensitivity of *Aristostomias* to its own longwave bioluminescence. If such a pigment exists, the most probable reason it has so far not been seen is that pigments have always been isolated using dim red illumination, which is likely to bleach such a pigment.

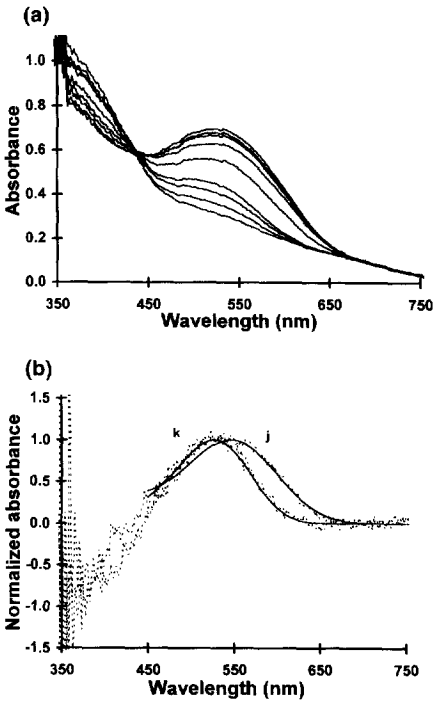

Fig. 16. (a) Absorbance spectra of a previously frozen retinal wholemount preparation from *Aristostomias tittmanni* following various bleaches (for method see Douglas *et al.*, 1995), in order of decreasing longwave absorbance (a) initial measurement of the unexposed wholemount 10 min after the addition of 100 µl 1M hydroxylamine to 1.5 ml of bathing medium, (b) after 60 min exposure to monochromatic light of 750 nm, (c) another 27 min 750 nm, (d) 60 min 709 nm, (e) 60 min 666 nm, (f) 30 min 634 nm, (g) 2 min 471 nm, (h) 4 min white light, (i) another 13 min white light. (b) Normalized difference spectra constructed using the curves shown in (a); (j) b–d, (k) f–g. These are best fit by a porphyropsin template with λ_{max} 546, and a rhodopsin with λ_{max} 527 nm respectively (smooth lines) (Douglas and Partridge, unpubl.).

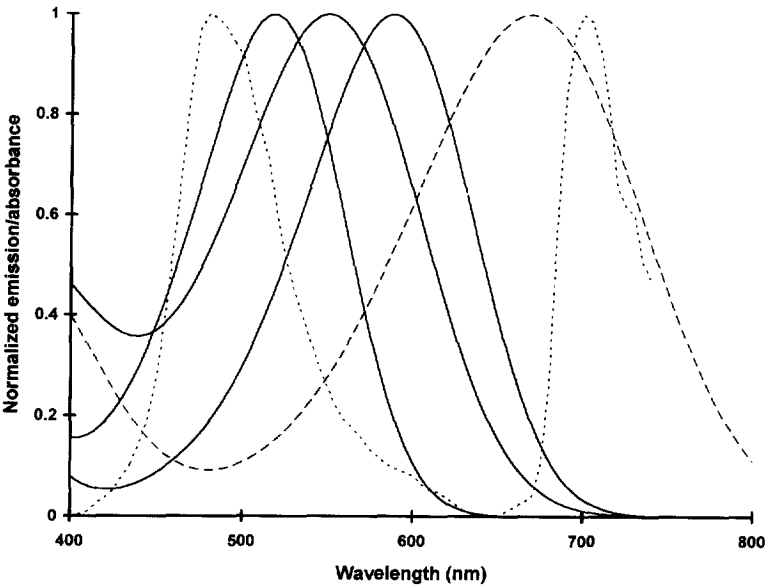

Fig. 17. Bioluminescence of *Aristostomias tittmanni* (dotted line; Widder *et al.*, 1984), and the best fit templates (solid lines) of the three visual pigments so far identified in its retinae (a rhodopsin/porphyropsin pigment pair with λ_{max} values 520 nm and 551 nm and a rhodopsin with λ_{max} 588 nm). The dashed line represents a theoretical porphyropsin with λ_{max} 669 nm, which is the analogue of the longwave sensitive rhodopsin (calculated using the formula of Whitmore and Bowmaker, 1989).

Aristostomias also possesses a pigmented lenses (Fig. 1; Douglas and Thorpe, 1992), which may further enhance the visibility of longwave bioluminescence by removing some of the downwelling background illumination (Section 2.3).

4.3.2.2. *Pachystomias*

The story for *Pachystomias* is similar to that for *Aristostomias*. Detergent extracts, as well as MSP, show the retina to contain two visual pigments longwave shifted compared to those of other deep-sea fish (λ_{max}; values *ca.* 515 nm and 540 nm; Fig. 18; Table 2), once again forming a rhodopsin/porphyropsin pigment pair. A partial bleach of a fresh wholemount from a single *Pachystomias microdon* retina, however, once again shows that the longest wave bleaches result in a difference spectrum that is best fitted by a longer wave sensitive rhodopsin with λ_{max} *ca* 595 nm (Fig. 19). Thus, the *Pachystomias* retina contains at least three visual pigments and, as for *Aristostomias* (see above), a fourth, even longer wave absorbing, porphyropsin may also exist. Interestingly, one previous study (Denton *et al.*, 1970) hinted at an unusually longwave sensitive visual pigment in *Pachystomias microdon* (λ_{max} 575 nm). This study also utilised retinal wholemounts.

While the lenses of *Aristostomias* (Fig. 1) and *Malacosteus* (Fig. 3) are heavily pigmented, appearing visibly yellow, the lenses of *Pachystomias microdon* are only slightly pigmented (Douglas and Thorpe, 1992). However, they may compensate for this by having a photostable, shortwave absorbing, yellow pigment within their retina. After complete bleaching of the visual pigments the retina of *Pachystomias* appears bright yellow, a colour that does not fade despite continued exposure to light. Such photostable retinal pigments are not uncommon in deep-sea fish (e.g., Douglas and Thorpe, 1992; Douglas *et al.*, 1995; Denton and Locket, 1989) and probably, like yellow lenses, serve to enhance further the visibility of longwave bioluminescence (see Section 2.3).

4.3.2.3. *Malacosteus*

As for the other stomiids, extracts and MSP once again reveal a rhodopsin/porphyropsin pair of longwave shifted visual pigments (λ_{max} values

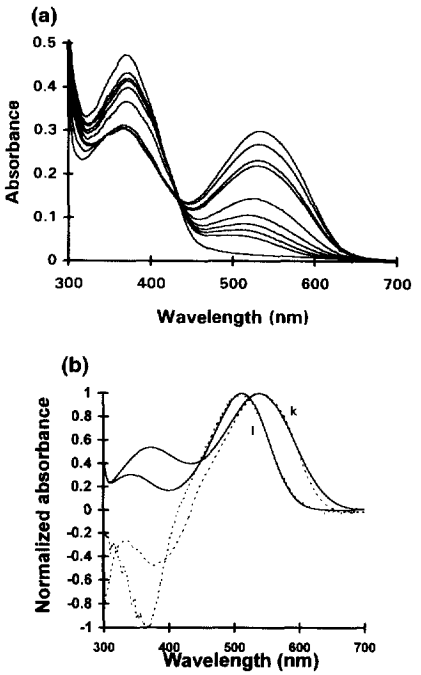

Fig. 18. (a) Absorbance spectra of a retinal extract of *Pachystomias microdon* following various bleaches (for method see Douglas *et al.*, 1995); in order of decreasing longwave absorbance (a) initial measurement of the unexposed extract 11 min after the addition of 50 µl 1M hydroxylamine to 1.0 ml of extract, (b) after 75 min exposure to monochromatic light of 709 nm, (c) 30 min 699 nm, (d) another 30 min 699 nm, (e) 15 min 666 nm, (f) another 15 min 666 nm, (g) another 15 min 666 nm, (h) another 30 min 666 nm, (i) 2 min 634 nm, (j) 5 min 521 nm. (b) Normalized difference spectra constructed using the curves shown in (a); (k) a–h, (l) i–j. These are best fit by a porphyropsin template with λ_{max} 540, and a rhodopsin with λ_{max} 513 nm respectively (smooth lines) (Douglas and Partridge, unpubl.).

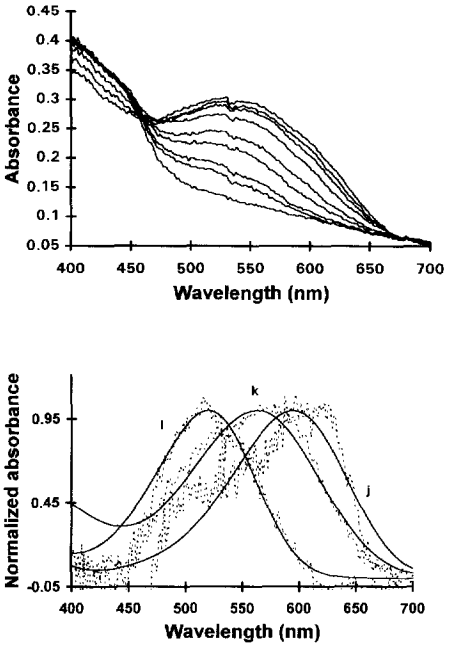

Fig. 19. (a) Absorbance spectra of a fresh retinal wholemount preparation from *Pachystomias microdon* following various bleaches (for method see Douglas *et al.*, 1995); in order of decreasing longwave absorbance (a) initial measurement of the unexposed preparation 90 min after the addition of 200 µl 1M hydroxylamine to 2.0 ml of bathing medium, (b) after 60 min exposure to monochromatic light of 709 nm, (c) 30 min 699 nm, (d) another 60 min 699 nm, (e) 10 min 666 nm, (f) 5 min 650 nm, (g) 2 min 609 nm, (h) another 2 min 609 nm, (i) 5 min 521 nm. (b) Normalized difference spectra constructed using the curves shown in (a); (j) a–c, (k) d–f, (l) h–i. These are best fit by a rhodopsin template with λ_{max} 595, a porphyropsin with λ_{max} 563nm and a rhodopsin with λ_{max} 520 nm respectively (smooth lines). (Douglas and Partridge, unpubl.). It proved difficult to isolate the intermediate visual pigment and the difference spectrum used to determine λ_{max} almost certainly represents a mixture of pigments.

ca 515 nm and 542 nm; Table 2; Fig. 20). Unfortunately, the high optical density of the longwave reflecting tapetum of this species (Section 3.3; and see below) would mask any visual pigment in a retinal wholemount preparation, which in the other species revealed the presence of a third, longer wave absorbing, visual pigment. As an alternative (Douglas *et al.*, in press) suspended rod outer segments in a 30% sucrose solution, as this, like the wholemounts, involves minimal disruption to photoreceptor membranes. Such suspensions, in contrast to the wholemounts of the other two species of stomiid, revealed only two visual pigments, similar to those observed in extract and by MSP (Fig. 21; Douglas *et al.*, in press).

Thus, the retina of *Malacosteus niger*, unlike those of other stomiids, appears only to contain two visual pigments, which on their own, are relatively insensitive to the animal's bioluminescence. However, the outer segents of *Malacosteus niger* contain not only visual pigments but also one or more photostable pigments that give rise to the unusual multipeaked appearance of retinal extracts (Fig. 20a; Bowmaker *et al.*, 1988) and MSP records (Partridge *et al.*, 1989) from this

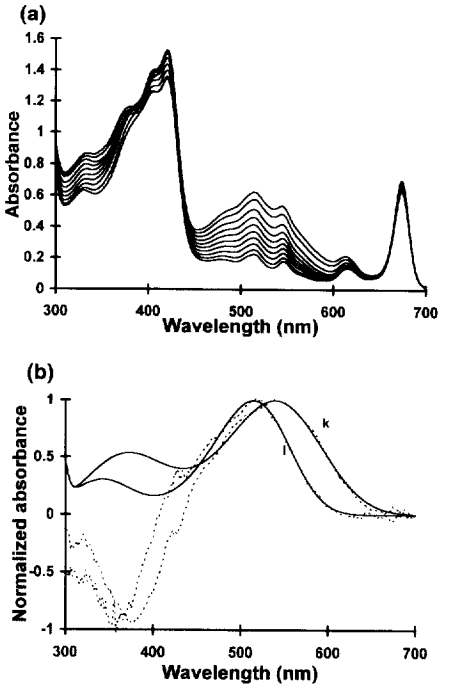

Fig. 20. (a) Absorbance spectra of a retinal extract of *Malacosteus niger* following various durations of exposure to monochromatic light; in order of decreasing longwave absorbance (a) initial measurement of the unexposed extract 10 min after the addition of 20 µl 1M hydroxylamine to 150 µl of extract, (b) after 5 min exposure to monochromatic light of 671 nm, (c) another 10 min 671 nm, (d) another 15 min 671 nm, (e) another 20 min 671 nm, (f) another 30 min 671 nm, (g) another 40 min 671 nm, (h) another 60 min 671 nm, (i) 2 min 501, (j) another 10 min 501 nm. (b) Normalized difference spectra constructed using the curves shown in (a); (j) a–b, (k) h–i. These are best fit by a porphyropsin template with λ_{max} 540, and a rhodopsin with λ_{max} 515 nm respectively (smooth lines). (Douglas *et al.*, in press).

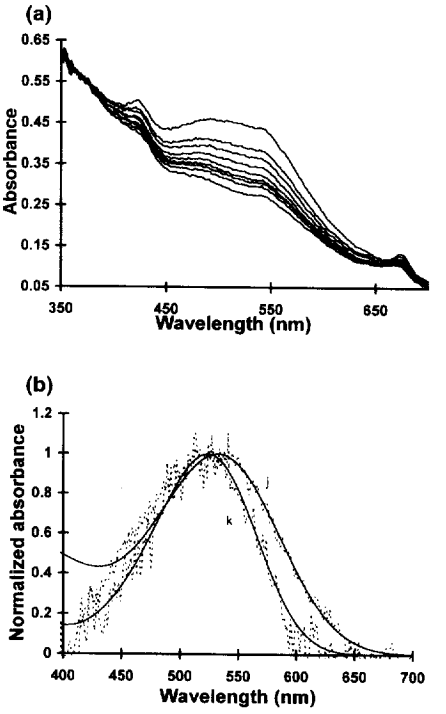

Fig. 21. (a) Absorbance spectra of a retinal suspension of *Malacosteus niger* following various durations of exposure to monochromatic light; in order of decreasing longwave absorbance (a) initial measurement of the unexposed extract 8.5 min after the addition of 200 µl 1M hydroxylamine to 200 µl of extract, (b) after 15 min exposure to monochromatic light of 671 nm, (c) another 15 min 671 nm, (d) another 30 min 671 nm, (e) another 45 min 671 nm, (f) another 60 min 671 nm, (g) another 60 min 671 nm, (h) another 60 min 671 nm, (i) 5 min 501. (b) Normalized difference spectra constructed using the curves shown in (a); (j) a–b, (k) h–i. These are best fit by a porphyropsin template with λ_{max} 533 nm, and a rhodopsin with λ_{max} 522 nm respectively (smooth lines) (Douglas *et al.*, in press).

species. This photostable pigment complex has recently been shown to consist of a number of chlorophyll-derived compounds, such as the pheophorbides bacteriochlorophyll *c* and *d* (Douglas *et al.*, in press). Bowmaker *et al.* (1988) suggested that this pigment complex might act as a photosensitizer, absorbing light at its main absorption peak in the red (*ca.* 670 nm) and in some way isomerising the shorter wave sensitive visual pigments. In order to test this possibility, we determined the sensitivity of both *Malacosteus niger* pigment extracts and outer segment suspensions to various wavelengths of illumination (Douglas *et al.*, in press).

In every case illumination with 671 nm light was more effective at bleaching the visual pigments than 654 nm radiation (e.g. Figure 22). If the bleaching lights were affecting the visual pigments directly the 654 nm light would be the more effective. The greater efficiency of the 671 nm illumination is consistent with the notion that the photostable pigment complex acts as a photosensitizer. At present we can only speculate as to the mechanism by which the photostable pigment(s) act as a photosensitizer. It is possible, however, for photon absorption by this pigment complex to generate a triplet state which could then be transferred to the visual pigment chromo-

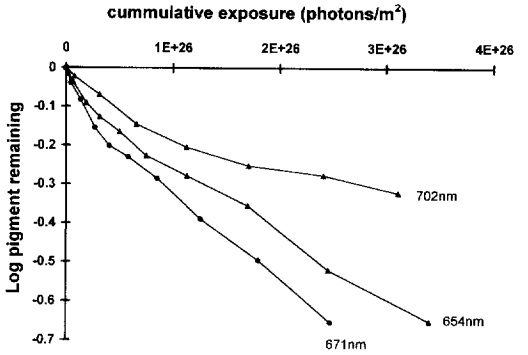

Fig. 22. Effectiveness of lights of different wavelengths at bleaching the visual pigments in a *Malacosteus niger* retinal extract. Three identical extracts were exposed to increasing durations of monochromatic light (e.g. Figure 21a). The sample was held in a cuvette in front of a bleaching light comprising a 75 W stabilised Xenon arc lamp and Balzer B40 interference filters (full bandwidth at half maximum transmission 10 nm). The overall integrated energy ($\mu W/m^2$) of each monochromatic bleaching light at the cuvette within the bleaching chamber was measured using a Spex Industries 1681 Minimate-2 spectrometer system with SCADAS software. These measurements were converted to photons/s/m^2, and multiplied by the total number of seconds that the preparation had been exposed to the light to give a measure of the total cumulative photon exposure. The total amount of photopigment in the preparation at the start of the experiment was estimated by calculating the area under the difference spectrum (450–650 nm) resulting from the subtraction of the absorption spectrum of the completely bleached pigment from that of the unbleached pigment following the addition of hydroxylamine. Similar calculations were performed for each bleach and the resulting area expressed relative to the total amount of pigment present at the start

phore. Energetic elevation to the triplet state has in fact been proposed as an intermediate step in normal visual pigment photoisomerisation (Tahara *et al.*, 1994). Interestingly, such a mechanism may generate damaging levels of free radicals within the photoreceptors, suggesting that mechanisms for active free radical scavenging might exist within the photoreceptors of *Malacosteus*.

Perception of long wavelengths is further enhanced in *Malacosteus*, by a yellow lens (Fig. 3; Somiya, 1982; Muntz, 1983; Douglas and Thorpe, 1992) which removes much of the background spacelight (Section 2.3) and through the possession of a longwave reflecting tapetum (Figs 5d and 6d; Denton and Herring, 1971; Somiya, 1982). A broad band reflecting tapetum would in fact be more efficient in terms of total photon capture than a more finely tuned one. However, such a tapetum would also result in eyeshine visible to other deepsea inhabitants (Section 3.3). From their jet black colour (Marshall, 1979) malacosteids are clearly concerned with remaining unseen. Restricting reflection to the far red using an apparently sub-optimal non broad band tapetum may thus serve to keep these animals hidden from potential predators and prey.

4.3.2.4. *Function of far red sensitivity*

Since stomiids are sensitive to the far red bioluminescence produced by their suborbital photophores, which other animals in the deep-sea cannot see, they have what could be regarded as a "private" waveband. This longwave light could therefore be used for intraspecific signalling immune from detection by potential predators or for the covert illumination of prey. Using published values for photophore emission, the spectral beam attenuation coefficients of water, the transmission characteristics of the ocular media and the visual pigment density, we have calculated that for *Aristostomias tittmanni* the longwave bioluminescence of conspecifics would be visible at a distance of around 2.0 m, while reflective prey would be visible from 1.3 m (Partridge and Douglas, 1995).The distances are about ten times greater than the range of the lateral line organs and demonstrate the great selective advantage of possessing such a private waveband.

4.3.2.5. *Summary of longwave sensitivity in stomiid dragon fish*

All three genera of stomiid, unlike all other deep-sea fishes have evolved a number of adaptations that enable them to see the far red bioluminescence produced by their own suborbital photophores. *Aristostomias* and *Pachystomias* have retinae containing at least three, and possibly four, longwave shifted visual pigments, while the *Malacosteus* retina possess only two visual pigments, but these are coupled to a photosensitizer. Thus, two solutions have evolved to solve the same problem. Perception of long wavelengths is further enhanced by the possession of short-wave absorbing filters in either the retina (*Pachystomias*) or the lens (*Aristostomias* and

Malacosteus), and by a red reflecting tapetum in *Malacosteus*.

The possession of multiple visual pigments in theory gives the stomiidae the basis to discriminate visual stimuli on the basis of hue (see Section 4.3.1). Whether they have this ability is of course not known, but it would surprising if it were not so, as this would be greatly facilitate the distinction between their own bioluminescence and that of other creatures. In the absence of hue discrimination, intraspecific recognition of bioluminescent signals would rely on some form of spatial or temporal coding.

5. FUTURE DIRECTIONS

5.1. Shortwave Sensitivity

Extreme shortwave sensitivity is common among terrestrial and shallow water animals (Bowmaker, 1995; Goldsmith, 1991; Jacobs, 1992; Tovée, 1995; for reviews). However, the electrophysiological (Frank and Case, 1988) and behavioural (Frank and Widder, 1994, 1996) demonstration of UV/violet sensitivity in some deep-sea oplophorid shrimps such as *Systellaspis debilis*, and the characterisation of a shortwave sensitive visual pigment in this species (λ_{max} 410 nm; Cronin and Frank, 1996), was surprising, since these wavelengths were traditionally assumed not to penetrate the deep-ocean. However, recent measurements have shown that enough UV reaches depths of up to 600 m during the daytime to be perceived by these animals (Frank and Widder, 1996). Such shortwave sensitivity has not yet been demonstrated in deep-sea fish, although it would not be surprising if it were found in the future.

5.2. Molecular Basis of λ_{max} Tuning

To date only one study (see Section 4.2.1) has addressed the question of how deep-sea fish visual pigments are "tuned" by their opsins. Further research is required to pin point the amino acid substitutions responsible for the variety of spectrally distinct visual pigments shown in Fig. 7. Perhaps of greatest interest are the structures of the opsins giving rise to extremely longwave sensitive visual pigments in the stomiidae (see Section 4.3.2).

5.3. Regeneration of Visual Pigments

Once a visual pigment molecule has been isomerised during the bleaching process (see Section 4.1) it rapidly regenerates. For several reasons it would be interesting to know the extent and speed of such regeneration in deep-sea fish. Firstly, due to the often high density of deep-sea visual pigments and the relatively low number of photons they are exposed to, it might be argued that the pigments of some deep-sea fish may have no need to regenerate, although calculations show pigment bleaching is probably appreciable even in the deep-ocean (Denton and Locket, 1989). Furthermore, since regeneration involves the retinal pigment epithelium (RPE), it is difficult to see how significant regeneration could occur in those species with several banks of photoreceptors, most of whose photoreceptors are not in contact with the RPE. In fact, it has been suggested that only the most vitread bank of photoreceptors in a multibank retina is functional, the visual pigments in the more sclerad layers having been bleached and not regenerated (Denton and Locket, 1989). Electrophysiological (Shapley and Gordon, 1980) and morphological (Fröhlich and Wagner, 1996) evidence supports the view that only the most vitread layer of rods is fully functional. Limited *in vitro* experiments (Crescitelli *et al.*, 1985; Crescitelli, 1991a) have shown the degree of visual pigment regeneration to vary widely between different deep-sea fish in vitro. It would be interesting to know how such variation is related to species' habitat depth and retinal structure.

5.4. Affect of Pressure on Visual Pigment Absorbance

Since the spectral characteristics of a visual pigment depend heavily on the tertiary structure of a protein (opsin), it is possible that the pressure experienced in the deep-sea will affect the absorp-

tion characteristics of the pigment (see Section 4.2.2.2.2). Future research should thus determine the properties of visual pigments at different pressures varying from those experienced at the surface to full ocean depth (ca. 1–1000 atmospheres).

5.5. Further Pigments in Longwave Sensitive Dragonfishes

The retinae of both *Aristostomias tittmanni* (Section 4.3.2.1) and *Pachystomias microdon* (Section 4.3.2.2) have been shown to contain a rhodopsin/porphyropsin pigment pair absorbing around 515 and 550 nm (Table 2), as well as an extremely longwave sensitive rhodopsin absorbing at around 588–595 nm. Since their retinae already contain the porphyropsin chromophore, 3,4-dehydroretinal, we have suggested that they might, in fact, have an even longer wave absorbing pigment based on the longwave opsin and the A_2-based chromophore. It would be interesting to attempt to isolate this pigment using infra-red illumination during preparation, which, unlike the dim red illumination normally employed, would not bleach such a longwave sensitive pigment.

5.6. *Malacosteus niger* Photosensitizing Pigment

Although the photostable pigment complex within the outer segments of *Malacosteus niger* has now been identified and shown to act as a photosensitizer (see Section 4.3.2.3), further research is required to define the mechanism of photosensitization.

5.7. Electroretinographic and Behavioural Investigations of Visual Function

As noted in Section 1, the most satisfactory way to characterise the properties of an animal's visual system is either some form of electrophysiological recording of preferably a psychophysical measure, both of which are difficult at sea. However, advances in recording technology and refined capture techniques mean that the description of, for example, deep-sea fish spectral sensitivity, response to flicker and absolute thresholds should soon be possible.

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